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"To the solid ground
Of nature trusts the Mind that builds for aye"—WORDSWORTH

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TRAINING AND RESEARCH IN FOOD SCIENCE

FOOD science, though the term is of recent origin, has travelled a long road since a hundred years ago when an Act of Parliament to prevent the adulteration of food and drink reached the Statute Book. Many side-roads—and perhaps not many cul-de-sacs—have been opened up on the way, and now the subject spreads itself across a bewildering complex of scientific disciplines; agriculture, chemistry, physiology, biochemistry, pharmacology, toxicology and pathology are only the more obvious. The subject has attained a position of prominence in the activities of Government, industry and higher centres of learning; and, again rightly, is arousing considerable public interest.

The nation spends £5,000 million annually on food—about a third of all personal expenditure in the United Kingdom. It is not easy to estimate spending by industry on food research, mainly because of the complexities already referred to and the diversity of effort, but it is unlikely to exceed 0·1 per cent of this, or £5,000,000 per annum. (The total estimated research and development expenditure on food, drink and tobacco in 1959 is given as £5·5 million in the Department of Scientific and Industrial Research *Report on Industrial Research and Development Expenditure* (H.M.S.O. 1960).) The amount spent on food advertising is estimated conservatively at £20,000,000 annually.

A number of larger food and allied firms have good research facilities, and Government and industry between them maintain five research associations dealing with food. In addition, there are other centres wholly maintained by Government and controlled through the Research Councils, and these and many departments of universities and other higher educational institutions are concerned directly or indirectly with research in food science. The productivity of all this effort is not inconsiderable, but some areas of inquiry are inadequately covered or wholly neglected. There has been no systematic study of natural foods or of a wide variety of 'traditional' food additives or contaminants, either from the point of view of toxicity or from the point of view of their other physiological or pharmacological effects. Legislation, much of which is of comparatively recent origin, has made it increasingly unlikely that anyone will wittingly adulterate or compound food with potent noxious substances, or with new potential toxins; but the paucity of knowledge concerning weak toxins and substances with peculiar or delayed toxic effects gives little ground for confidence in the complete safety of our food. Lack of finance is only partly, and not primarily, to blame for these wide gaps in knowledge; lack of organization on a sufficiently large scale (which could mean failure to perceive the importance, or even to recognize the existence, of many problems), lack of positive directives in situations where responsibility for tackling problems is divided and lack of suitably qualified investigators have been the more important factors.

The rapid pace of advance in food technology and in food handling practice, sociological and commercial pressures, and the dictates of fad and fancy, all tend towards increasing sophistication in the preparation of food prior to retail sale More and more food is artificially treated with preservatives, flavourings, colourings, and other agents; the calculated results of labour-saving and added appeal may, therefore, be accompanied by increasing and not so readily ascertained toxic risks. There is no doubt that understanding of the long-term biological effects of these practices is not keeping pace Industry. in all its ramified operations from primary production to presenting food on the consumer's table, no doubt does the best it can within present limits of knowledge to ensure a harmless and wholesome result. But this best often falls a long way short of the desired standard. The crucial question is whether conventional test methods are safe, consistently reliable, and their results sufficiently applicable to man.

The Government and industry are advised in these matters by a group of expert bodies the terms of reference of which effectively cover all the possible hazards likely to arise from what is done to food and put into food, and from food itself. There is a Food Hygiene Advisory Council which advises the Ministry of Health and the Ministry of Agriculture, Fisheries and Food on matters concerning the handling, transport, labelling and advertising of food. The Food Standards Committee, recently reconstituted, works largely through a Food Additives and Contaminants Sub-Committee which considers problems in relation to all substances added to food, whether deliberately or not. A Standing Advisory Committee on Poisonous Substances used in Agriculture and Food Storage ensures that food produced from treated crops contains no harmful residues, and a special research study group is also concerned with pesticides and herbicides and "the needs for further research into the effects of the use of toxic chemicals in agriculture and food storage". The most recent of these bodies to be set up is a Standing Panel on Carcinogenic Hazards in Food Additives and Food Contaminants. The field appears to be under continuous and thorough scrutiny in Great Britain; there are also the regular joint conferences between the Food and Agriculture Organization (F.A.O.) and the World Health Organization (W.H.O.) of the United Nations on the subject of food additives, and much knowledge and advice are available from other countries, some of which, notably the United States, have as yet a more rigorous legislative. if not scientific, approach.

This whole edifice of advisory machinery and safeguards rests ultimately on a foundation of fundamental knowledge of general human physiology and, in particular, of human digestive and metabolic processes, but it is difficult to escape a feeling that this foundation is inadequate. There is no case for lessening the load of the structure; it is the foundation which must be strengthened by greater efforts to widen and deepen the extent of knowledge about what really happens biologically when man assimilates his food with, on occasion, some component which may have immediate or delayed deleterious effects.

A joint F.A.O./W.H.O. Expert Committee on Food Additives meeting in 1957 examined procedures for the testing of food additives to establish their safety for use. It was pointed out that as additives may be taken for the greater part of a lifetime and produce hitherto unsuspected reactions, relatively much higher doses of the additives than human beings are likely to receive should be fed to experimental animals for most or all of their lives. No single pattern of tests will do for all the many additives—hence the Committee could make only general recommendations—and even so, it admitted that absolute proof of non-toxicity could not be established since there may also be occasional cases of idiosyncrasy. The Committee recognized that the best evidence for the safety of food additives must come from human studies, but that because of the obvious difficulties reliance would have to be placed on other methods, among them animal experiments. The Committee suggested that the use of high dosage-levels and the spread of investigations over a number of different species made it reasonable to extrapolate the data to man; but it allowed that there would need to be some margin for species differences in susceptibility, the numerical difference between the relatively small numbers of test animals and the relatively large human population at risk, the greater variety of complicating disease processes in human beings, the difficulty of estimating human intake, and the possibility of synergistic action between different additives and between additives and other environmental factors.

It is possible that the F.A.O./W.H.O. Committee is here putting too little emphasis on the allowances which need to be made for species differences in susceptibility and the validity or otherwise of extrapolation of findings to the human case. In another and closely related connexion, the screening of pharmaceutical products by resort to laboratory animal tests, it has been argued that this is of doubtful value in relation to assessing safety of administration to humans. It has been said that the pharmacological activity and toxicity of drugs vary, both qualitatively and quantitatively, between the widest limits in the diversity of animal species used in medical research; and that similar wide differences occur even between different strains of the same species, and between the sexes.

What will undoubtedly go far to repair deficiencies in our understanding of these problems, and to provide a broader and sounder base for advice and legislation, was the creation in 1960 of the British Industrial Biological Research Association (B.I.B.R.A.). More recent proposals for a food research institute at Norwich may also contribute much in this direction. B.I.B.R.A. has been formed to study the possible effects on health, and to ensure the harmlessness, of substances which may be ingested in food, drink and cosmetics. The Association's main object is the establishment of a biological research station for investigating the effect of substances used in food manufacture, either as processing aids or for flavouring and colouring food, as well as those which may get into food from pesticides, from plant used in food manufacture, from packaging materials, or from utensils.

The new Association, supported equally by industry and by the Government, will clearly have to devote much of its effort to dealing with industry's immediate needs for tests and advice on the safety of particular substances and processes; in the case of smaller firms this is primarily what the industry is paying for; in the case of larger firms, having research facilities of their own, the prevention of costly duplication of basic investigations is probably equally important. Of great interest to industry also is the part the Association can play in simplifying, speeding up and reducing the cost of testing procedures; a comprehensive test of a substance, using several species of laboratory animals and designed to show up any carcinogenic as well as other toxic hazards, can at present cost upwards of £20,000. But the Association is rightly taking a much wider view of responsibility for ensuring that food is safe and known by the public to be safe; a responsibility not in the nature of things so easily attached to any particular section of the food industry—producer, chemical and plant manufacturer, converter and distributor. For apart from the more routine testing function, the Association will engage in research into the basic biological mechanisms underlying the testing methods themselves, and into topics such as comparative toxicology. This programme of more fundamental inquiry might have the very desirable result of improving present testing procedures and devising new ones which will be safer and more reliable. It is very much to be hoped that industry, and indeed other appropriate sources of funds, will recognize the importance of this wider function and be willing to help finance it adequately.

But problems are accumulating more rapidly than they can be investigated and B.I.B.R.A. cannot hope to tackle more than a fraction of them. Other research associations concerned with food might, therefore, consider devoting more attention to fundamental studies. University departments might also help, and the colleges of technology, some with long-established interests in food science and much good relevant research to their credit, could increase the significance of their contribution. Many other centres—Governmental, industrial and private—could also aid in the better understanding of matters such as the biochemistry of food metabolism, comparative toxicology and pathology. The whole methodology of testing procedures, as already implied, needs much closer study, and, since the facilities for animal experimentation available to B.I.B.R.A. are likely to be limited for several years, this is a field which should commend itself to centres already well equipped for animal studies.

Yet more pressing still than the inadequacy of material facilities is the acute shortage of scientists with the necessary qualifications and experience, particularly in the fields of pathology and pharmacology. Furthermore, a good grounding in any one discipline is rarely adequate, by itself, for grappling effectively with the many complex problems which arise in this general field. Often it is necessary for teams of specialists from different disciplines to work together on such problems. From co-operative efforts of this kind individuals with some specialist knowledge in several disciplines eventually emerge, but at present these constitute a *rarum genus*.

Able specialists in physical, biological, medical, or veterinary science have seldom shown a special inclination to engage in fundamental food science studies, nor to fit themselves better for these by seeking appropriate training or experience. They may often harbour the unfounded suspicion that subsequent career prospects may not be especially attractive. It is timely, therefore, that the Nuffield Foundation has now announced that it is prepared to offer a number of scholarships to encourage science, medical and veterinary graduates to acquire wider experience and expertise in fundamental food science. Details of these awards appear on p. 20 of this issue, and it must be hoped that suitable graduates will avail themselves of these new opportunities. Those who do can certainly look forward to finding ample scope for fundamental research, and to progressively improving career prospects. Industry, Government agencies, and the academic world are likely to make increasing demands for high-calibre people with wide experience; the Nuffield scheme should succeed in stimulating a greater flow of able people into the field to meet this demand.

The scheme can do little to alleviate the general shortage of food scientists as such, designed, as it is, to meet a critical need only in the higher echelons where it is increasingly important and urgent for more work to be done on basic metabolism, toxicology, and pharmacology, and the methodology of testing. The shortage of food scientists and technologists in general is another and very great problem, and it is quite clear that present facilities for training greater numbers of graduates are inadequate. A symposium at last year's meeting of the British Association for the Advancement of Science showed that the present output, plus planned extensions in the United Kingdom, would by no means keep pace with the increasing demand over the next few years, and this assessment takes no account of the vast training needs overseas which, at least so far as the Commonwealth is concerned, should be partly met by Britain. The scale of the requirement for more training facilities in the United Kingdom, even for internal needs, is quite beyond the resources of any private finance and must be a charge on the Government, but the Nuffield scheme could well provide some of the inspiration and leadership which will be essential.

The Nuffield scholarship awards should also be helpful to existing research institutions concerned in one way or another with food science. It is sometimes observed that Government research establishments and the research associations suffer from the evils of having such high proportions of permanent staff; there is all too little mobility among the established staff and too few opportunities for temporary attachment of visiting research workers. These factors tend to lead to a poverty of fresh ideas. A woeful reluctance, sometimes refusal, on the part of universities to allow graduates working in these organizations to register for higher degrees can mean further impoverishment. It will be possible, however, for holders of the Nuffield awards to receive part of their training in a research institute or a research association (whether or not they can there study for a Ph.D.), and being selected for their ability and imaginative outlook they should be an asset to any host institution. Their numbers, necessarily small, will perhaps have little enough overall impact; but other ways should be found to follow the Nuffield lead and create more opportunities for temporary attachments of this reciprocally beneficial kind.

NATURAL RESOURCES AS POPULATION PROBLEMS

The Exploitation of Natural Animal Populations
Edited by E. D. Le Cren and Dr. M. W. Holdgate. (The British Ecological Society, Symposium No. 2.) Pp. xiv + 399. (Oxford: Blackwell Scientific Publications; Springfield, Ill.: Charles C. Thomas, 1962.) 60*s*. net.

FOR thirty years the British Ecological Society has sought to encourage a common outlook on population ecology among workers on different groups of animals, many of whom tend to be partly isolated by their special economic interests in pest control, game management, fisheries and the like. This has been done through the agency of the *Journal of Animal Ecology*, and latterly also through special conferences.

The present Symposium attracted more than a hundred people of very varied backgrounds, from ten countries. Of the twenty-one papers printed in this volume, there are two on land mammal populations (Denmark and Africa), four on seals (Canada and Britain), two on whales (Norway and Antarctic), three on wildfowl and gamebirds (Britain), seven on marine or freshwater fish (mainly from Europe), and three on invertebrates. The last three are on very diverse subjects: British marine benthos, Canadian insects, and American laboratory experiments on waterfleas and algæ. There is also a general discussion, of far less value than the individual papers. The latter also range widely in emphasis from the directly economic manipulation of populations at one end, to quite complex mathematical discussions and models at the other. But there is a fairly coherent skein of common interests running through them: the ways in which populations may be regulated naturally, as well as by man, their fluctuations, age distributions, input and mortality, and so on. The usefulness of such a common forum for such a variegated field of effort is clear enough. The papers are rather unusually clear, full and business-like for conference contributions, and carry most informative bibliographies in their special fields, and the editing has been very capable. The renderings of questions and answers after the papers, though adding a little to clarity, are probably not worth the great labour of rescuing them from oblivion. There is a good index, which is worth perusal in any event as an indication of the remarkable concourse of subjects involved in population ecology: for example, "Giraffe, Glen Esk, *Glossina*, Goats, Gompertz Curve, Goose . . . Grilse, Grimsby, Ground-nut, Grouse".

It might be supposed that the easiest part of population research would be making a complete census of part of the population. But in many instances this is not only the most laborious but also the most difficult technically. An investigation of a Danish roe-deer population, in which individual deer were caught and then released with special marked collars to wear, revealed that something like three-quarters of the whole population would need to be marked, in order to get accurate estimates of the total. The age distribution of a species recurs in these studies as one of the crucial components of the whole picture, and to get this it is necessary to find age indices (such as fish-scales, plumage stages, ear-plugs or annual shell-rings), or else mark animals over some years. To get any notion at all of the changing population structure and movements is a very long and exacting task. Nearly all the investigations described still have large blank patches. The mathematical models being developed, though useful and promising, are mostly far from fitting known facts, let alone predicting unknown ones. The rational exploitation and management of wild populations, therefore, will require many more years of basic research.

C. S. ELTON

THE ATLANTIC AND GULF COAST GEOSYNCLINE

Geology of the Atlantic and Gulf Coastal Province of North America

By Grover E. Murray. Pp. xvii + 692, illus. (New York: Harper's Geoscience Series, Harper and Brothers, 1961.) 24.00 dollars.

THE Atlantic and Gulf Coastal Province of North America is a well-marked physiographical and geological entity extending from Newfoundland to Central America, the part north of Cape Cod being submerged, but the rest partially emerged. Its surface geology is well known, and its subsurface geology is known better than that of anywhere else in the world, because of the intensive drilling for oil which has taken place and because of the numerous geophysical investigations carried out Prof. Murray has brought together the information gleaned from 10,000 papers published on the Province, as well as that from numerous private reports to which he has had access, so that fundamentally this is a factual account with only rare theoretical discussion.

This great coastal entity is an extensive "geosyncline consisting of an irregular mass of Mesozoic and Cenozoic sediments, grossly lenticular in the land-sea dimension and superimposed upon Palæozoic and Precambrian rocks, the surface of which dips generally seaward". These sediments, commonly unconsolidated, vary from some 20,000 ft. along the Atlantic portion to from 40,000 to 50,000 ft. around the Gulf of Mexico. Rightly, the author discusses the areas flanking the Province, since their geology has had a profound influence on it. The basement in fact, is essentially a continuation of those areas both stratigraphically and structurally, and folded and faulted metamorphic, igneous and Palæozoic and Triassic sediments are present. In a chapter on structural geology, the initiation, development and growth of the geosyncline are thought to have been connected with the elevation of the continental platform following the late Palæozoic orogenies and the destruction of the Palæozoic geosynclines. The sedimentary load was probably intimately related to its development—"downbuilding of the geosyncline . . ., once an initial site for deposition was established, seems to have been continually abetted by the load of sediments, a process that has continued to the present day". Thus structurally the Province is essentially one of vertical movement (though compressional movements in Mexico produced the folded area adjacent to the Sierra Madre Oriental) The major fracture zones, which occur in much o the Province, trend north-east–south-west and north-west–south-east, and are aligned with the fracture patterns in the older areas bordering it Control by Precambrian and Palæozoic structure and trends is thus evidenced, and is doubtless rendered by major fractures in the basement rocks.

The salt structures, which occur in the 'deep south and in Mexico, are domes, anticlines and ridge-like diapiric folds of great structural magnitude and o varying height and extent, for example, up to six miles in diameter at the surface. They are described in some detail and their relationship to sediments cap-rock, etc., discussed. The author considers that clusters of them seem to be related to thick salt and extra thick salt overburden and to zones of strike faulting and flexing. There is a long and useful section on regional stratigraphy, with the emphasis on lithology. Upper Cretaceous and later deposit make up most of the surface rocks of the Province especially of the part in the United States, though Jurassic and Lower Cretaceous rocks occur ther below ground and outcrop in Mexico and in a few smal areas in Arkansas, Oklahoma and possibly Alabama The account of the mineral resources is very much condensed, but they are abundant and very important, especially oil and gas. The book concludes with two geographical chapters. The first, on physiography, deals with both the submerged and th emerged areas of the geosyncline, the former with a subdued topography, and the latter with its topography clearly related to its alternating types o rock, the seaward progression of depositional centre and axes, and the progressive tilting of the olde strata. Once again Mexico, with mountains carved out of the uplifted strata, shows contrasts with th rest of the Province. The final chapter deals i summarized form with climate, vegetation and soil —the climate, ranging from tropical wet to humid microthermal, produces different results on th landscape, soils, drainage and vegetation of th various regions into which the coastal plain is divided and hence on the human geography.

With such a comprehensive factual account, covering so large an area and so many aspects, compression the presentation of data is necessary. This does make for easy reading, but the numerous, clear grams facilitate this, and the author has carried his task well. In the result, this detailed study great geosyncline is of importance not only to erican geologists, but also to all who are interested e origin, composition and structure of such major tures of the Earth's crust.

The book is provided with a selected bibliography each section as well as a comprehensive biblio-raphy at the end. It is well produced and clearly rinted.

H. Dighton Thomas

TEXTURES IN SHEET METALLURGY

Textures in Metal Sheets

By Dr. F. A. Underwood. Pp. xii + 148. (London: Macdonald and Co. (Publishers), Ltd., 1961.) 28*s*. net.

The technological importance of texture has recently been emphasized by several new developments. Twenty-five years ago anisotropic properties became commercially useful in silicon–iron transformer core material; about six years ago cube texture was also produced in this alloy. Furthermore, in this past six years many details of processing have become known generally. Less common metals, such as bismuth and uranium, are being used commercially; there is strong interest in their anisotropic properties, particularly where they are subjected to cyclic heat treatment.

Control of texture is important and might arise, for example, through alloying, by the control of a separate phase or by the control of the annealing atmosphere. Much of the information on these aspects has become known recently. While this is not covered in any detail by this book, it does give a useful introduction to the subject. The book is intended either for beginners, who hope to get more involved in the detail, or for those with peripheral interests such as managers, who primarily want to understand results in investigators' reports. Nevertheless, a small extension to the book would have presented a more complete, up-to-date and useful contribution. The lack of very recent references is apparent in several instances.

The elementary level of starting is clear from the introduction and first chapter on preferred orientation. Efforts to keep the book short are laudable but might leave some bewilderment; for example, When are Wulff or stereographic nets used? Methods for determining pole figures are very well described, with the exception of optical techniques. It should be pointed out, however, contrary to p. 55, that instruments are available commercially for constructing pole figures.

Textures are discussed in separate chapters for rolling and recrystallization. They are good summaries but omit some important points. The author pointed out that the work of Bishop and his colleagues gave a reasonably rigorous treatment of rolling textures, but otherwise gave it only a brief mention. The effects of alloying, impurities and atmospheres on recrystallization textures are almost completely ignored.

The last chapter on anisotropy reports measurements on mechanical properties. Earing is discussed in detail. Only in the last few pages is the torque magnetometer mentioned. This is surprising since it is such a useful tool.

It is easy to criticize somebody else's efforts, but it should be clear that most of these comments imply that the book should be longer and more expensive. In fact, the author is to be congratulated on keeping size and cost down to a level at which students can afford to buy it. It is a useful starting point for students in this field; all metallurgical libraries should have a copy.

G. M. Leak

LIFE: NORMAL AND ABNORMAL

La Vie

Par Jean Rostand et Andrée Tétry. Pp. 468 (960 illustrations). (Paris: Librairie Larousse, 1962.) n.p.

Anyone glancing through this comprehensive, abundantly illustrated and well-produced volume could be forgiven for thinking life was very odd. Scarcely a page is passed that does not contain a picture of the abnormal, from the slightly unusual to the chimerical. So we run the gauntlet from the inevitable mutant *Drosophila* through Siamese twins, the two-headed newt and fork-tailed lizard to albinism and children born with neither hands nor feet. Since the natural freaks are relatively limited, there is a strong reinforcement of the monstrosities produced by selective breeding, experimental zoology and the nuclear device exploded over Hiroshima.

This macabre display of pictures seems scarcely in keeping with the sober and well-informed textual matter, and there may be two reasons for this apparent emphasis on the teratological. First, as every student of taxonomy knows, it is often the exception that sheds more light on, or even lays bare, the truth about the common run. Secondly, as the anatomist and the morphologist have learned, and the behaviourist is beginning to learn, the exceptional or the abnormality is more common than the text-books would lead us to believe. Perhaps there is a third reason, that when the life sciences are treated as exhaustively as they are here there is bound to be a fair proportion of space devoted to these freaks.

Another unusual feature of the book, which has everything to recommend it, is that not only are the various evolutionary theories set forth but so are the arguments against them, even those against the most orthodoxically acceptable. Nothing could be fairer. It is this, perhaps more than anything else, that reveals the true purpose of the book: to bring together within a single volume, as objectively as possible, knowledge that elsewhere tends to be spread over many publications and which, as often as not, reflects the bias of a particular author. The specialist scientist no less than the untutored layman wishing to be abreast with present-day knowledge and theory can expect to find in this book the answers to questions outside his competence. In this, the text is no disappointment. It begins with morality and religion, passes to the consideration of the living cell and the latest knowledge on this important topic, proceeds to sexuality, growth and development, senescence and death, regeneration, the many aspects of genetics, evolution, including that of the Hominidae, and ends with some speculations on the future of the human race. One can but envy the authors their erudition—or the amount they must have learned in compiling the volume—and congratulate them on the fruits of their labours.

Maurice Burton

Progress in Organic Chemistry

Vol. 5. Edited by Dr. J. W. Cook and Dr. W. Carruthers. Pp. viii+172 (Progress Series). (London: Butterworth and Co. (Publishers), Ltd., 1961.) 50*s*.

THE present volume contains a mixture of topics drawn from the fields of natural products, synthesis and reaction mechanisms. Dr. W. A. Waters's lucid chapter, "Homolytic Oxidation Processes", includes sections on autoxidation and one-electron oxidations by ions containing transition metals. "Developments in Hydroxylation of Phenols", by Dr. J. D. Loudon, is a beautifully presented survey of recent preparative methods in this field, including the author's elegant ortho-hydroxylation sequence. "The Chemistry of Dextrans", by Dr. C. R. Ricketts, summarizes present knowledge of the structure and biosynthesis of this polysaccharide which finds important application in blood transfusion. "The Chemistry of the Higher Terpenoids", by Drs. J. A. Barltrop and N. A. J. Rogers, includes some diterpenoid topics of recent interest and an excellent summary of the now very fully explored biosynthesis of squalene and some of its cyclization products. Prof. T. Nozoe contributes the final chapter, "Tropylium and Related Compounds", dealing with the tropylium ion and recent syntheses of tropones and tropolones, including some naturally occurring tropolones. The interesting photo-chemical transformations of colchicine and simpler tropolones and a postscript on the chemistry of heptafulvene conclude this chapter.

Progress in Organic Chemistry aims to summarize areas of the subject where a vantage point has been reached and a degree of order can be seen to emerge. The present volume is a good example. Viewing the series as a whole, the editors' choice of topics has in many cases been timely and the list of contributors includes an impressive number of acknowledged authorities. The non-specialist reader, to whom these reviews are addressed, will look forward to future volumes. He may, however, in view of the price, regard the drastic reduction in size of the present volume with some misgivings. K. H. OVERTON

Advances in Agronomy

Vol. 13. Edited by A. G. Norman. (Prepared under the auspices of the American Society of Agronomy.) Pp. xi+386. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 86*s*.

THE general agronomist, whose chemistry may not be very near the surface, may find some of the articles in this volume specialized or difficult. Thus, the contributions on physical chemistry of clay–water interaction and that on iron chlorosis in plants are rather heavy going, while the article by the late Dr. Alex. Muir on podzol and podzolic soils will be fully appreciated mainly by pedologists and soil surveyors.

Compared with the above the treatment of subterranean clover is almost bedside reading. This paper naturally comes from Australia, where, thanks to sound experimental work, this crop has put a new face on millions of acres in that country. All aspects of this fascinating crop have been dealt with, and some 250 references quoted. Equally readable is the summary of the barley yellow dwarf virus disease of small grains. The widespread virus diseases of cereals are not well known outside pathology circles. The cause of the disease, an aphis-borne virus, was only discovered in 1951. A very clear pictur
the follow-up of this discovery is given. As so c
happens with the diseases of cereal crops the
hopeful remedy appears to be in the hands o
plant breeder. Stubble-mulch farming is ty
associated with wheat monoculture in arid or
arid areas of the United States, where one of it
functions is to stabilize the soil against erosion
review shows that there are still plenty of prob
to be solved, especially if the practice is to be extenc
to areas with greater rainfall.

There are shorter articles on earthworms, the effe
of which on soil productivity are still somewh
uncertain; and the rather specialized problem of tl
agricultural aspects of the contamination of soils b
petroleum hydrocarbons. H. V. GARNER

An Introduction to Statistical Science in Agricultur

By Dr. D. J. Finney. Second edition. Pp. 21
(Edinburgh and London: Oliver and Boyd, Ltd
1962.) 30*s*. net.

IN the course of the preface the author state
"The book is intended to supplement . . . a
elementary course of lectures for agricultural st
dents who are without mathematical training"–
"The book is designed for consecutive reading, n
for occasional reference"—" . . . my chief aim is
show statistical science as a whole—as a single att
tude of mind to quantitative problems, and not
a series of isolated methods . . .". In all these ai
and intentions Dr. Finney has succeeded admirabl
This second edition extends the discussion of sampli
and sample surveys and adds a chapter on gener
questions of experimental planning; there are al
other additions and revisions. The author is partic
larly successful at indicating the backgrounds
experimental situations, wherein lie all the difficu
problems that so rarely yield a cut-and-dried soluti
for the conduct of an experiment. At the same tim
basic statistical ideas are lucidly dealt with, with
the necessarily restricting framework of the boo
Students and research workers alike, who are worki
in this field, will scarcely fail to find something
interest them here. J. A. NELDER

Teach Yourself Atomic Physics

By Dr. J. M. Valentine. (Teach Yourself Book
Pp. 192. (London: The English Universities Pre
Ltd., 1960.) 7*s*. 6*d*. net.

THIS little book is intended to give the laym
an introduction to the ideas of atomic and nucle
structure and of the experimental methods used
atomic physics. The language is simple and math
matics are confined to a few elementary equation
The subject is developed along orthodox lines, begi
ning with kinetic theory and the gas discharge, an
proceeding through the Rutherford–Bohr model
the atom to an outline of nuclear structure an
stability. There follow chapters on particle acceler
tors, detectors, nuclear reactors and nuclear weapon
and finally on the industrial, medical and biologic
uses of isotopes.

The treatment is clear and perhaps more full the
is usual in popular books. But a book with this titl
published in the 1960's, ought to give its reader som
notion of wave mechanical concepts. As it is, he
left with a firm impression that electrons move
circular orbits about a nucleus which can be pen
trated only by particles capable of surmounting t
potential hill around it. K. E. B. JAY

FAITH AND FACT*

By THE RIGHT REV. W. L. GREER

Lord Bishop of Manchester

ow faith is the substance of things hoped for,
e evidence of things not seen." *Hebrews XI.*

nd what is faith? Faith gives substance (or assurance) our hopes, and makes us certain of realities we do ot see." (N.E.B.)

IN 1951, during the meeting of the British Association for the Advancement of Science held in Edinburgh, my friend the late Prof. Donald Baillie gave an address to the Association and others in St. Giles Cathedral. The theme of his discourse was that the most significant part of the conflict between religion and science was not between the scientists and the theologians as two different sets of people, but rather etween two diverse tendencies in the same person, two parts of one man—between the scientist and the heologian existing in the same human breast. t was a tension in the mind of the intellectual etween the honest consideration of factual evidence n one hand, and the haunting claims of faith on the ther.

Now I would wish to pursue this theme a little rther.

We are, I think, all familiar with the fact that in very one of us there are two areas of experience which frequently seem to be in conflict. Indeed we might say they are incommensurate. I refer to the rea of fact and the area of faith.

The area of fact is open to those who have the skill, he intelligence and the insight to observe and o-ordinate natural phenomena. But the area of faith—that is, the area in which God becomes a known eality—is open only to those who (in Prof. Baillie's rase) are "simple-hearted and honest, with something of the wide-open eyes of little children gazing t reality". It is open to those who are not sophisicated by pride, but are obedient and dedicated. The knowledge of God is an assurance, given to humble, honest, obedient hearts. To them, the universe discloses its most vital secrets. What is hidden from the mere intellectual is "revealed unto babes".

Science concerns itself with fact, with the outward, the visible (or potentially visible), with the temporal; and its language is mathematics. Religion deals with faith, with the inward, the invisible, the eternal; and *its* language is poetry. Both are areas of true knowledge, and both are essential to a full human life.

I am, of course, drawing a rough contrast. For these two areas interpenetrate. Science has a faith in the rationality of the universe and in the uniformity of its material constituents, though its faith is a heuristic principle rather than a final commitment. Many of its greatest discoveries have been made in realms for ver invisible to the human eye. Christianity, n the other hand, is vitally concerned with facts, h daily bread, with human actions, with justice, with a historical person who was crucified under a certain Roman Governor, Pontius Pilate, whose name (it is interesting to note) has for the first time been recently discovered on a contemporary Palestinian inscription.

Nevertheless, although these two areas of human experience interpenetrate, the contrast still remains. It is a disturbing, bewildering and perplexing contrast. We venture out on the sea of faith, and fear lest the white canvas of our intellectual integrity may be torn from the mast. We confine ourselves to the study of material things, and the fear takes hold of us that the mind may become a machine, and we may lose something vital in our human inheritance. There is a passage in Darwin's *Autobiography* where he says: "Up to the age of thirty or beyond it, poetry of many kinds, such as the works of Milton, Gray, Byron, Wordsworth, Coleridge or Shelley, gave me great pleasure. I have also said that formerly pictures gave me considerable, and music very great, delight. But now, for many years, I cannot endure to read a line of poetry. My mind seems to have become a kind of machine for grinding general laws out of a large collection of facts, but why this should have caused the atrophy of that part of the brain on which the higher tastes depend, I cannot conceive. The loss of those tastes is a loss of happiness, and may possibly be injurious to the intellect, and more probably to the moral character, by enfeebling the emotional part of our nature". Charles Darwin's experience must have many counterparts to-day when specialization is almost pre-natal.

On the other hand, we can see in the history of religion the disastrous effects of a faith uncontrolled by facts or by rational reflexion. It is recorded of one religious enthusiast that she refused to believe in the physical existence of the city of Jerusalem until it was revealed to her by the Spirit. In poetry and the arts, uncontrolled and undisciplined imagination easily leads to the chaos and the madness of a private world. It is not without significance that Wordsworth was keenly interested in chemistry, and sent the first proofs of his *Lyrical Ballads* to the young scientist Humphrey Davy to correct.

So then, we have these two areas of human experience, distinct, yet not divisible, separate, yet interpenetrating, and in these areas, the pathway to experience presents a contrast, a contrast well described by Pascal when he says "Human things must be known to be loved—Divine things must be loved to be known".

The area of faith, of course, has to do not only with divine things, but also with all things of the spirit, and in particular with human personal relationships. Here we observe at once the inapplicability of scientific method. A young scientist decides that he wishes to get married; he sets about this highly important experiment scientifically and methodically. Having purchased a card index, determined his system of marking for certain desirable qualities, he canvasses all the feminine possibilities. Then, having put the cards in the appropriate machine, he presses the button and obtains the answer. Unfortunately he

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Substance of the sermon delivered on September 2 in Manchester thedral during the service attended by the Lord Mayor, the Vice-ncellor of the University and the President and members of the tish Association for the Advancement of Science.

discloses his method of procedure to the lady whom the machine has chosen, and she, so far from being pleased, calls him inhuman and sends him about his business. Persons ought not to be treated as things. They, too, must be loved to be known.

I have said that the language of religion is poetry. It is common knowledge that much of the Old Testament is written in Hebrew poetry, and a great deal of the teaching of Jesus Christ seems to have been given in poetic form. But there is more to it than that. There is, I believe, an inner and necessary relationship between religion and poetry. Wordsworth in his Preface to *Lyrical Ballads* expresses well what I have in mind. He writes: “Aristotle has said that poetry is the most philosophic of all writing; its object is truth, not individual and local, but general and operative, not standing upon external testimony but carried alive into the heart by passion. It is the breath and finer spirit of all knowledge; it is the impassioned expression which is in the countenance of all science”. He continues: “The concerns of religion, refer to indefinite objects, and are too weighty for the mind to support them without resting a great part of the burden upon words and symbols. The commerce between man and his Maker cannot be carried on but by a process where much is represented in little. In all this may be perceived the affinity between religion and poetry”. Precisely here is the point of greatest difficulty. The language of religion, which is poetry, is so utterly different from the language of science. We must learn to become bilingual. Perhaps we would, if we were convinced that the other language has real truth to convey.

In the area of experience covered by faith comprising the spiritual relationship between persons, the methods of science have little or no place But that does not mean that the truths there revealed or discovered are illusory or less important. For human happiness they are indeed incomparably more important. They are certainly less exact, less precise, less easily communicable, and less subject to proof. That is but to be expected, for here we are attempting to understand not the part, but the whole. As Walter Bagehot says: “a clear, precise, discriminating intellect shrinks at once from the symbolic, the unbounded, the indefinite The misfortune is that mysticism (and by that he means Christianity) is true”. We can, as some Eastern philosophers do, write off our experience of the material world as illusory—or (and this is the temptation of Western man), write off the Christian Faith as a fairy tale; in either case, human life is impoverished. The two areas of experience must be held together, and the validity of neither denied. That is no easy task in a world dominated by power politics, greed and materialism; but unless it is done deliberately, persistently, and with cost, there is no future eithe for scientific progress or for humanity.

I have spoken of the area of fact and the area faith. “But what is Faith ?” asks my text . . . “Fait gives substance to our hopes and makes us certai of realities we do not see”. It is not belief in spite evidence, but life in scorn of circumstance. I involves committing ourselves and throwing ou actions behind our beliefs. As Hartley Coleridge says “Faith is an affirmation and an act That bids etern truth be present fact”.

That, at a particular time in history, the etern truth became a present fact is precisely what w as Christians, believe. “The word was made flesh an dwelt among us and we beheld His glory (the glor as of the only begotten of the Father), full of grac and truth”. The author of the *Epistle to the Hebre* recognizes this. for in the first verse of the very nex chapter he tells his readers that they must run wit resolution the race for which they are entered, the eyes fixed on Jesus, on whom faith depends fro start to finish.

The secret of this strange and wonderful univers in which we live cannot be contained in any equation only in a person; uniquely, in One Person. An faith depends on that Person, because He was and Himself, the assurance of things hoped for, th evidence of things not seen. It is as we keep our eye fixed on Him, hating the things He hates, loving th things He loves, looking to Him for light to guide u and courage to sustain us—it is as we do these thing that experience validates faith, and we come t know with deeper assurance that He is not only th Way, but also the Truth—a truth no other in kin than that which we seek in our laboratories, yet a a deeper level, on a grander scale, and at a greate cost. That must be so, since it is concerned wit God and Man; God in his glory, love, and power; an Man, his humble origin, and unimaginable destiny.

The British Association has as its object th advancement of science. That is to say, science a commonly understood in its limited meaning. Bu science, of course, really includes all knowledge, an in this age of dazzling discovery, so great are the socia and ethical responsibilities of scientists that I d not see how they can be properly discharged unles we humbly and patiently set ourselves to advanc not only in the accumulation of material facts, bu also in that knowledge that comes through faith Might we not say that—social investment in scienc demands personal investment in faith.

If we do, we shall surely find, as countless other have found, that wisdom is added to our knowledge and that faith (though at the outset a venture increasingly gives assurance to our deepest aspiration and makes us certain of realities we do not at presen see.

MEETING WITH MACHINES*

By PROF. AUREL DAVID

École Pratique des Hautes Études, Sorbonne, Paris

THERE is no difficulty in defining cybernetic medicine; it is a medicine using cybernetic knowledge for diagnosis, treatment or simply for understanding the process of an illness. The weakness of such a definition lies in the fact that it i practically impossible to define the term ‘cybernetics itself. Yet, lack of definition has in no way im paired the promotion of other sciences. If w are able to enumerate a certain number o subjects, apparently dissimilar, connected wit cybernetics, time will show the thread connecting these problems.

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* Substance of an address delivered at the Second International Congress of Cybernetic Medicine, Amsterdam (1962).

It is already possible to isolate certain more general parts, and specifically cybernetic methods[1]. But these methods are for the present applied to some well-defined and curiously dissimilar points. One could try to give an idea of what cybernetics are by considering those points and examining the spirit in which they are considered.

Cybernetics are connected with questions of:

(*A*) *Loss of determinism*, especially in simple and macroscopic mechanisms connected with an act which is beyond computation, not to be foreseen by its builder himself and giving an irresistible feeling of 'liberty'.

(*B*) *Simulation of functions in a living being* (M. N. Wiener's classical work dealt with this) with an unmistakable preference for those functions which seem the most obscure and farthest from usual material physics. Artificial organs exert an attraction, proportionate to their degree of complexity. The much more difficult question of 'learning' (creation, by the finalized machine, of new parts of itself, new organs or parts of organs, in short, the 'growing' of a machine under pressure of its finality and surrounding) engrosses everybody's mind. The nervous system has been, at the very first, a choice ground. Lastly, the questions which we cannot escape are the subtlest and more immaterial ones: information, negative entropy.

One tries then, so far as possible, to build explicit simulators of the living body: machines that proclaim their name of machine.

(*C*) *Investigation of total efficiency.* This is the definition which Couffignal[2] proclaims, and it can be seen how different this point of view is from the other two and how diverse the definition of cybernetics can be. I shall return to this.

What gives life to the whole study of cybernetics (points *A*, *B*, *C* here) can be expressed in two steps.

(1) Tending to a complete explanation of man, to revealing the miracle, at the risk of causing the miracle itself to disappear.

One can be sure that students of cybernetics have closely followed works about nucleic acids, proteins, submicroscopic morphology of protoplasma, regretting that this insight so near the central mystery has come from radiocrystallography and electronic microscopy and not from cybernetics. The beginning of the destruction of the essential miracle, protoplasm, ought to have fallen by right to the cybernetician.

Then we may remember M. Louis de Broglie's profound remark, declaring that cybernetics will not give entire explanations because of their macroscopic mind when all explanations belong to the microphysical order. Undoubtedly, cybernetics cannot account for everything, and even this subject must invoke the aid of physics, biology, etc. But predilection of cybernetics for what is macroscopic, simple, explainable, throws light on the spirit of this subject.

Perhaps some authors exist who not only try to destroy but also to laugh at the human miracle. There exists a tale—the author's name has slipped my memory—in which a half-insane philosopher is building in a shed a larynx and buccal cavity, set in motion by bellows with huge pieces of leather imitating tongue and cheeks, a mechanism which he hoped to hear some day pronounce in a thundering voice: "Then the word was". It would, no doubt, be possible to do it to-day, but no cybernetician would take any interest in it; for phonation has no longer any semblance of a miracle or mystery. He would be interested in it only if it was a question of improving on Nature (point *C* here), for example, to build a larynx for a wounded person.

The macroscopic vocation of cybernetics does not in any way come from an inclination to laugh at a human miracle by imitating it with pulleys and strings; it comes from a desire of imitating the microphysical, elusive mechanisms of the living being by tangible pieces. If ever the cybernetician is to build a larynx he will not lodge it in a shed. He will make an admirable model of which the scheme, the plan for fitting, and the list of the attendant parts he will possess.

(2) This gives the last precise details about the cybernetic spirit: it does not aim at destroying the miracle for the pleasure of destroying, but to put an end to the impossibility of taking care of an organism which we are not able to understand.

This is, then, real scientific research: the curiosity of knowing and need of knowing, in order to act. Above all, if we can replace the organ which is missing or in danger of being destroyed, by an apparatus built in a factory, that is immortal and ever replaceable. One prefers a machine one can understand to a man one must allow to die because he delights in an unfathomable and capricious miracle.

There is in such an enterprise something passionate and neurosthenic, something of the rescuer who stuns a man who has fallen into deep water in order to quieten him and thus rescue him.

So medicine and cybernetics ought to meet.

How could the meeting be arranged? The physician is, even before being a man of science, a contemplator of the total human miracle which he tries to heal and save. What would remain of the physician if he was a simple machine repairer? The cybernetic spirit seems, on the contrary, to tend to the destruction of man. Each of the three points *A*, *B*, *C* here shows this in its way.

(*A*) The 'losses of determinism' brings the idea of human liberty into ridicule.

(*B*) When a vital function is counterfeited by a machine, either the corresponding human organ is itself only a machine or the living organ—simulated but not reproduced—has indeed something inimitable and miraculous. Then it is a wasted miracle, for the machine obtains, without a miracle, the same results. Why talk of witches' brooms or a flying carpet when aeroplanes exist?

Moreover, the macroscopic simulator goes further in 'dehumanization'. Claude Bernard thought of man as an indivisible whole. Civilized man to-day still follows this medical way of thinking. We do not ignore that (functionally, at least) the eye, or neurone or heart, taken separately, is nothing miraculous. But by adding them together we cross over the threshold to a miracle. There is not here a well-worked-out *gestalt* system, but only this: the eyes, the heart, the nervous system are imperfectly known, and, moreover, bathe in the miracle of their protoplasmic structure and their onto- and philogenetic mode of appearance, which still remains mysterious (at least for some time to come) even if functionally the organ is understood and can be simulated. All organs have a dark zone which increases by addition. A large number of comparatively little-understood organs are merged into an unknowable whole.

Things are not the same with artificial organs. A machine plus a machine is nothing but a larger machine. If the size of the whole surpasses our present power of pre-vision, our ignorance, and not the miraculous character of the whole, must be accused. Never would a judge agree to call a robot 'Sir' (however learned, well educated, loving and clever it may be), the mechanical details of which are thoroughly known and understood. Cybernetics do not acknowledge the *gestalt* and have no dark spots.

(*C*) The study of efficiency is the attribute of techniques; these form an attempt to organize means tending to an end; they look for 'the best way to do work', the most effective way leading to the proposed end. But we may find out in cybernetic efficiency the idea that in a living being, as in a machine, one is always able to calculate the 'best way' where we often see a matter of inspiration, of tricks of the trade, of flash of genius.

If the cybernetician acknowledged this statement, he would seem to set two metaphysical postulates:

(1) There is one 'best way'; a route towards the aim which is better than any other.

(2) It is possible to calculate this route. Then it is calculation which will give the assurance of a better route and not the process of a more human aspect such as inspiration or cleverness. On that score cybernetics, like all other sciences, arrives at the limits of metaphysics.

Yet, just as no cybernetician has ever expressly denied the human miracle, but endeavours to undermine it, inch by inch, so one would not find anywhere the assertion that any technique is calculable. Cybernetics only try to study, one after the other, the ways of working out so-called human routes and replace them—where it is possible—by calculations.

Cyberneticians do not at all question the possibility of choosing aims. (It is, in fact, the attack on this faculty which is the real concern of cybernetics, and this precisely in the indirect manner of the study of means. I am dealing with this point in a book now being written, but we cannot go into it within the limits of this article.) Cyberneticians apply themselves to dismantling the humblest part of the act: the realization of the aim already chosen.

Then, if the techniques used to rely and still often rely on the human being, these also take for granted many data. Starting from this possibility of working up some 'ways to do work' cybernetics try to calculate other ways and itineraries. They do not have to grapple with the techniques of the staff engineer, for, here, the idea has spread for a long time that many things are calculable and are deprived of human mystery. Nevertheless, they are interested in techniques similar to those of the engineer; techniques that set in motion a great number of data, very long-dated pre-visions, ambiguous informations, etc., which until now were entrusted to the inspiration and 'flair' of the great administrator, captain of industry, leader of men, strategist. The success of this cybernetic intrusion in a zone apparently quite human has considerable scientific and philosophical implications.

But now there exist techniques which have a direct effect on human, mysterious techniques, the existence of which puts forward questions next to those set by cybernetics. Such techniques try to shape the man, with the aim of bringing him nearer to perfection: for example, education, law, medicine, etc.

We acknowledge in our heart that only man can touch man. Not that it is not necessary, even here, to surround oneself with all the possible calculations. But we believe that when all has been calculated, when all analyses have been made: (1) no certitude will be reached; (2) the final decision must be taken by the technician himself, a man who will decide—to use simple words—'according to his inspiration and heart'.

Only man can educate, make laws, judge, cure.

Such an axiom is still responsible for the fact that there is no opportunity for the application of human techniques towards the cold cybernetic method. Manufacturers, generals, administrators have accepted this method, perhaps an inhuman but a particularly efficient one. But there exists no such thing as cybernetic law or education. and cybernetic medicine is still in its infancy.

Perhaps there is some methodological impossibility in mixing science with the human factor; the feeling that one cannot unite the heart with the cold calculating machine, in the same way as an artist may feel that the least calculation would throw into confusion the whole of his inspiration.

But we do not reach this point in the techniques of man. They all include—in different proportions—an important calculable part. In medicine this part is so important that, seen from afar, medicine may appear as clear as the technique of an engineer. There is much science in medicine to be written and taught. and this science can, with impunity, find profit in the cybernetic contribution. Compared with law and education, medicine is much more 'cybernetized' in its calculable part. Before letting the human factor speak, laboratory analysis has a word to say.

Education and law are far from having reached this attitude. Instinctively they elude calculation, as would a musician or a painter. Still, law is less defiant of medicine (itself a handler of men, so linked to law on that point) than it is of 'cold' physics. It is through the expedient of medicine that law received the first elements of cybernetic efficiency. Two examples are well known: (1) Law is often led to solve questions relating to health. Formerly these questions were tinged with morals and humanity. Nowadays, the legislator has lost ground to medical techniques (as many people do, who see medicine from the outside, the legislator believes it to be still more scientific, unerring and able to calculate than it really is). The law-giver goes on signing texts referring to infectious diseases or inoculations, but the text is already established before he sees it; the calculations are made by the physician. Would one dare to put to the vote in Parliament medical precepts that are scientifically established? (2) It was a physician who, in 1876, claimed that a criminal was not an ordinary offender but a sick man. Many things have changed since Lombroso's great work. But the whole of criminology still remains, that is to say, the affirmation that a criminal must be nursed and not judged. Here, penal law (at least for what concerns blood, sex and juvenile crimes, etc.) dwindles a little each year; the physician's science taking the place of juridical methods.

To what extent would the physician be ready to do to himself the service done to law? Nothing seems to be against it on scientific grounds, and so long as the human factor holds the decision in the last resort.

Yet things are not quite as simple as they seem. The cybernetic spirit goes towards destroying all human factors. Cybernetics are an alarming partner, and the physician may have the feeling he is losing something in this game. The limits between cold and scientific calculation and the human factor are nowhere distinct, and development in calculation may displace them, by 'de-humanizing' both medical work and the sick man, and even the idea we have of man.

But will the physician really run a risk? It would perhaps be to exaggerate the present power of cybernetics and, above all, minimize the confidence the physician has in the human being. Debate with the cybernetician will sometimes show the human factor as a sham mystery; but if we wanted to approach with love and inspiration what depends on calculation, if it were really a question of sham mystery, here would be a gain in truthfulness and efficiency. The medical aim is human in itself. What does it matter to save a man with a little more or a little less calculation? In our present ignorance, the physician will never find enough in all his heart and in all his calculations; and it is always the human factor which will have the last word.

In truth, this contact with the inhuman and the insensible widens still more the medical horizon.

To consider an organ as a finalized apparatus, to accept the replacement of this organ by a more efficient artificial machine, involves new problems and questions. One sacrifices or modifies a machine to save the life of the man who uses it. One sacrifices a hand, a kidney, an artery, a small part of cornea, to save what? If medicine is a proof of love, to whom does this proof apply[3]?

Just as Socrates, son of Phenarete the midwife, practised the art of 'delivering' the minds, so this fight with the cybernetic angel is in itself maieutic.

The physician must be led, as so many others, to ponder over man. Let us hope he will make his thoughts known.

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ORIGIN OF PETROLEUM AND THE COMPOSITION OF THE LUNAR MARIA

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ADOPTING a cold accretionary hypothesis for the origin of the solar system[1], it is proposed that the primitive material from which the Earth and Moon were formed contained a small percentage of high molecular weight hydrocarbon, as do some of the meteorites falling on the Earth at the present time (carbonaceous chondrites). During the agglomeration of this material it would have been heated, by release of gravitational energy and by radioactive heating. As the temperature approached 400° C the hydrocarbon would have undergone thermal cracking. The products of this reaction would flow and be distilled to the surface regions of the planet (or Moon). It is suggested that the maria on the surface of the Moon are the residue of this material after the lighter hydrocarbons have evaporated from the exposed surfaces (that is, vacuum-reduced crude oil or asphalt). It is proposed that on Earth this material is petroleum and that it has moved around through porous strata picking up from sedimentary deposits, by solvent extraction, small quantities of biogenic materials. I consider that Nagy, Meinschein and Hennessy[2] have recently carried out a crucial test of the theory by producing a 'petroleum-like' product by the destructive distillation of a portion of the Orgueil meteorite.

Of the meteorites which have fallen, and been recorded, 3·6 per cent are carbonaceous chondrites[3] containing a few per cent (1–5) of carbon[4]. Of the carbonaceous chondrites which have been investigated some of the carbon, perhaps half, occurs as high molecular weight hydrocarbon[2,5–7]. To take a very conservative estimate let us assume that the Earth and Moon were made from material the composition of which was similar to that of the average of all meteorites found. That is to say, that this original material was at least 0·01 per cent hydrocarbon. This does not seem an unreasonable assumption when the cosmic abundance of carbon and hydrogen is considered (2·7 and 2·94 × 10^4 times that of silicon respectively)[8]. The mass of the Earth is 6×10^{24} kg and 0·01 per cent of this is 6×10^{17} tons. If this hydrocarbon were cracked to liquid hydrocarbons with a 10 per cent efficiency, 6×10^{16} tons would be produced, sufficient to cover the Earth to a depth of 40 metres. Such a source is adequate to provide all the petroleum deposits of the Earth. By similar reasoning, such a source would be adequate to provide all the material in the lunar marias.

Petroleum contains large quantities of hydrocarbon in a straight-chain configuration, so a basic assumption of the foregoing proposal is that straight-chain hydrocarbon can be produced by a non-biological mechanism. It is interesting to speculate on the mechanism by which hydrocarbon in a straight-chain configuration could be synthesized in free space. Fig. 1 illustrates how such a material might be produced, via a free radical mechanism, from methyl radicals. The methyl radicals themselves could be produced from solar photons on methane. Such a reaction might be expected to favour addition to the 'end' groups to produce straight-chain hydrocarbon. Small particles of inorganic material would become coated with a layer of high molecular weight hydrocarbon. This has been found by Mueller, who found that some of the silicate of the Cold Bokkeveldt meteorite was insoluble in hydrogen fluoride[5]. High molecular weight hydrocarbon has been produced on surfaces by electrical discharges in a methane–ammonia atmosphere by Wilson[9].

Undoubtedly some of the minor constituents of some petroleums are of biological origin; but it is suggested that petroleum is largely of the origin described here 'contaminated' to a greater or lesser

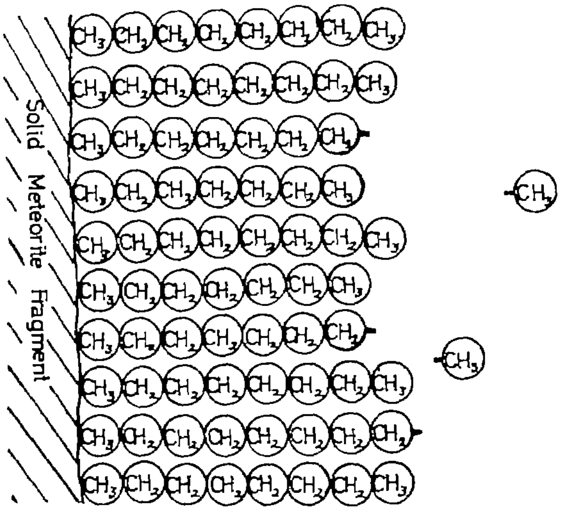

Fig. 1. Proposed mechanism for synthesis of hydrocarbon in a straight-chain configuration

extent by oil-soluble material extracted during its migration through sedimentary deposits.

It is difficult to obtain conclusive evidence to distinguish between a biogenic or the non-biogenic theory of the origin of petroleum. The extra-terrestrial hypothesis suggested here would imply that petroleum could occur on the Moon, whereas a biogenic theory would predict that it would not (unless, of course, life has also 'flourished' on the Moon). It might be expected that some of the liquid products produced by the thermal cracking of this 'cosmic hydrocarbon' would flow to the surface and form great lakes of petroleum. The surface layers of these lakes would lose their volatile constituents and form a skin of vacuum-reduced crude oil or asphalt. The surface of this skin, in turn, would be converted to petroleum coke under the influence of photons and corpuscular irradiation from the Sun.

The maria on the Moon have puzzled observers for many years. Could they be bigger versions of the asphalt lake in Trinidad ? They are extremely smooth and are very dark in colour (albedo = 10 per cent). They have the general appearance of a liquid flow. It has been suggested that they are lava flows; but they have no characteristic terminal faces, and there are objections to any volcanism in the normal sense on the Moon[10]. The temperature properties of their surface are consistent with that of petroleum coke. Radar measurements indicate that the surface is smooth down to a few inches[11]. One might expect debris from meteorite impacts to be lying on the surface of the maria. If the maria were asphalt lakes it would explain the lack of debris since the large fragments would slowly sink into the asphalt. Only rock dust can be supported as, for example, in the case of the ray systems associated with Tycho and Copernicus. It would also explain why the 'seaward' walls of the craters on the edges of the maria disappear—for example, Mare Crisium and Mare Humorum, and the 'seaward' wall of the Imbrium crater. On the foregoing model the 'seaward' wall would have been composed of fragments of asphalt and would have flowed slowly back into place, whereas the 'landward' wall consists of rock debris sitting on a solid bottom. One can see craters in the maria in stages of slumping back; some, for example, in Mare Cirisium, are in an advanced stage and form a lacy network of 'ghosts'. Some of the craters in the maria seem to be filling up—perhaps by asphalt percolating through the rock rubble walls. Areas of no craters (Maria Imbrium and Serenitatis) can be explained by the lake being so deep that asphalt only is displaced in meteor impacts or that any crater wall thrown up from the bottom does not break the surface. Some indication can be gained of the depth of these asphalt lakes from the presence or absence of craters. For example, that part of Mare Imbrium in the vicinity of Archimedes is not nearly as deep as Sinus Iridum. There is evidence that the asphalt is moving slowly, or has moved in the past. For example, some of the craters, such as Ross, seem to be distorted. Some of the craters seem to have been filled to a breach in the wall, for example, Julius Caesar and Posidonius. Some of the asphalt may have come from the impacting bodies themselves. One might imagine a large body of similar composition to the Orgueil meteorite crashing into the Moon. The heat of impact would be expected to crack thermally some of the hydrocarbons present to produce liquid hydrocarbon products.

The low dielectric constant of hydrocarbon is consistent with the very low radar reflexions from the Moon[12].

Is it perhaps possible that the oxidizing environment in which we live on the Earth has blinded us to the cosmic abundance of hydrocarbon ? Is the asphalt lake in Trinidad a last vestige of 'terrestrial maria' ? As has been shown here, even very small cosmic abundances of hydrocarbon could modify the lunar surface considerably, as has the relatively small cosmic abundance of water modified our 'terrestrial surface'. In any event, we probably have only a few years more to wait before we can observe samples of the lunar maria at first hand. If these should prove to be vacuum-reduced crude oil, it will force a major revision in the present-day accepted ideas on the origin of petroleum.

Note added in proof. Since the preparation of the manuscript of the above article a paper by A. Giraud[13] has come to my attention. Giraud has critically reviewed the literature on the exploration of the Moon using electromagnetic radiation. He has calculated the physical properties of the Moon's surface. In Table 1 Giraud's estimates are compared with those postulated in the article here.

Table 1

	Values calculated by Giraud (ref. 13) from electromagnetic radiation received from Moon	Pertinent values (ref. 14) postulated from the hypothesis presented here
Dielectric constant	1–1·5	Petroleum 2·13 Asphalt 2·68
Thermal conductivity (cal sec^{-1} cm^{-1} deg C^{-1})	Of the order of 5×10^{-4} to 10^{-5}	Lampblack 7×10^{-5}
Specific heat	0·1 or 0·2 (cal deg C^{-1} cm^{-3})	Charcoal 0·165 Carbon (amor.) 0·168 (cal deg. C^{-1} gm^{-1}) Density ≐ 2

The specific heat and thermal conductivity measurements would be related to the surface and so should be compared with radiation damaged or cracked petroleum. Dielectric constant measurements would be related to the bulk material and therefore on the proposed hypothesis should be related to asphalt or petroleum.

It has also been concluded from measurements on moonlight that the surface has a vesicular structure with dimensions of granules and interstices in the range 10–300 microns. Such a structure is entirely compatible with petroleum coke[15].

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Comment by Prof. G. Mueller*

THE great cosmic abundance of carbon, hydrogen, oxygen and nitrogen leads us to the conclusion that the crust of the Earth must have contained an original non-biogenic carbonaceous phase. What was its composition, and to what extent is it at present preserved, remain open questions.

It should be stressed that all the known deposits of petroleum and related substances, including the asphalt lakes of Trinidad and Venezuela, are within sediments in general and shallow sea deposits in particular. A considerably greater volume of metamorphic and igneous rocks have proved so far to be barren of oil, etc., although the layers of ashes within the older, often folded, volcanics would serve as quite ideal reservoirs covered by the highly impermeable lava-flow traps. On the other hand, the few reliably abiogenic organic mineraloids found in pegmatite veins (tucholites, etc.) have a quite uniquely high oxygen/hydrogen ratio of around 1·0 as against the range of from 0·0 to 0·25 of petroleums and asphalts. It is unlikely that the former substances would yield much of the liquid low oxygen/hydrogen substances on cracking. Indeed, it seems that the main role of life was to differentiate this original high oxygen/hydrogen phase into carbon dioxide and the correspondingly lower oxygen/hydrogen ratio organisms, the partially biochemical decomposition of which yielding still lower oxygen/hydrogen products.

It appears more likely, therefore, that our petroleums would be essentially distillates, etc., of biogenic substances, which in turn picked up small quantities of abiogenic residues.

It appears from our scanty experience that the most probably abiogenic organic substances from the meteorites are also high oxygen/hydrogen complexes, which yield on distillation relatively little hydrocarbons per carbon, and mostly organic acids on extraction. It seems, therefore, that the supposed parent asteroid of the meteorites contained a similar essentially 'acid' carbonaceous phase, as are our terrestrial tucholites. It would be unlikely that such types of substances could freely flow and form the extensive maria. However, it should be stressed that our knowledge of the terrestrial and extra-terrestrial abiogenic carbonaceous phases is based on too few specimens or up-to-date investigations. Ultimately, only the actual exploration of the lunar surface could give us the reply as to the validity of the most interesting observations and their interpretation given by Dr. A. T. Wilson.

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STABILITY OF VIRUSES ON *DISCOVERER* FLIGHTS *XXIX* AND *XXX*

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THE imminence of space travel suggests the need for an increased effort in determining effects of physical and physiological stresses on host–parasite interrelationships. Synthesis of known and speculative conditions encountered during space exploration and the possible influence of infectious disease processes have recently been reviewed[1]. Included among those stress factors are: radiation, hypoxia, temperature, endocrine, nutrition, weightlessness and various combinations. It was of interest, therefore, to ascertain whether or not viruses could be safely orbited and returned to the laboratory, prior to any attempt at experimentally simulating these conditions. The purpose of this report is to give results demonstrating the successful flight and recovery of two animal viruses.

The viruses included in this work were laboratory stock strains of influenza (*PR* 8) and *ECHO* 1. These viruses were selected because of their known inability to produce disease in man or laboratory animals. Furthermore, they differ in their response to deleterious conditions from an unstable virus (influenza) to a relatively stable virus (*ECHO* 1). Influenza stock virus was obtained by inoculation of 9-day-old chick embryos and *ECHO* 1 stock virus from monolayers of monkey kidney cells. Both viruses were collected at the time of maximal replication. Each virus was pooled, lightly centrifuged, and sealed in glass ampoules which were maintained at −70° C. Titrations at various intervals were performed for EID_{50} and $TCID_{50}$ using standard laboratory procedures[2].

A number of successful *Discoverer* flights containing biopacks have been reported[3,4]. Briefly, these biopacks are small air-tight metal canisters, which ride 'piggy-back' on the *Discoverer* rockets. The various biological specimens are tightly fitted into different compartments of the biopacks at the laboratory prior to shipment. In addition to these viruses, other biological specimens included: various tissue and organ cultures, bacteria, algae, insect larvæ, serum and radiation dosimeters.

In general, flights *XXIX* and *XXX* are similar regarding their flight profiles. Table 1 lists an approximation of these data indicating the essential information on both flights. It is obvious, however, that these flights are not critical tests of space conditions inasmuch as the van Allen belt was not

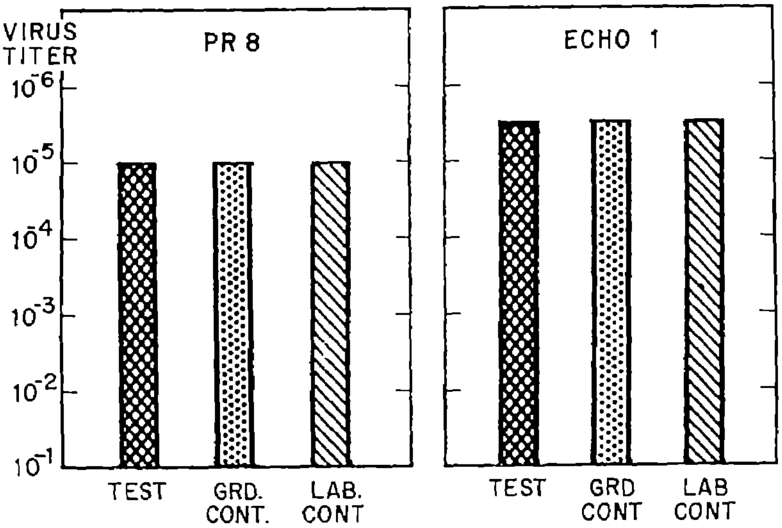

Fig. 1. Effect of *Discoverer XXIX* flight on influenza (*PR 8*) and *ECHO 1* viruses

penetrated nor were detectable radiation effects from any other source readily apparent.

On return to the laboratory, the specimens were frozen (−20° C) and afterwards titrated. Simultaneous tests were performed on the laboratory controls (original virus preparations maintained in the laboratory at −20° C), ground controls (specimens packed similarly as the test but kept at ambient ground-temperatures at the launch site), and the test material. The results of flight *XXIX*, which were identical to flight *XXX*, are shown in Fig. 1.

Table 1. *Discoverer XXIX* and *XXX* Profiles

	Flight	
	XXIX	*XXX*
Launch date	Aug. 30, 1961	Sept. 12, 1961
Recovery	Sept. 3, 1961	Sept. 15, 1961
Site of recovery	Air catch	From sea
Apogee	303·68 N.M.	312 50 N.M.
Perigee	81·49 N.M.	125·00 N.M.
Inclination	82 02°	82·70°
Time of orbit	91·55 min	92·41 min
No. of orbits	33	33
Max. acceleration	9 0*g*	8·0*g*
Duration	450 sec	450 sec
Temperature in capsule	?	16°–37 5° C

It may be noted that excellent agreement was obtained between test and controls with both viruses. Hæmagglutination tests with influenza virus were also performed without any apparent detectable alterations in titre.

The significance of these experiments is the fact that the viruses did withstand the rigours of space flight. Thus vibration, temperature fluctuations, alteration in *g* forces, weightlessness, etc., as produced by these flight conditions do not alter the stability of the virus utilized. Since virus function is not altered, maintenance of functional structure apparently is not impaired by zero gravity. The maintenance of virus structure tends to indicate that gravitational forces as encountered do not contribute appreciably to the stability of intermolecular binding at least for the virus under consideration. Unfortunately no radiation stresses sufficient to alter the virus particle were encountered. However, it is recognized that large radiation dosages are necessary before any effect on the virus particle can be detected by present techniques[5]. Additional investigations are obviously necessary, primarily in the laboratory, to determine the effect of combined stresses on viruses. Furthermore, the ability of these virus suspensions to survive these flights gives impetus to the need for expanding studies on problems associated with the role of viruses as a public health hazard in future space-travel.

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ANIMALS OF WESTERN SZECHUAN

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A FIVE-MONTH survey of the animal life in the highlands of western Szechuan Province in south-west China has recently been made.

Traversed by high north–south mountain ranges, western Szechuan has snow-covered peaks as well as deep river gorges; the slopes between are often heavily forested. The Minya Konka, the tallest peak in this region, is 7,700 metres high. While high up on the mountains are glaciers and tundra, in the valleys below the climatic conditions match those of the warm Yangtse Delta. Despite its rich wild life, western Szechuan was visited by few Chinese naturalists in the past.

A preliminary estimate shows that more than one-fourth of the known species of mammals of China and one-third of her known birds are located in western Szechuan. Their species cover a wide range known in the arctic, temperate and tropical zones of the world. The region produces excellent furs and feathers. Tibetan otter furs are generally regarded as the best in China; not until our visit did I realize that the western Szechuan type is equal to the best of the Tibetan species.

Western Szechuan abounds in spotted, black, snow and clouded leopards; reddish golden, grey golden and marbled tiger cats; and lynxes. Their furs are usually very fine, soft and beautiful. Other animals of great economic value are the highland red foxes, silver weasels, beach martens and Himalayan marmots.

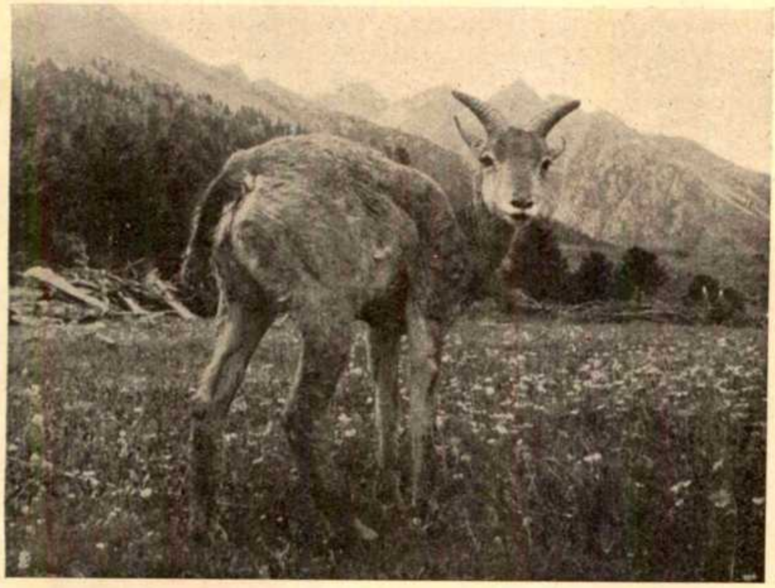

Fig. 1. A blue sheep of Minya Konka

The highlands are the home of a great variety of pheasants, including the golden, Tibetan eared, blood, Stone's, Reeve's, Lady Amherst's, and Chinese moonals and Temminck's tragopans. Their brilliant tail feathers are much sought after as decorations for women's hats and theatrical costumes.

Deer are plentiful around Patang, a county on the western border of the area. They inhabit the barren rocky zone at an altitude of 3,000 metres in the mountains, in the lower grassland and bush, in the forest and down the valley of the Chinsha River at an elevation of 2,000 metres. The alpine grassland abounds in Tibetan gazelles the size of domesticated sheep. In Shichu, in the westernmost part of the region, we found the Asiatic wild asses in herds of hundreds. Beneath the snow-line of Minya Konka the blue sheep ran in flocks of 30–80 (Fig. 1).

Western Szechuan is China's major producer of antlers and musk, both of which are important ingredients in Chinese medicine, while the latter is also used in the perfume industry. Among the deer found in this region were sambars, white-lipped deer and white stags. The two types of musk deer known in western Szechuan are the common musk deer and the Chinese musk deer. Under the skin of the abdomen of the male is the musk gland. The prized secretion of this gland is extracted, often at the expense of the animal's life. The recent success in domesticating the animal in Markang in the northern part of western Szechuan preserves the animal, and steps up the output of musk, for the secretion increases with the animal's age.

Of all the big-game areas in western Szechuan the Yunwu Mountain and the Hsinlu Sea (Fig. 2) are the most outstanding.

A V-shaped mountain range extending some 10 km, the Yunwu Mountain, affords asylum for many of the rarest animals and birds in the world, namely, giant pandas, lesser pandas, golden monkeys, takins, serows, sambars, tufted deer, Reeve's muntjacs, gorals, musk deer, Temminck's tragopans, blood pheasants, Stone's pheasants and Lady Amherst's pheasants. Guided by the local hunters, we traced the giant panda's earthern cave by the tree where the animal passed the winter. In summer, however, it moved to sunken ground covered with moss and grass, as was shown by spoor and droppings. We also identified the pond and the ground where the musk deer, takins and sambars watered.

Hsinlu Sea is a highland lake in the midst of the mountains in western Szechuan (Fig. 2). Around the lake the ruddy sheldrakes and Tibetan eared pheasants breed in profusion, while the shore is dotted with a multitude of caves of otters and Himalayan marmots. The surrounding mountains cover a wide range of faunal elements known in north, south and west China, as shown by the presence of blue sheep, sambars, white stags, spotted leopards, grizzly bears, snow leopards, stump-tailed macaques, serows, gorals, Chinese musk deer and common musk deer.

The first reports of the mammals of western Szechuan by Europeans date from 1869, when Père Armand David, a French missionary, travelled to Paohsing (Muping) County and made an extraordinary collection. The region has since been world famous as a treasure house for mammals and birds.

One of the factors contributing to the rich and remarkable fauna is the region's varied topographical conditions, ranging from the margin of the basin at an elevation of no more than 1,000 m to the 7,700-m high mountain ranges. The north–south running mountain chains set a barrier against the westerly winds, and, by condensing moisture from these air

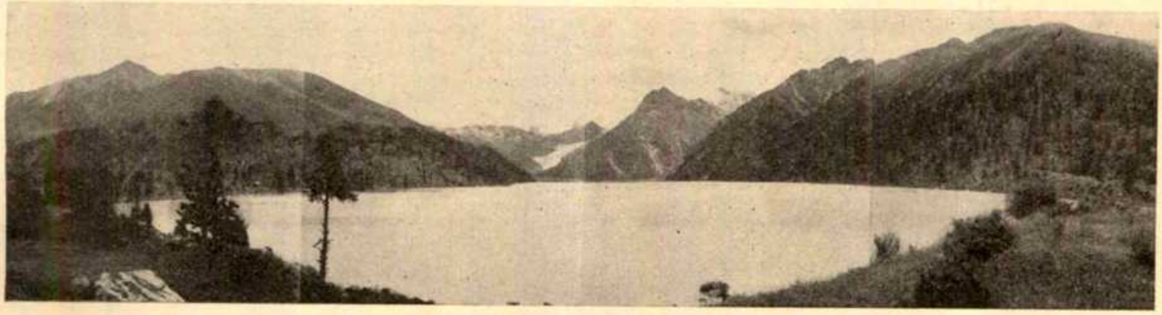

Fig. 2. The Hsinlu Sea of western Szechuan

currents, produce plentiful rainfall, with its consequent forest growth. Along the Chinsha, Yalung and Tatu Rivers are rich warm valleys. The wide range of climatic and geographical conditions makes it favourable as habitation for a great variety of species.

Another factor is the region's geological age. The north–south ranges of western Szechuan are believed to have been established during the last stage of the Pleistocene period, which makes them older than the Himalayas, with which they are contiguous. The mountains seem to have afforded asylum for many primitive types of mammals, including the giant panda, the lesser panda, the golden monkey, the Szechuan jumping mouse and the common Chinese zokor.

The third factor is that the region lies at a latitude where the Palæarctic and the Oriental of the world's zoogeographical regions meet, with the north–south mountain ranges serving as a kind of corridor for the mammals of the two regions. Contact of the north–south ranges with the east–west Himalayas and Kunlun allows a certain interchange or extension of range for some of the faunal elements.

OBITUARIES

Dr. S. T. Ma

THE death occurred in Sydney on January 27, 1962, of Dr. Shih Tsun Ma, senior lecturer in theoretical physics in the University of Sydney.

Dr. Ma was born in Peiping, China, on June 29, 1913. He attended the National University of Peking, first as a student and then on the staff, during 1929–37, obtaining his B.Sc. degree in 1935. From 1937 until 1941 he was a postgraduate student of Queens' College, Cambridge, obtaining his Ph.D. degree in 1941. During this period he worked under and was deeply influenced by Prof. P. A. M. Dirac.

He was then appointed professor in the physics department of his old University, the National University of Peking, and held this position during 1941–45. During this period two of his students were C. N. Yang and T. D. Lee, destined themselves to become distinguished theoretical physicists. In 1945, Dr. Ma left China to become a member of the School of Mathematics, the Institute for Advanced Study, Princeton, where he stayed for two years; he spent the succeeding two years (1947–49) as a scholar in the School of Theoretical Physics, Dublin Institute for Advanced Studies, and the next two years again (1949–51) as a post-doctorate Fellow in the Institute for Nuclear Studies, University of Chicago. During this time he married Mrs. Helen Lin, secretary to Prof. Enrico Fermi. In 1951 he took up a post-doctorate fellowship with the National Research Council of Canada, Ottawa. Finally, in 1953, he joined the Theoretical Physics Department of the School of Physics, University of Sydney, as a lecturer, to be soon promoted to senior lecturer, which position he held until his death at the age of forty-eight years. His stay of almost eight years in the University of Sydney, broken only by a sabbatical study leave in 1960 and spent at the University of Cambridge, was his longest stay in any one place in the Western world.

Dr. Ma's first scientific article, "Variational Wave Functions of Doubly Excited States of Helium", was published, in collaboration with T. Y. Wu, in the *Journal of the Chinese Chemical Society* in 1936. After several such publications in Chinese journals his first article in a Western journal was "Photo-magnetic Disintegration and Magnetic Moment of the Deuteron in the Meson Theory", published in the *Proceedings of the Cambridge Philosophical Society*, 1940. During the ensuing years his thirty-five scientific papers found global distribution in the world's scientific journals; most of these were devoted to subjects in quantum electro-dynamics or meson field theory.

Staff and students alike in Sydney knew Dr. Ma not only as a research leader but also as a man devoted to his work and who prepared and delivered his lectures with meticulous care and a thoroughly sympathetic comprehension of student problems. By his unobtrusive manner and quiet sense of humour he endeared himself to all with whom he came in contact. Dr. Ma's life was obviously influenced by the teachings of Confucius and the ancient Chinese love of learning and devotion to duty. The rigid rules—self-imposed—to which he forced himself to subscribe included the belief that a day is wasted when something is not added to one's store of knowledge. His sole ambition was to contribute to man's scientific knowledge and understanding; it is thus fitting that his contributions to physics should form the monument to Dr. Shih Tsun Ma.

He is survived by his widow and stepson Kenney.

S. T. BUTLER

Prof. Gabriel Bertrand

PROF. G. BERTRAND, who was approaching the age of a hundred, passed away at Paris on June 20, 1962. He was a professor of biochemistry in the Pasteur Institute, Paris, from 1908. There he worked extremely systematically and regularly for sixty years.

During the First World War, I followed his lectures and it was known at that time that Prof. Bertrand advised the French War Office to introduce gas warfare by the application of chlorine. The Germans took possession of his Laboratory during the Second World War, when Prof. Bertrand continued his researches.

He was an extremely gentle and kind man. He created a large School of Biochemistry. He was a versatile research worker and a pioneer in several lines of investigations. This is indicated in the Bertrand Medal, when the following lines occur:

"Venin and vaccination and anti-venin, cellulose and wood, sugars, Sorbose bacteria, lac, laccase, oxydases, diastases and co-diastases, manganese and infinitely small amounts of catalytic chemical fertilizers (trace elements), biocatalysts, chloropicrin, destruction of parasites, etc. . . ."

Prof. Bertrand by his research work established that plant growth and health do not depend only on carbon, nitrogen, oxygen, hydrogen, soda, phosphorus, potash, lime and sulphur, but also on a host of other elements like manganese, iron, copper, zinc, cobalt, boron, iodine; even rubidium and cæsium are present in the plant ash.

He was deeply interested in the history and philosophy of science and attended many congresses. He was a great believer, like Pasteur, Metchnikoff, Roux, Duclaux and others, in the progress of humanity and maintenance of world peace through the experimental method of science. His research work was of great practical value in agriculture and medicine.

Bertrand was a member of the French Academies of Sciences, Agriculture and Medicine.

His only son, Dr. Didier Bertrand, who also is a well-known medical man, is following in the footsteps of his father and pursuing investigations on trace and oligo elements in the Pasteur Institute of Paris.

N. R. Dhar

NEWS and VIEWS

Colour Chemistry and Dyeing at Leeds :
Prof. W. Bradley

The premature retirement of Prof. William Bradley from the chair of colour chemistry and dyeing at the University of Leeds in September 1961, owing to ill-health, was a severe loss to his department. An exceptionally gifted lecturer, he was able to impart to many of his students his own enthusiasm for research, and during the thirteen years (1948–61) in which he occupied the chair, he succeeded—aided by the generosity of industry—in building up a research school that was very large in proportion to the size of his department. Prof. Bradley's name will always be associated with the chemistry of anthraquinonoid vat dyes, a subject on which he is the leading authority. Since 1951 a stream of highly important papers on this subject has appeared in the *Journal of the Chemical Society* under his name and the names of his research students. In particular, he has been interested in the mechanism of formation of vat dyes, a hitherto almost neglected topic. Other fields which he has explored include the chemistry of heterocyclic bases and their metal derivatives, the oxidation of leuco-esters of vat dyes, and azo coupling in non-aqueous media. He has also contributed to the theory of dyeing through his study of the adsorption of mandelic acid (an optically active compound) by wool. He gave every encouragement to his staff to undertake research and was always generous in the allocation of funds and research students. Another notable feature of this period was the introduction into the undergraduate course of lectures and laboratory work on pigments, thus filling a serious gap. It is some consolation to know that Prof. Bradley's health is now much improved, and all those connected with the department, in particular his colleagues and his former students, hope that Prof. and Mrs. Bradley will enjoy many years of happiness in their new home in Paignton, Devon.

Mr. I. D. Rattee

Mr. I. D. Rattee, who is to succeed Prof. Bradley to the chair of colour chemistry and dyeing at Leeds, was educated at the Imperial College of Science and Technology, where he graduated with honours in chemistry in 1945. He is an associate of the Royal College of Science, and in 1960 was awarded the Gold Medal of the Society of Dyers and Colourists. Following graduation, Mr. Rattee was appointed technical officer in the Dyehouse Department of Imperial Chemical Industries, Ltd. (Dyestuffs Division). From 1946 until 1950, he carried on the work initiated by the late Dr. F. Townend on the level dyeing properties of acid and chrome dyes on wool and the relation between these properties and constitution. In 1951, Mr. Rattee departed from the normal lines of wool dyeing research and began work on the use of dyes capable of reacting with the fibre substrate in wool dyeing. He made extensive investigations of the mechanism and chemistry of dye–fibre reactions and examined a class of reactive dyes, containing 2 : 4-dichloro and 2-chloro-4-amino triazin-6-yl groups because of their particular properties for reactivity towards cellulose. This work resulted in the discovery of the reactions which led to the introduction of the Procion dyes in 1956 and the subsequent appearance of an entirely new dyestuff class. Since that time, his work has been almost exclusively devoted to the study of reactive dyes for cellulosic fibres, with particular reference to the development of novel reactive systems, novel techniques of application, and also examination of the fundamentals underlying the behaviour of this type of dye. Mr. Rattee has lectured at the Manchester College of Science and Technology.

The Third Fritz London Award :
Prof. J. Bardeen

Prof. John Bardeen, professor of electrical engineering and physics at the University of Illinois and 1956 Nobel Laureate in physics, has been awarded the third Fritz London Award for distinguished research in low-temperature physics. In collaboration with Prof. Leon M. Cooper, now of Brown University on Rhode Island, and Prof. J. R. Schrieffer, of the University of Illinois, Prof. Bardeen developed the first successful microscopic theory of superconductivity, which is the characteristic loss of all electrical resistance by some metals at extremely low temperatures. In 1957 they published the now well-known Bardeen–Cooper–Schrieffer theory of superconductivity, which has played an outstanding part in the clarification of one of the principal problems of solid-state physics. Prof. Bardeen graduated from the University of Wisconsin, received his doctoral degree from Princeton University in 1936 and an appointment as Junior Fellow from Harvard University. He was with Bell Telephone Laboratories from 1945 until 1951, where he carried out research with Walter H. Brattain and William Shockley on semiconductors, in the course of which they discovered the transistor effect. For this achievement they received the 1956 Nobel Prize in physics. Prof. Bardeen joined the faculty of the University of Illinois in 1951.

The Fritz London Award, established in 1957 and awarded at two-year intervals, is named after the first scientist to elucidate the nature of superconductivity. He also made significant theoretical contributions towards an explanation of superfluidity in liquid helium, the state, unique to this liquid, in which normal viscosity disappears and the liquid 'climbs' the side of its container. The Awards, accompanied by an honorarium of 1,000 dollars, have been sponsored by the ADL Foundation, of Arthur D. Little, Inc., Mass. The first two recipients were Dr. Nicholas Kurti of the University of Oxford (1957) and Lev Davydovich Landau of the Academy of Soviet Sciences (1959).

Reorganization of the Atomic Physics Division at the National Bureau of Standards, Washington

DR. KARL G. KESSLER has been appointed chief of the Atomic Physics Division of the National Bureau of Standards, U.S. Department of Commerce, and Dr. William C. Martin, Dr. Robert P. Madden, Dr. Harold Boyne and Dr. Wolfgang L. Wiese have been named to head reorganized sections of the Division. Dr. Kessler will direct a programme of basic research on wave-length standards, spectroscopy, solid-state physics, interactions of the free-electron, atomic constants, and laboratory astrophysics. He succeeds Dr. L. M. Branscomb, who has joined the National Bureau of Standards–University of Colorado Joint Institute for Laboratory Astrophysics at Boulder, Colorado. Dr. Martin will succeed Dr. Kessler as chief of the Spectroscopy Section. A Far-Ultra-violet Physics Section, headed by Dr. Madden, has been formed to develop radiation standards in the far-ultra-violet spectral region and instrumentation for the investigation of this region. The Section will also investigate the effect of far-ultra-violet radiation on the properties of materials and conduct basic research on the physical phenomena which involve quantitative measurement of far-ultra-violet radiation. Dr. Wiese will be in charge of the new Plasma Spectroscopy Section. This Section will make experimental and theoretical determinations of atomic transition probabilities and lifetimes of excited atomic states, prepare bibliographies on atomic transition probabilities, and tabulate the available numerical data. In addition, spectroscopic techniques for the diagnostics of dense plasmas will be studied and refined, and the shape and shift of spectral lines will be investigated. The Atomic Physics Section will now be headed by Dr. Boyne. He replaces Dr. S. J. Smith, who has also joined the Joint Institute for Laboratory Astrophysics. No changes have been made in the remaining sections. Dr. E. K. Plyler will continue to be in charge of the Infra-red Spectroscopy Section, Dr. H. P. R. Frederikse heads the Solid State Physics Section, and Dr. J. A. Simpson heads the Electron Physics Section.

New Research Laboratory for Reactor Centrum Nederland at Petten

THE firm of Bredero's Bouwbedrijf Nederland is to build a general laboratory on the Reactor Centrum Nederland site at Petten in collaboration with the Engineering and Architect Bureau. The building and furnishing of this laboratory, shortly to be started, will involve about fl. 2·5 million. The building is of modern design and use has been made of the most recent developments in this field. The laboratory will give facilities to about 140 research workers and will consist of two large wings in which the Physical Department will be housed. Further room has been reserved for members of the management and some administrative groups. The general laboratory will be built in the western cup in the dunes of the Reactor Centrum Nederland site at Petten, next to the Fermi building, which is now ready. When finished, a central shop and storehouse building, a lecture theatre and a library will be built. At present, work on the building of a laboratory for highly radioactive materials is in progress. This building will be ready for use by the beginning of 1963. In the spring of this year a temporary building for general services (technical services with drawing-office and administrative departments) was finished. It is expected that, by the end of 1963, 430 research workers will be housed in the various buildings at Petten. At the moment, 385 persons are employed there.

Space Science at the University of Rochester

A CENTER for Space Sciences is to be established at the University of Rochester. The Center will serve as a focus for the interdisciplinary research activities of the many University departments active in space studies and will prepare graduate students for research and teaching careers in the space sciences. The director will be Dr. Wallace O. Fenn, senior professor of physiology at the University. The new unit will be one of the first multi-disciplinary space centres established at a university. Among the academic units which will participate in its activities are physics, engineering, optics, chemistry, biology, psychology, geology, physiology, and radiation biology. The Center is expected to facilitate the work at present being carried out in separate departments and also will encourage new experiments related to space exploration. One of its primary purposes will be to train future space scientists, and initial projects will include a series of seminars for faculty, students, and area industrial scientists working in various phases of space science. An orientation course in space science for graduate students also is being organized. Dr. Fenn has been associated with the University of Rochester School of Medicine and Dentistry as professor of physiology since 1924, and was head of the Physiology Department until 1959. He is well known for his contributions to respiration physiology.

The Scientific Documentation Centre, Ltd., Halbeath, Dunfermline

A NEW research association, the Scientific Documentation Centre, Ltd., is now in operation at Halbeath, Dunfermline. The present organization is continuing the activities of the Scientific Documentation Centre, set up there in 1960. The new unit is carrying out research into the means of improving access to scientific information and is assembling collections of scientific data and operates information services from these, which will be available to research organizations throughout Great Britain and abroad The constitution of the Centre is modelled on those of existing research associations and the Centre functions as a non-profit making company. The Council, which has been formed to control the unit first met in August of this year. The chairman is Prof. P. T. Landsberg, of University College, Cardiff and the members of the Council are Mr. C. E. Kendall Dr. D. M. McIntosh, Mr. J. R. McNally, Mr. M. C Pottinger, Mr. J. F. M. Scholes, and Dr. D. W. A Sharp.

The first service project undertaken by the Centre the *Spectra Index*, is now growing rapidly, and som 3,500 spectra or sets of numerical spectral data—including spectra of several hundred inorganic compounds—are held. All types of spectra are collected but effort is primarily directed to the collection o infra-red and ultra-violet data. Plans to collec nuclear magnetic resonance and related types o spectra will be implemented shortly. Experimental spectra services are being offered, and searches by compound identity and structure will be accepted; spectra can be purchased from those held by the Centre. The Centre also has a substantial collection of specialized data relating to the methods of storage and retrieval of information, and in particular to the use of electronic and photo-electric methods. Documentation advisory services are now being offered, partly on the basis of this. The publications of the Institute for Scientific Information and the Chemical Abstracts Service can be obtained through the Centre. Application for membership of the Centre is open to organizations and individuals requiring research information (annual subscriptions are £15 for an organization, £3 for individuals). Subscribing members receive substantial reductions in the charge for services. A publication, *Spectra Index*, listing the broad types of compound the spectra of which have been copied, is issued to members several times a year.

Studland Heath National Nature Reserve, Dorset

Studland Heath, which covers the more southerly of the twin promontories almost barring access from Poole Harbour in Dorset to the English Channel, has been declared a National Nature Reserve by the Nature Conservancy. It is an intricate complex of sand-dunes, freshwater lagoons, lowland heaths and bog covering the seaward fringes of the ridges formed by the sandy Bagshot Beds. Its flora and fauna were the subject of a comprehensive biological survey begun in the 'twenties by Capt. C. Diver, the first director-general of the Nature Conservancy (1949–52). The establishment of this Heath as a National Nature Reserve was officially recommended in the *Report of the Wild Life Conservation Special Committee* (Cmd. 7122, July. London: H.M.S.O., 1947). This emphasized the overlapping at Studland of distributional limits of many south-eastern and south-western species and the educational importance of the 'diagrammatic' zoning of maritime, sub-maritime, dune, heath, marsh, freshwater and woodland vegetation types on acid soils. The Conservancy has leased 429 acres, including the freshwater lagoon called the Little Sea and the representative range of both old and younger sand-dunes except for their seaward frontage within a hundred feet of high-water mark. These, with the low-lying woodlands and marshes extending back to the Ferry Road and one detached bog with heathland to the west of the toll-gate, have been declared as the Studland Heath National Nature Reserve.

Translocation of Game Animals in the Kruger National Park

During recent years a number of scientists, game wardens and trappers have conducted investigations on the immobilization of game by means of a dart-syringe containing paralysing or hypnotic drugs. This is a completely new field of research for the veterinarian, the zoologist and the nature conservator, but its value and importance in nature conservation cannot be over-estimated. It enables the scientist to study the migration of game by marking suitable individuals, facilitates the transport and re-introduction of game into areas where they have become extinct, and makes possible veterinary research without the shooting of game. The Department of Nature Conservation of the National Parks Board of Trustees, Republic of South Africa, has recently initiated research on the immobilization and transportation of game. J. W. Van Niekerk, State veterinarian, and Dr. U. de V. Pienaar, biologist, Skukuza, Kruger National Park, have described some of the results (*Journal for Scientific Research in the National Parks of the Republic of South Africa*, No. 5; 1962). Work was commenced on the blue wildebeest (*Gorgon taurinus taurinus* Burxhell) for the object of migratory studies, the impala (*Aepyceros melampus melampus* Lichtenstein) for physiological observations and diagnostic purposes in diseases, such as foot and mouth disease, and in the case of the hippopotamus (*Hippopotamus amphibius capensis* Linnaeus) for re-introduction to the Eastern Cape.

The Atomic Gardening Society

The Atomic Gardening Society is a "scientific, educational, non-profit-making body" which carries out research on plant breeding using irradiated and chemically treated seeds and plants. Samples of seeds are exchanged between members and any variations which occur are recorded and, if possible, developed. The Society publishes a *Digest*, the latest issue of which contains information about chemical mutagens, radioactive irradiation of grain in the U.S.S.R., irradiation of African violets and peas and lettuce treated in Sweden (3, No. 3; June 1962. Pp. 10. The Atomic Gardening Society. Dr. Thomas Gray, "Winnipeg", Winchelsea, Sussex, 1962).

Sir Henry Tizard

Mr. R. W. Clark, of 10 Campden Street, Kensington, London, W.8, writes to the Editor of *Nature*, stating that he is preparing a biography of the late Sir Henry Tizard (with approval of Sir Henry's family). The Tizard papers, to which he is being granted full access for use in the biography, are very considerable. He would, nevertheless, be grateful to hear from any readers who have reminiscences of Sir Henry's life and work, or who possess letters from him.

Announcements

Mr. Walter Binaghi has been re-elected president of the Council of the International Civil Aviation Organization. The Council is the permanent governing body of the Organization, and is elected by the Assembly for a three-year term. Mr. Binaghi was first elected to the presidency of the Council in 1957.

A conference on "Hydraulics and Fluid Mechanics", sponsored by the Faculty of Engineering, University of Western Australia, is to be held in Perth during December 6–13. Further information can be obtained from the Conference Convener, School of Engineering, University of Western Australia, Nedlands, Western Australia.

An exhibition of plastics and machinery, entitled "Centenary of Plastics 1862–1962" and arranged by the North-Eastern Section of the Plastics Institute, will be held at the Rutherford College of Technology, Newcastle upon Tyne, during November 6–10. Further information can be obtained from Mr. P. C. Oliver, 45 Worcester Way, Newcastle upon Tyne 1.

A symposium on "Recent Developments in Iron and Steel-making with Special Reference to Indian Conditions" has been jointly arranged by the Indian Institute of Metals, the National Metallurgical Laboratory (India) and the Iron and Steel Institute (U.K.) during February 4–8, 1963. Further information can be obtained from the Director, National Metallurgical Laboratory, Jamshedpur-7, India.

THE NUFFIELD FOUNDATION

SCHOLARSHIPS IN FOOD SCIENCE

THE Nuffield Foundation has become increasingly interested in the field of food science, with special regard to what it sees as a need for more fundamental research and for more people capable of carrying it out (see also p. 1 of this issue). Simplification and labour saving in the preparation and storage of food involves the greater use of food additives and of various processing techniques, and toxic risks may therefore be increasing. The Foundation, guided on these matters by an advisory committee, is aware of considerable gaps in knowledge in this field; limitation of knowledge of the pathology of test animals appears to be a basic deficiency.

While there appears to be a reasonably satisfactory position in the training and supply of food scientists and technologists to carry out standard procedures, the numbers available for fundamental research are entirely inadequate; in particular, among pathologists and pharmacologists with toxicological interests and experience, and among food scientists with postgraduate qualifications who have adequate training and experience in more than one discipline.

The Foundation is anxious to encourage entry into this field by means of a scholarship scheme. It is hoped that these awards may attract science and medical graduates who might not otherwise contemplate a career in food science research. Career prospects in this branch of science are now good; official and public interest in the field, as with academic and industrial interest, is quickening and the demand for highly trained scientists will continue to grow. The recent setting up of the British Industrial Biological Research Association is a practical expression and measure of this quickening interest.

At a later date the Foundation may be able to give further support to this developing career structure by the offer to selected post-doctoral candidates of senior research fellowships.

With the aim of helping to advance research into the more important problems of food science the Nuffield Foundation is prepared to offer annually up to five research scholarships for science graduates and up to three research scholarships for medically qualified graduates.

The awards, which may be held for periods of up to four years if necessary, are intended to provide suitable experience and knowledge to qualify graduates in science and medicine for subsequent research in food science.

Science graduates will be able to receive, as necessary, a year of more or less formal training in selected subjects and to study for the Ph.D. degree in the field of food science and toxicology. The value of the awards will be between £650 and £900 per annum if taxable, or the equivalent tax-free, according to age, qualifications, and experience, and subject within these limits to annual increments of £50. The value will be supplemented by the amount of fees, an allowance of £50 per annum if married, and a further £50 per annum for each child up to the first two. Additional expenses likely to arise in connexion with a scholar's research programme, other than normal bench fees, which will be automatically covered, will be sympathetically discussed on an *ad hoc* basis.

Graduates in human or veterinary medicine will be able to receive, as necessary, a year of more or less formal training in selected subjects and to study for a Ph.D. degree in pathology or toxicology or other higher degree approved by the Foundation. The value of the awards will be between £800 and £1,200 per annum if taxable, or the equivalent tax-free, supplemented and subject to the same conditions as for science graduates.

The scholarships will be open to men or women preferably under the age of thirty-five, who hold a science, medical or veterinary degree of a United Kingdom or other Commonwealth university, who are of high merit and whose ability and inclinations fit them for a career in food science research. A candidate for a scholarship must be recommended by an appropriate professor or other authority of the university where the candidate graduated, and of the university or other institution at which the candidate would wish to receive training.

Scholarships will not normally be tenable for longer than four years.

If a scholar is allowed to spend part of his period of study abroad or to travel in Britain in connexion with his work, travelling expenses will be paid.

A scholar may not hold any other award concurrently with his scholarship, nor undertake other work, paid or unpaid, without the permission of the Nuffield Foundation.

Both the programme of work and training, and the institute at which it is to be carried out, must be approved by the Foundation.

Should the Foundation at any time find that a scholar neglects or has neglected the obligations of his appointment, it shall have power immediately to terminate his scholarship.

Selection for scholarships is to be made twice a year. Forms of application are obtainable from the Director of the Nuffield Foundation, Nuffield Lodge, Regent's Park, London, N.W.1.

SPECIAL CERAMICS

THE development of new technologies and the increasing tendency to seek greater operating efficiencies at higher temperatures create a rapidly growing demand for special ceramics, to withstand conditions too severe for metals or traditional ceramics. Nuclear engineering and the supply of power at microwave frequencies are two fields where progress in design and construction now requires the development of new high-performance materials. The engineer looks to the ceramist to provide these materials. The impetus of this demand is reflected in the growth of the small group working on special ceramics at the British Ceramic Research Association of the ceramic industries into a major division of the

Association in but a few years. The terms of reference of this group are wide, covering the preparation and testing of new materials, mostly non-oxides, for specialized electrical, nuclear and high-temperature applications, including rocket nozzles, and the assessment of existing materials as potential valve components; its research is carried out in close co-operation with various Service departments and industrial concerns. The first symposium on special ceramics, held by the British Ceramic Research Association in 1959, received enthusiastic support, and the proceedings were edited by P. Popper and published by Heywood and Company in 1960 under the title *Special Ceramics*. Its success led to the holding of a second symposium, under the same heading, at the Association's Laboratories during July 11–13, 1962. This was attended by 170 delegates from seven countries, representing universities, Government departments, research associations and industrial concerns. The twenty-five papers contributed were grouped into sessions under the headings: preparation and properties of non-oxides; oxides; pyrolytics; measurement; techniques; applications of ceramics.

Dr. N. F. Astbury, director of the British Ceramic Research Association, opened the symposium and welcomed the delegates.

The research described in the opening paper, read by Dr. E. D. Lynch (Argonne National Laboratory) on behalf of his colleagues J. H. Handwerk, P. D. Shalek and G. D. White, was stimulated by the need for new nuclear fuels to give greater reactor operating efficiencies. Refractory uranium compounds other than oxides were being investigated, with emphasis on uranium monosulphide and uranium monocarbide, their preparation and their properties. The closely related paper by M. C. Regan and J. W. Isaacs (Atomic Energy Research Establishment, Harwell), read by H. J. Hedger, described the effect of various additions of uranium monocarbide, uranium dicarbide, uranium metal and plutonium monocarbide on the reaction-sintering behaviour of uranium/carbon mixtures and on the density of the monocarbide produced.

The synthesis, purification and hot pressing of silicon hexaboride and silicon tetraboride and the assessment of their mechanical, thermal and electrical properties and resistance to oxidation were described by R. S. Feigelson and Prof. W. D. Kingery (Massachusetts Institute of Technology). Both borides were reported as semiconductors with high resistance to oxidation. Aluminium nitride is a highly refractory non-oxide which, like silicon nitride, is chemically inert and should prove a useful material, especially for applications in contact with molten aluminium. The preparation of pure aluminium nitride by levitating aluminium in a radio-frequency field in an atmosphere of nitrogen was described by Drs. C. F. Cooper, C. M. George and S. W. J. Hopkins (Morganite Research and Development, Ltd.), who also showed that its oxidation was controlled by the rate of diffusion of aluminium ions through the oxide layer. The appearance of profuse whisker growths on aluminium nitride was described by Dr. P. E. Evans (Manchester College of Technology) in the discussion.

The formation of protective coatings on graphite to improve its oxidation and erosion resistance is to-day the subject of considerable study and, in a combined paper, A. E. S. White, Dr. R. F. Deacon, J. Chown and Dr. N. Singer (Morganite Research and Development, Ltd.) described two methods of coating graphite with silicon carbide, composite coatings containing zirconium diboride, and the testing of these in an oxidation furnace, at 2,300° C in a high-temperature flame and in a liquid oxygen/kerosene rocket motor. In the discussion F. C. Carpenter (S. Smith and Sons, Ltd.) pointed out the usefulness of a fluidized bed technique in building up coatings on sharp edges of articles. Beryllium oxide is another material of interest for nuclear power applications. The sintering characteristics of various beryllium oxide powders, the effects of particle size and impurities, and the conditions that must be satisfied if high density is to be achieved were detailed by A. W. Hey and Dr. D. T. Livey (Atomic Energy Research Establishment, Harwell).

Pyrolytic decomposition is sometimes the only means of producing a pure, dense material in bulk form, since many refractories do not sinter and can be hot-pressed only by the incorporation of additives. It is often used to form dense coatings on a heated substrate: good, adherent coatings require that the thermal expansion coefficients of coating and substrate should match closely. The session on pyrolytics opened with a description by P. Popper (British Ceramic Research Association) of the preparation of silicon carbide and the nitrides of aluminium, boron, phosphorus and silicon by pyrolysis, from gases or vapours containing the constituent elements in the correct proportion. Pyrolytic carbon was discussed in two contributions. The first, by Dr. K. W. Carley-Macauly and M. Mackenzie (Atomic Energy Research Establishment, Harwell), indicated the development of new types of very low permeability carbons and graphite for high-temperature gas-cooled nuclear reactors, by impregnation of a porous bed in which a large temperature-gradient is maintained. The second, by Drs. J. Harvey, D. Clark and J. N. Eastabrook (Royal Aircraft Establishment, Farnborough), considered how the structure and crystallographic orientation of pyrolytic carbon varied with the temperature of deposition, surface roughness and graphitization. There was a considerable discussion of the mechanism of growth of the observed cones in the layers.

The electrical properties of rutile, with particular reference to its anisotropy and breakdown, were discussed by M. G. Harwood (Mullard, Ltd.), who showed that the conductivity along the c-axis could increase without affecting that in the perpendicular direction and that small filamentary regions carrying high current were present. The frictional properties and deformation of refractory borides and carbides at high temperature were discussed by C. A. Brookes (University of Cambridge), with reference to the adhesion theory of friction. Slip was observed along (111) planes of titanium carbide single crystals and the mechanism of deformation of polycrystals was considered.

In the session on measurement and techniques, measurements of the thermal conductivity of pyrolytic graphite at temperatures up to 2,100° C were reported by W. Johnson and W. Watt (Royal Aircraft Establishment, Farnborough). The accurate measurement of thermal conductivity is always very tedious, and great interest was shown in the report by Dr. R. W. Powell and R. P. Tye (National Physical Laboratory) of measurements made very rapidly with a new, direct-reading form of thermal comparator and agreeing well with other methods. An automatic dilatometer for the measurement of thermal expansion up to 1,500° C in controlled atmospheres was

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described by K. N. Preece (Morganite Research and Development, Ltd.).

Dr. S. Scholz (Philips, Aachen) discussed the rate of densification of refractories during hot-pressing and the effect on this of activating impurities, such as iron in tantalum carbide. A. W. Moore and Dr. D. A. Young (Imperial College of Science and Technology, London) showed how the anisotropy of electrical and thermal conductivity of well-oriented graphite had been utilized in the design of susceptors for induction heating, in the attainment of temperatures up to 3,400° C. The radial pressure produced in a powder during its compaction in a die and its relation to compacted density were discussed by W. M. Long (Atomic Weapons Research Establishment, Aldermaston). The application of the electron microscope to ceramics, in the study of grain structure and surface texture, in the identification of crystalline fibres and fine particles by electron diffraction, and in following reactions, including the nitridation of silicon, was considered by Dr. S. N. Ruddlesden (British Ceramic Research Association).

The final session of the symposium was devoted to the applications of ceramics. Dr. A. J. Moulson and P. Popper (British Ceramic Research Association) first outlined the physical properties required of a ceramic for use as valve envelopes and windows, and the methods of measuring these properties in order to assess the suitability of a ceramic for valve applications. J. Free and Dr. D. Walsh (University of Oxford) described new uses of ceramics at ultra-high frequencies (cm and mm wave-lengths). Ceramics of low dielectric loss could be used as reflectors of microwave power, with the reflexion exceeding that of a pure silver surface, and experiments using titania were discussed. With non-linear dielectric materials it is theoretically possible to generate microwave power. The succeeding paper, by Dr. K. H. Kreuchen (Electrical and Musical Industries, Ltd.), considered the special ceramic problems arising with the growing use of very-high-power valves in the transmitters of television stations, radar systems and particle accelerators, including the need for low surface resistivity to avoid charge build-up from the plasma, new shapes for wave-guide windows to achieve broad-band performance, and secondary emission qualities. T. D. Davidson and J. Watkins (Morganite Research and Development, Ltd.) described the preparation of high-power microwave absorbing loads from silicon carbide and the variation of its semiconducting properties by the addition of silicon.

The theoretical and practical advantages of using ceramics in microwave tubes for particle accelerators and amplifiers, and the impressive performance obtained on a laboratory scale, were demonstrated by J. Free (University of Oxford), who stressed the need for the commercial development of new materials and predicted that accelerators using ceramics would eventually outclass those of conventional type, on a cost basis, offering higher efficiencies and higher operating frequencies. In the magneto-hydrodynamic generation of electricity, materials are subjected to unusually severe conditions at temperatures above 2,000° C. Dr. E. G. Wolff (Central Electricity Research Laboratories, Leatherhead) described the preparation and testing of some refractory borides, carbides, oxides and silicides as possible electrode and duct materials for such generators. The session closed with an illustration by W. F. Gibbons (Ferranti, Ltd.) of the ceramic-to-metal sealing technique and its application to problems in the field of nuclear engineering.

At the conclusion of the symposium the visitors toured the laboratories of the British Ceramic Research Association. The proceedings of the symposium will be published by the Academic Press in due course.

S. N. RUDDLESDEN

SCIENTIFIC RESEARCH IN BELGIUM

THE annual report of the Institute for the Encouragement of Scientific Research in Industry and Agriculture, Brussels, for 1961 records 65 grants totalling some 244·5 million francs distributed to some 229 investigators and 423 technicians*. A new grant of 21·5 million francs went to the Committee for Establishing the Soil and Vegetation Map of Belgium for its systematic study of the pedological character of the soil and completion of the pedological map of the kingdom. The Committee for the Study of the Solid State received a grant of 11·69 million francs towards research in progress in Prof. Dekeyser's laboratory at the University of Ghent on semi-conductors, in the Laboratory of the Electrical Construction Works at Charleroi on the study of magnetic steel sheets, and in Prof. Brasseur's laboratory at the Gevaert factory on the improvement of photographic supports by perfecting the method of acetylation: a further grant of 2·15 million francs was for continuation of the Committee's work on the welding of resinous materials, 1,787,500 francs for research on the preparation of hard magnetic materials, and one of 9 million francs for continuation of research on the mechanism of photographic processes and for work on new photographic processes. The Carbochemical Society of Tertre received a grant of 3·9 million francs for continuation of work on textile colours, and the Gevaert Photo Products Society 7·225 million francs for the study of photographic dyes and processes of colour photography.

A grant of 10·19 million francs went to the Committee on the Application of Isotopic Methods in Agronomic Research for research on the utilization of radioisotopes, and one of 9 million francs to the Research Centre of Gorsem for continuation of work on diseases and insect pests of various cultivated fruits with the view of a system of control, and also of research on the maturation and conservation of fruit and the influence of insecticides and fungicides on the physiology of the vegetables to which they are applied. The Technical and Scientific Centre of the Belgian Glass Industry received some 8·5 million francs for its research on the properties and homogeneity of glass, the mechanism of action of abrasives and on enamels for glass, while a grant of 7,386,000 francs to the National Centre for Metallurgical Research was for analytical research by spectrochemical and X-ray methods, physical metallurgy investigations, fundamental research by microcinematography on the mechanism of fracture, and various applied investigations; further grants of 2.795,000 francs and 5,050,000 francs were for continuation of research on the mechanical properties of steel and on methods of utilization of steel, and one of 6.500,000 francs for continuation of research on cobalt.

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* Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Bruxelles. Rapport Annuel-Exercice 1961. Pp. 266. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1962.)

The Committee for the Study of the Problem of Nitrogen received a grant of 6,660,000 francs for research on nitrogenous fertilizers in Belgium soils, including investigations on nitrification and absorption of nitrogen by vegetables A grant of 6,027,000 francs to the Centre for Biological Chemistry and Soil Colloids was for investigations on the transformation of organic matter in the soil, mineral colloids of the soil and on the cationic and organic manuring of soils. The Centre for Research into Forestry Biology received 5,590,000 francs for pedological, entomological and genetic research devoted towards improving the exploitation of forests and their conservation by natural regeneration. The Centre for Scientific and Technical Research on the Conservation of Vegetables received a grant of 5,222,000 francs for scientific and technical research on the cultivation of vegetables for conserving and on the conditions of conservation; 4,165,000 francs went to the Centre for Research on Agriculture for work on the mineral alimentation of vegetables, 3,700,000 francs to the Centre for Research on Vegetable Hormones, 3,210,000 francs to the Centre for Research on Parasitic Diseases of Domestic Animals, 2,835,000 francs to the Centre for the Study of the Forest Soils of Ardennes and Gaume; 5,470,000 francs to the Scientific and Technical Research Centre of the Explosives Industry and 5,174,079 francs to that of the Belgian Textiles Industry. Other grants included 3,350,000 francs to Dr. C. Janssen's Research Laboratory for research on the synthesis of ring systems with 3 or 4 atoms of carbon; 2,456,450 francs to the Laboratory of the Varnish, Paints, Printing Inks and Art Colours Industry and 2,835,000 francs to the Centre for the Study of Ornamental Plants for work on plant diseases and the cultivation of chrysanthemums and cyclamens. Other grants included 2,162,000 francs to the Technical and Scientific Centre of the Woodworking and Related Industries; 1,980,000 francs to the Pedological Service of Belgium for researches on magnesium and on minor elements in vegetables; 1,840,000 francs to the Foundation for Potato Research; 1,770,000 francs to the Committee for the Study of Apiculture; 1,525,000 francs to the Committee for Strawberry Research; 1,160,000 francs to the Committee for the Scientific and Technical Study of Lactation; and 1,086,000 francs to the Belgian Institute for High Pressures.

THE AUSTRALIAN DEFENCE STANDARDS LABORATORIES

THE function of the Australian Defence Standards Laboratories is outlined in the annual report for the year ended June 30, 1961*. Its main concern is the application of scientific knowledge and research within the fields of chemistry, physics, metallurgy and engineering to problems arising in the design, manufacture, inspection and use of defence material. In addition, as its name implies, it is concerned with standards of measurement and standards of quality. The report deals with the general activities of the establishment and with some of the more important projects and investigations either completed or in progress in the fields of chemistry, metallurgy and physics.

In the Chemistry Division research has continued on the mechanism of the initial film formation on lead alloy anodes in sea-water, and the initial film has been shown to contain lead sulphate and some carbonate, in addition to lead chloride. Infra-red spectroscopy has been used for the study of a series of *bis*-pyridinium oximes. The cæsium bromide prism of the double pass spectrometer was calibrated for the far infra-red and a curve fitted to twenty points by orthogonal polynomial methods. The best fit was given by a cubic expression. The application of the Stokes parameters of electromagnetic radiation to the description of the state of polarization of Raman lines has been considered, and a procedure has been developed for the calculation of the intensity ratios and depolarization factors. The new apparatus of Lench and Martin for low-pressure gas analysis, used in conjunction with improved apparatus for vacuum fusion gas extraction, collection and measurement, has been in constant use during the period under review for the determination of the gas content of chromium metal and chromium alloys by the vacuum fusion method.

During the year, major improvements were made to the control system of the air-conducting plant of the metrology laboratory in the Physics Division to enable temperatures in the Length Standards Room to be maintained at 20° C ± $\frac{1}{4}$° C irrespective of external conditions. Considerable developments in the application of interferometry to measurements of length are reported. A Varian wide-line nuclear magnetic resonance spectrometer with associated electromagnet and power supply has been installed, and is being used to investigate the concentration of the fluoride catalyst in the chromic acid solutions used in the production of chromium. Electron spin resonance has been used to study the defects produced by γ-rays on a commercial polymethyl methacrylate. New values for X- and γ-radiation absorption coefficients have been obtained, and designs for counters for the detection of soft X-rays have been developed and constructed.

The Metallurgy Division has been largely concerned with the evaluation of one of the Australian chromium-based high-temperature alloys processed under quasi-industrial conditions. This includes ductility studies, internal friction measurements and observations of the creep behaviour. Work has continued on cleavage in cast molybdenum of high purity and in wrought chromium. As a contribution to the understanding of the abrasion of metals, the sliding of a metal specimen on silicon carbide abrasive papers has been studied, and it has been found that the majority of abrasive particles make a groove in the surface of the metal, piling up a ridge on either side, but removing little or no metal. An idealized model of the process has been devised and an expression obtained for the rate of removal of material with allowance made for the deterioration in use of the abrasive paper. Using metallographic techniques it has been established conclusively that the damage, produced in the surface layers of slices of germanium for use in semiconductor devices when cut from large single crystals and lapped to size, is due to cracks, and new methods of preparation of the slices which will considerably reduce the extent of the damage are being developed.

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* *Commonwealth of Australia: Department of Supply.* Australian Defence Scientific Service. Annual Report of Defence Standards Laboratories for the year ended 30th June, 1961. Pp. 49. (Maribyrnong, Victoria: Defence Standards Laboratories, 1962.)

The annual report lists the various personnel of the Laboratories and their status, and gives details of the publications by members of the staff during the year under review. The staff numbered 687 on June 30, 1961. Open days were held at Maribyrnong during April 1961, and during the four days some 4,000 persons visited the Laboratories.

S. WEINTROUB

MENTAL HEALTH IN THE WORLD

It is difficult to estimate the prevalence of mental illness, but in countries where services for the mentally ill are relatively highly developed, psychiatric cases account for almost half the total number of patients occupying hospital beds. Apart from cases in hospital, too, a considerable proportion of the world's population suffers from minor forms of mental ill-health which do not require hospitalization but may disorganize social relationships and productive capacity. It has been suggested that to provide satisfactory treatment for all cases of psychiatric disorder one psychiatrist is needed for every 20,000 people—a requirement that has so far been met in only a few areas.

The main concern of the World Health Organization in mental health has been to develop knowledge that can be applied in public health administration by its Member States. The reports of its Expert Committee on Mental Health and of its study groups on related subjects, for example, provide a conspectus of international views on policy in this field as they have developed over the past twelve years. Laying special emphasis on preventive work, the Organization has assisted the development of psychiatric services in Member States through seminars, conferences and fellowships and by providing consultants and expert advice. Its educational influence in mental health has been further extended by its publication of a number of authoritative studies—on topics from the architecture of psychiatric hospitals to the epidemiology of mental disorders—by leading international authorities. The World Health Organization has also done much to foster both basic and applied psychiatric research.

A new booklet, which is a reprint of a series of articles that appeared in the *WHO Chronicle* between March and July 1962, surveys those aspects of mental health work with which the World Health Organization has been particularly concerned *. These include: the development of psychiatric services; the mental health tasks of the public health services; problems of prevention and treatment; education, training and research, and programme development. There is also a bibliography of World Health Organization reports and studies on mental health.

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* World Health Organization. WHO and Mental Health, 1949–1961. Pp. 48. (Geneva: World Health Organization; London. H.M. Stationery Office, 1962.) 1 Swiss franc; 1*s*. 9*d*., 0 30 dollar

WIDESPREAD IONOSPHERIC DISTURBANCES DUE TO NUCLEAR EXPLOSIONS DURING OCTOBER 1961

By PROF. TATSUZO OBAYASHI

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A SERIES of Soviet nuclear tests made during October 1961 at Novaya Zemlya reveals the evidence of strong blast waves propagating through the ionosphere over a considerable distance. Two large nuclear tests were carried out at 08h 28m on 23 and at 08h 33m on October 30, 1961, which were probably of 30 and of 50 megatons, respectively. Although the exact situation of the tests at Novaya Zemlya is not known, the detonations were made probably in the troposphere. Some preliminary investigations on the remarkable ionospheric disturbances associated with these explosions have already been reported. At Scandinavian stations, the ionograms showed a sudden heavy disturbance in the *F* region of the ionosphere about half an hour after the detonation[1,2]. A large increase of f_0F_2 and subsequent oscillatory variations with a period of 1½ h were found at Lindau, Germany, being delayed about 2 h[3]. Short-wave radio communications between Europe and Japan (*GBJ*–34, 14·80 Mc/s, and *DGP*–862, 15·86 Mc/s) deteriorated immediately and rather poor conditions lasted several hours[4].

Similar ionospheric disturbances have been known at the high-altitude nuclear explosions over Johnston Island during August 1958. The travelling F_2 disturbance was found which spread several thousand kilometres over the Pacific area[5-7]. Photographs of the explosion taken from Hawaii indicated clearly the existence of an expanding luminous front through the upper atmosphere[8]. Since these disturbances may possibly be due to shock waves spreading out from the explosion, the investigation of the present effect by the world-wide ionosonde network would be of great value. Thus, ionospheric data at about 30 stations in the northern hemisphere have been analysed.

The results for two events on October 23 and 30, the time variations of f_0F_2 at several key stations in Europe and in Asia, and the field-strength record at the route Hamburg–Osaka (15·86 Mc/s) are illustrated in Fig. 1. The great-circle path of the Hamburg–Osaka is deviated about 1,000 km from the shot point. It is shown that an anomalously large increase and subsequent fluctuations of f_0F_2 were observed at all European stations, and smaller effects in Japan. The peak of increased f_0F_2 was delayed progressively from nearer to distant stations. This travelling disturbance was confirmed in India and in South-East Asia. Both

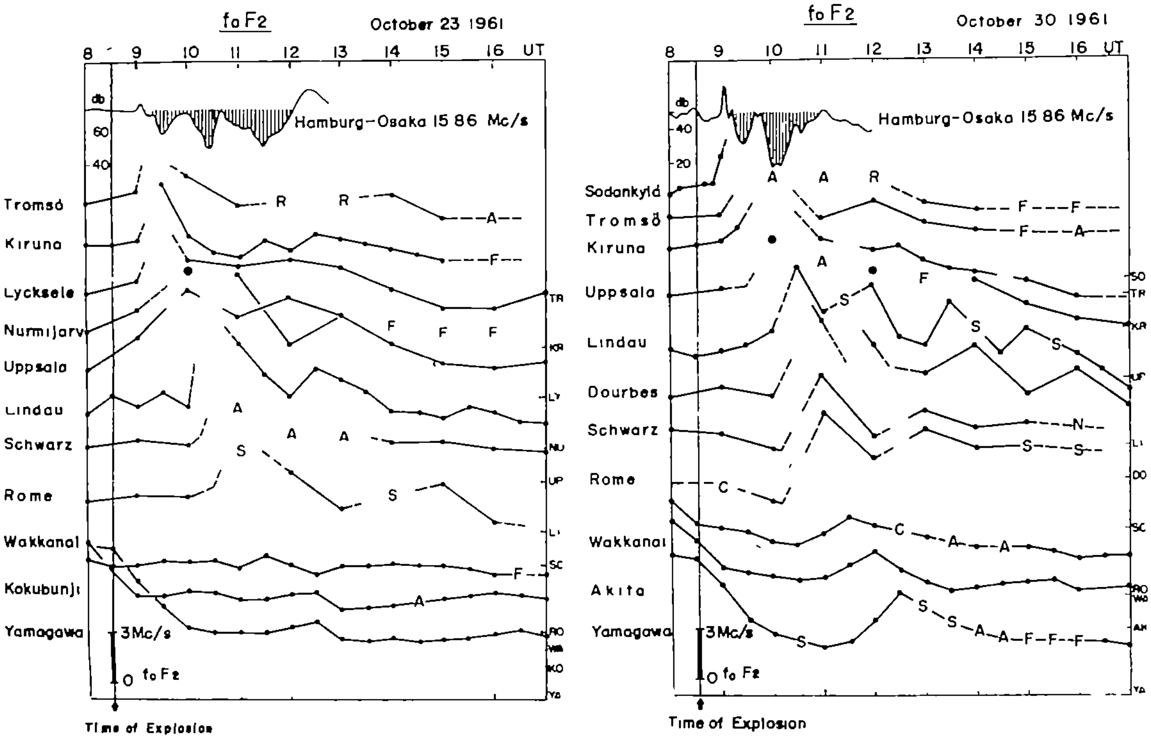

Fig. 1. Time variations of f_0F_2 and the field-strength *DGP*-862 (15 86 Mc/s) during the periods of nuclear explosions on October 23 and 30, 1961

events of October 23 and 30 are quite similar, except a more turbulent condition for the second one.

Travel-time versus distance diagrams of F_2 disturbances are given in Fig. 2. It is evident that the disturbance spread out with nearly a constant speed over several thousands of kilometres. The speed of these travelling disturbances was of 400–500 m/s, significantly faster than the speed of sound in the stratosphere. In spite of such widespread effect over the Eurasian continent, little or no effect was noticed at the North American stations, although their distances are comparable with those of European stations.

On the other hand, it has been reported that the pressure waves were propagated all over the world and were observed by microbarographs[1,2,9,10]. The mean velocity was about 310 m/s with wave-length of the order of 100 km. Round-the-world echoes of the F_2 disturbance were also noticed by Rose, Oksman and Kataja[2] and Dieminger and Kohl[3]. It has been inferred, further, that the travelling disturbance wave would have an excess velocity near the detonation, but be propagated later with nearly constant velocity of 420 m/s. The wave-length is estimated to be about 200–300 km, and hence a dominant period of such a wave would be 10 min or somewhat more.

The nature of such a travelling disturbance wave is a matter of speculation. It appears, however, that the disturbance may have its origin in propagating atmospheric waves controlled by gravitational and compressional forces. The internal atmospheric wave has two types, depending on its wave frequency[11,12]. In the high-frequency range the behaviour is that of an acoustic (sound) wave, while in the very low frequency the gravitational forces introduce a profound anisotropy and essentially it may be called the gravity wave. The characteristic frequencies separating these two domains are known as the Vaisala-Brunt frequency (gravitational stability

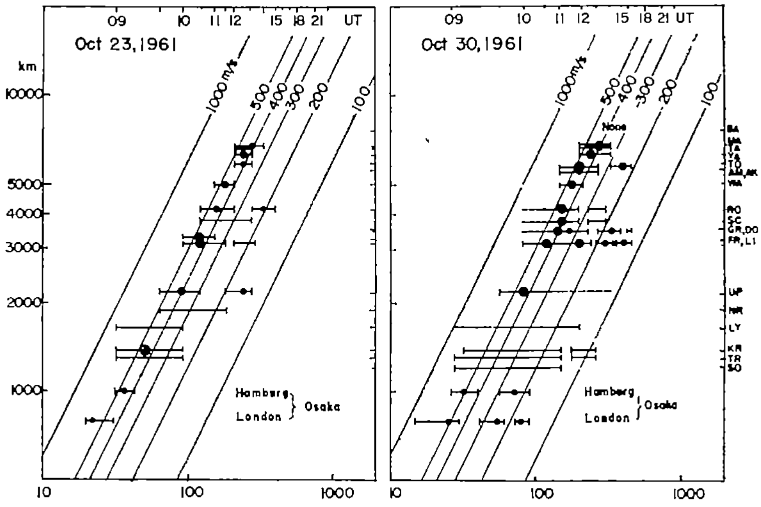

Fig. 2. Travel-time versus distance diagrams of the ionospheric F_2 disturbances for the events on October 23 and 30, 1961. Oblique lines show the travel-time curves for constant speeds

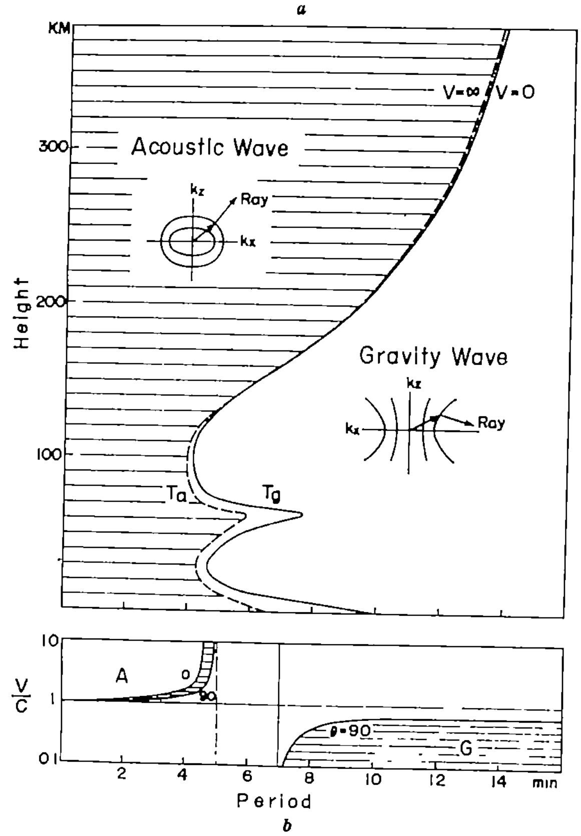

Fig. 3. *a*, Altitude profile of the stability frequencies T_g and T_a in the upper atmosphere; *b*, phase velocity of atmospheric waves at the height of 60 km

frequency) ω_g, and modified (acoustic) stability frequency ω_a. For the atmosphere in hydrostatic equilibrium with density $\rho(z)$ and the speed of sound $c(z)$, they are given[13] by:

$$\omega_g^2 = -g\left(\frac{g}{c^2} + \frac{1}{\rho}\frac{\partial\rho}{\partial z}\right) = \frac{g}{H}\left[\frac{\gamma - 1}{\gamma} + \frac{\partial H}{\partial z}\right]$$

and

$$\omega_a^2 = \left(\frac{c}{2\rho}\frac{\partial\rho}{\partial z}\right)^2 = \frac{\gamma g}{H}\left[1 + \frac{\partial H}{\partial z}\right]^2$$

where g is the gravitational acceleration, γ is the ratio of specific heat and H is the scale height. The phase velocity V of the wave is given:

$$\left(\frac{V}{c}\right)^2 = \frac{1 - Y\sin^2\theta}{1 - X}$$

where $X = (\omega_a/\omega)^2$, $Y = (\omega_g/\omega)^2$ and θ is the angle between the gravity field and the wave normal.

Two characteristic periods ($T = 2\pi/\omega$) in the upper atmosphere and horizontal velocity of the wave at approximately 60 km height are depicted in Fig. 3. The propagation-surfaces at two domains are also shown. They indicate that the acoustic waves propagate more or less isotropically, while the gravity waves. exhibit a strong anisotropy, tending to propagate in the horizontal direction.

Since the wave produced by the explosion at high atmosphere has the period of 10 min or more, the atmospheric gravity wave must be most conspicuous in the region of the upper atmosphere. The attenuation of waves through the upper atmosphere is due mainly to the air viscosity and the thermal conduction. The linear attenuation loss due to viscous damping is given approximately by $\mu/\rho c(2\pi/\lambda)^2$, where μ/ρ is the kinematic viscosity in which μ is (2–5) × 10^{-3} c.g.s. in the ionospheric region. Then, the attenuation distance should be of the order of 6,400 km at 150 km height ($\rho \simeq 2 \times 10^{-12}$ g/cm^3) for the wave-length of 250 km. Therefore, the wave suffers negligible attenuation below the F region, although the attenuation increases rapidly above this height owing to the exponential decrease of the air density.

On the other hand, it is shown theoretically that the wave tends to increase its amplitude of oscillation with increasing height, in proportion to $\exp(z/2H)$. Then the region of most intense disturbance will be determined by the competing effects of dissipation and the upward growth of wave amplitudes. This would place the largest amplitude of waves in the lower F region. Furthermore, the gravity waves of their period of 10 ~ 15 min are bounded only in the region below 200–300 km, and the phase velocity of wave becomes much slower than the local speed of sound near the upper fringe. This may be that an intense shock wave is inevitably generated in this region, since the speed of the disturbing agent may easily exceed the phase speed of the medium.

It will also be noted that, for the gravity wave of its period well above T_g, the ratio of horizontal and vertical wave-lengths is very large, and the movements of gas particles are followed in the horizontally elongated ellipse. However, in the F_2 region the motion of particles, especially of electrons, is affected profoundly by the existing geomagnetic field. This implies that the atmospheric gases may move with the waves, but electrons can execute their movements only along the field lines.

Taking account of these factors, a somewhat schematic illustration of the wave motions together with the horizontal velocity profile of the gravity waves at some representative periods are shown in Fig. 4. The amplitude of wave grows exponentially with increasing height until the dissipation dominates over this, and a shock wave may develop near the upper fringe. This

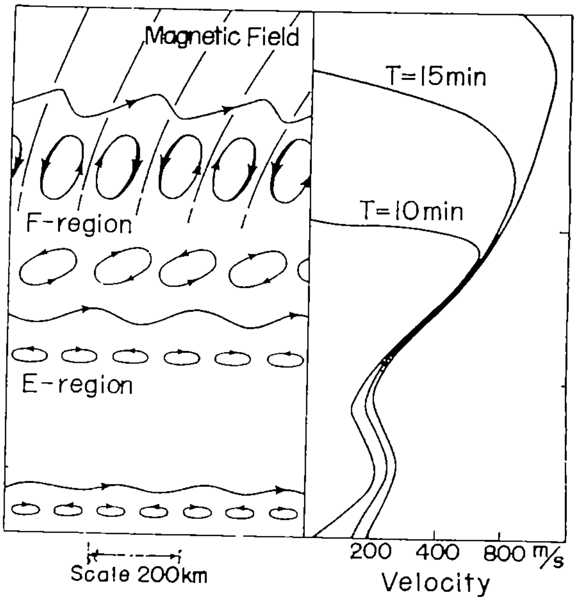

Fig. 4. Pictorial representation of the behaviour of the gravity waves and their speed in the upper atmosphere. Motion of air particles and electrons, and the wave-forms at different altitudes are depicted

may explain why a strong disturbance is observed in the F region but not in the E region. Sharp shock fronts produced by the convergent movement of ionization may attribute specular reflexion of radio waves, and result apparently in the temporal increases of penetration frequency (f_0F_2).

The transportation of the wave energy to very long distances warrants further discussion. As has been pointed out by Hines[12], there must exist a strong ducting of the energy in the lower ionosphere below the thermal incline. The main flow of the energy may propagate quasi-horizontally in the duct, although the duct is admittedly leaky and attributes the disturbance in the F region. Since the viscous dissipation of the wave is negligible at the wave-length of a few hundred kilometres, it would appear that the wave propagates greater distances without excessive attenuation, being reflected at the antipodal point of the explosion. This has been well demonstrated by the observed round-the-world echoes of travelling disturbances[2,3].

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AMBIENT XENON: A KEY TO THE HISTORY OF METEORITES

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THE existence of isotopic anomalies in meteoritic xenon has been firmly established by Reynolds and other investigators[1–6]. Xenon in many meteorites exhibits a 'special' anomaly, in which the isotope xenon-129 is greatly enhanced with respect to terrestrial xenon, as well as smaller 'general' anomalies affecting the remainder of the xenon isotopes. Conflicting explanations of the general anomalies have been offered[7,8], but it does seem clear that the xenon special anomalies are a result of the meteorite parent bodies having formed quickly enough after the last synthesis of iodine-129 to permit an excess of the radiogenic daughter xenon-129 to accumulate in the iodine-bearing minerals. Jeffery and Reynolds[9] have shown that the 'excess' xenon-129 and the iodine in the Abee chondrite are almost certainly located at the same sites, thus supporting the above interpretation. The research initiated by Reynolds has therefore established 'xenon chronometry' as a fundamental tool in studies of the early solar system, because it provides a measure of the interval from iodine synthesis to the cooling of the meteoritic minerals. Since it is not certain which processes contributed to the nucleosynthesis of iodine, one can only derive relative rather than absolute decay intervals.

We believe that Reynolds's data concerning meteoritic xenon carries, in addition, information about the interiors of meteorite parent bodies, information which rivals in importance the xenon chronometry. In discussing these data, we shall suggest an improvement on one unsatisfactory aspect of xenon chronometry—that the special anomalies have been determined by comparison with unsuitable reference standards.

The first characteristic of the interiors of the meteorite parent bodies that we must consider is their isolation from the surrounding primitive solar nebula. In our model, the outer layer of the meteorite parent body represents both a thermal wall and a barrier to gaseous diffusion. The portion of this barrier that lies at temperatures below 300° K would scarcely be less than 1 km in thickness for parent bodies 50 km or more in radius, barring unbelievably large heating-rates much in excess of 0·05 cal/g/yr.[10]. DuFresne and Anders[11] have suggested that the interiors of the parent bodies contained water which would freeze in the barrier layer as on a cold trap, and thereby form a 'wall' of ice. While this barrier might be temporarily ruptured during the evolution of meteorites, any such ruptures would be self-sealing, as DuFresne and Anders have pointed out. Both the existence of such an icy wall and the strong tendency for xenon to be adsorbed at low temperatures would tend to inhibit exchange between the interiors and the surrounding nebula. Thus, there appear to be at least two mechanisms for the isolation of the interiors of meteorite parent bodies. Furthermore, evidence does exist both for such isolation and for mixing within the internal atmospheres. In exploring this evidence, we can show that higher pressures of volatile materials were maintained inside the meteorite parent bodies than outside, and that significant evolutionary changes in the xenon mass spectrum occurred before the meteorites completed their physical and chemical development.

One meteorite of particular importance in this connexion is the Sardis iron. An analysis of a sample of troilite from Sardis indicated that it has an iodine content of 3·6 p.p.m., a factor of 100 greater than that typical of ordinary chondrites[12]. Preliminary measurements by Reynolds, Merrihue and Pepin[13] show that Sardis troilite contains radiogenic xenon-129 accompanied by 'primordial' xenon, with a total xenon content of $4{\cdot}2 \times 10^{-10}$ c.c./g (Table 1). Using data of Jeffery and Reynolds for Abee[14,15], it is possible to estimate the difference between the decay intervals of these two meteorites by assuming only that the ($^{129}I/^{127}I$) ratio was the same for these meteorites at any given time. It is not necessary to postulate a definite value for this ratio, so that one

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Table 1. XENON ANOMALIES IN SARDIS AND ABEE

Sample	δ_M‰ normalized to ^{132}Xe ^{129}Xe	 ^{131}Xe	Total Xe c.c.STP/g
Sardis troilite	318 ± 10	81 ± 5	4·2 × 10^{-10}
Sardis metal	160 ± 25	31 ± 10	2·6 × 10^{-11}
Abee	4400 ± 100	4 ± 16	8 × 10^{-9}

δ_M‰ = 1000 [(^{M}Xe/^{132}Xe)/(^{M}Xe/^{132}Xe)atm—1]

need not rely on specific models of iodine nucleosynthesis. We have used the procedure of Reynolds *et al.* to compute 'excess' xenon-129 (by subtracting the atmospheric xenon spectrum) and find that Abee has the shorter decay interval by about 190 m.y.

The interpretation of this difference requires some caution. The assumption of identical (^{129}I/^{127}I) ratios in the two meteorites is valid in general only if they were derived from a homogeneous nebula, or if they both represent material from the same well-mixed element of an inhomogeneous nebula. Since the general anomalies at xenon-131 differ for the two samples (Table 1), it might be argued that this is not the case. However, even a variation in iodine isotopic composition by a factor of two will only give rise to an error of 16·4 m.y. in this calculation. While our estimate of the difference in these decay intervals may be modified when sufficient data on Sardis troilite become available, it seems likely that the difference is a real one under any interpretative scheme and due to different times required for cooling these meteorites. (In the following sections it will be shown that the 'conventional' model is essentially justified for Abee. For Sardis, however, the proportion of xenon-129 produced by the decay of indigenous iodine-129 is not known. Nevertheless, if one assumes in the extreme case that all xenon-129 in Sardis troilite was produced by decay of indigenous iodine-129, the difference in these two decay intervals would be reduced by no more than 35 m.y.) In Fig. 1 we have given volume-average and central cooling times for meteorite parent bodies of various dimensions containing the complement of long-lived radioactivities which they possessed about 4·5 × 10^9 years ago. The difference in Abee and Sardis decay intervals supports the view that Sardis was once part of the well-insulated core of a parent body of perhaps 100-km radius, while Abee may have evolved in the mantle of a similar object. It is also evident that for asteroidal-sized bodies the volume-average cooling times are comparable to the entire xenon decay intervals characteristic of meteorites. (Goles, Fish and Anders[16] arrived at the same conclusion previously, from a discussion of argon diffusion in stone meteorites.)

Let us now consider whether the observed 'primordial' xenon in these and other meteorites may be considered to be a relic of internal atmospheres of parent bodies, or whether it is a sample of the gases of the solar nebula. Murthy and Patterson[17] have reviewed the lead isotope data now available and have concluded that the primary lead–lead isochron of zero age for meteorites and that for the Earth agree to within a standard deviation of 1·7 per cent. Thus, the date of the primary uranium–lead differentiation for any meteorite and for the Earth may be assumed not to differ by more than 150 m.y., applying a 95 per cent confidence limit. Radiogenic xenon could only have been retained in Abee some time after the uranium–lead differentiation in the materials of that meteorite, which from the argument of Murthy and Patterson can scarcely have occurred as much as 150 m.y. prior to the corresponding differentiation for the Earth. Thus it is evident that the retention of radiogenic xenon in Sardis troilite, and perhaps in Abee as well, was an event approximately contemporaneous with or possibly later than the formation of the Earth's crust. For Abee, at least, xenon-132 is lost on heating more easily than is xenon-129. Therefore, the retention of 'primordial' xenon probably began later than that of radiogenic indigenous xenon-129, whether one interprets the data with the conventional model or with that to be developed. Although data on Sardis troilite are lacking, exchange of 'primordial' xenon in Sardis with xenon in its environment probably was rapid at high temperatures, so that retention of indigenous xenon-129 and the fixation of its 'primordial' xenon should have occurred nearly simultaneously.

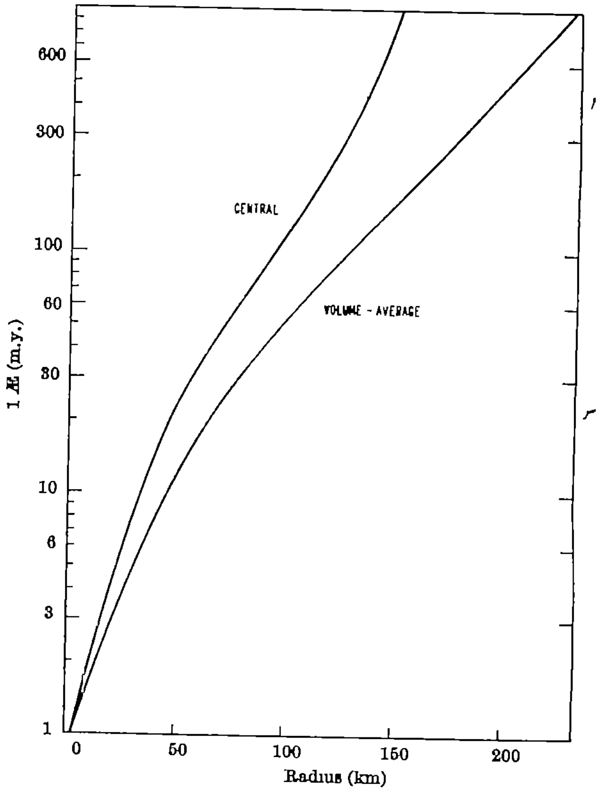

Fig. 1. Cooling times versus radius for meteoritic parent bodies. The times required for central and volume-average temperatures to fall from 700° K to 200° K were computed from the relations given by Allan and Jacobs[20]. The following assumptions were made: (1) the only heat source during cooling was ^{40}K, 1·45 × 10^{-9} deg. y^{-1}; (2) thermal diffusivity $K \equiv 1 \times 10^{-2}$ cm^2 sec^{-1}; (3) the surface temperature was fixed at 100° K by radiation

Suess[18] and Urey[19] have presented compelling evidence that when the Earth was formed, the solar nebula was already grossly depleted in noble gases. It is difficult to believe that Sardis troilite, which cooled at very nearly the same time, obtained its 'primordial gases' from the nebula at a time when the Earth was unable to do so. Instead, it seems much more plausible that Sardis was isolated from the exterior of the parent body and cooled under a substantial partial pressure of highly evolved gases. These gases, absorbed on cooling, were not necessarily identical with the gases that were expelled on first heating the primitive materials of the parent body and so it would be misleading to call them 'primordial', despite their large abundance.

We shall refer to this absorbed gas as 'ambient', to emphasize that it does not necessarily have the same composition as the gases of the primitive nebula. In particular, we suggest that radiogenic xenon-129 (from iodine-129) and xenon-131–136 (from fission of heavy elements) was produced in those regions of the parent body that were at relatively high temperatures and mixed freely with the ambient xenon, so that the isotopic abundance in ambient xenon of xenon-129 and xenon-131–136 increased with time. The progressive enrichment of the ambient gas in xenon-129 is a most significant characteristic of our model, because it is confirmed by a direct experimental check that will be described in the next section. But it should be noted that evolutionary changes in the ambient gas have been anticipated in earlier papers[10,11]. Fish *et al.* propose that the initial state of the parent bodies was that of a cold, small object, perhaps 50–100 km in diameter, containing a large amount of condensed volatiles such as water, CH_4, H_2S, and ammonia. (Subsequent work by Miller[20] has shown that these compounds might have entered the parent bodies in a matrix of gas hydrates). On heating, it is argued, volatile materials in the central regions of these bodies were vaporized and participated in oxidation-reduction reactions. These volatile compounds may have played a part in the extraction of chalcophile elements which was termed the 'sulphur cycle' by Fish *et al.* Physical fractionation of these gases might have occurred during transport within the parent body, their escape to the nebula, and the eventual re-absorption of a surviving portion by the minerals as they cooled. DuFresne and Anders give detailed evidence for the action of water on the minerals of the carbonaceous chondrites and the absorption by the aqueous solutions of sulphur dioxide and carbon dioxide, and show how appreciable pressures of gas—consistent with xenon partial pressures of 10^{-5}–10^{-6} atmospheres—might have been retained in the interiors of the parent bodies during their cooling era.

If one supposes that each meteorite's xenon originated in a single, closed environment in which some degree of gaseous mixing could take place, it becomes possible to monitor the evolutionary stage of the ambient gas, and also to gain new insight into the xenon dating method. We shall use data obtained with the experimental approach utilized by Jeffery and Reynolds[9], although we shall place a different interpretation on the results. Jeffery and Reynolds sought to establish the association of anomalous xenon-129 with meteoritic iodine by means of neutron irradiation of the meteorite, followed by heating experiments. 'Anomalous' xenon-129 in their sense is the xenon-129 in excess over the amount of xenon-129 that corresponds to the terrestrial or carbonaceous chondrite $^{129}Xe/^{132}Xe$ ratio. In the meteorite Abee, an excellent correspondence of the thermal release patterns of pile-produced xenon-128 (which we call xenon-128*) with anomalous xenon-129 was found. In several other meteorites such a correspondence was also apparent but not as convincing.

If we regard the terrestrial $^{129}Xe/^{132}Xe$ ratio as having no special cosmochemical significance, we must find a more general way to separate the total meteoritic xenon-129 into a radiogenic component, xenon-129 (indigenous), associated with iodine-127 and a component associated with the ambient gas. We shall assume that only these two components exist and that the ratio xenon-129 (indigenous)/xenon-128* is independent of temperature in the heating experiments. Xenon-129 (indigenous) is therefore equal to a times xenon-128*, where a is a constant, for each xenon fraction released on heating. Similarly, xenon-129 (ambient) is equal to b times xenon-132 for each fraction, where b is another constant. The total xenon-129 in any fraction is then:

$$^{129}\mathrm{Xe} = a\,^{128}\mathrm{Xe}^* + b\,^{132}\mathrm{Xe} \qquad (1)$$

On normalizing to ^{132}Xe we obtain:

$$\frac{^{129}\mathrm{Xe}}{^{132}\mathrm{Xe}} = a\left(\frac{^{128}\mathrm{Xe}^*}{^{132}\mathrm{Xe}}\right) + b \qquad (2)$$

Note that for three or more fractions, equation (2) should define a straight line if our assumptions are valid. This relation also is of a particularly convenient form, because the intercept b should represent the ratio $^{129}Xe/^{132}Xe$ in the ambient xenon at the time when the meteorite became retentive of xenon, and the slope a is related to the decay interval Δt through the formula:

$$a = \left(\frac{^{129}\mathrm{I}}{^{127}\mathrm{I}}\right)_0 \left(\frac{^{127}\mathrm{I}}{^{128}\mathrm{Xe}^*}\right)_{\mathrm{monitor}} \exp\left(-\Delta t/\tau_{129}\right) \qquad (3)$$

The ratio $(^{127}I/^{128}Xe^*)$ in the monitor (used to calibrate the neutron flux) is a measurable quantity, and $(^{129}I/^{127}I)_0$ signifies the ratio of radioactive to stable iodine at the beginning of the decay interval. It is a quantity that is fixed by the model adopted for iodine synthesis. τ_{129} is the mean lifetime of ^{129}I (23·7 m.y.).

Equation (3) fixes the decay interval Δt uniquely, once $(^{129}I/^{127}I)_0$ is assumed, the monitor activation-level has been measured, and a has been determined by a least squares fit of equation (2) to the irradiation and heating data. Since this technique separates the ambient from the indigenous xenon-129 component, no external normalization is required. Freedom from normalization to a terrestrial xenon mass spectrum or a xenon mass spectrum characteristic of the carbonaceous chondrites is a gain in three respects. First, xenon of both the Earth and the carbonaceous chondrites is only remotely related to 'primæval' xenon, since in one case there is evidence for strong physical fractionation[18], and in the other for advanced chemical evolution[11], probably accompanied by fractionation. Secondly, equation (3) makes it possible to date the carbonaceous chondrites, as cannot be done if they are used as a basis for normalization. Thirdly, even samples showing negative xenon-129 'anomalies' can in principle be dated as they could not under the earlier procedure.

We have described an idealized case. A real meteorite would have required an appreciable time to cool from those temperatures at which exchange with the ambient xenon is rapid for all sites to those temperatures at which exchange is greatly inhibited for all sites. Thus, high-temperature sites (presumably those contributing to the high-temperature xenon fractions of the heating experiments) may contain ambient xenon which is discernibly 'younger' than that of the low-temperature sites. Similarly, the high-temperature sites might be expected to have an anomalously high content of indigenous xenon-129, compared with low-temperature ones. Fortunately, since the measured quantity in question is the $^{129}Xe/^{132}Xe$ ratio in xenon from each group of sites, these two effects tend to cancel. Only if the average $^{132}Xe/^{129}I$ ratio in the source of the ambient xenon had been very different from that in the meteorite during its cooling would any large error be introduced. In real meteorites there is presumably more than one iodine-bearing mineral, which conflicts with the assumption that the ratio ^{129}Xe (indigenous)/^{128}Xe* is independent of temperature in the heating experiments. This problem will not always be unmanageable however, for it may be that a single mineral will contain most of the iodine and other minerals will only introduce a certain degree of scatter in the points. In other cases two minerals may be important, but will release xenon at such different characteristic temperatures that the two sets of data will be essentially independent of one another. In general, the most refractory iodine-bearing mineral may be treated by our procedure if only high-temperature points are considered. As noted here, fission of heavy nuclides adds xenon-132 to the ambient xenon and so might have changed the basis of normalization during cooling. Owing to the probable low abundance of fissionable nuclides, this effect will be assumed to be negligible. Finally, problems of pre-terrestrial diffusion losses, surface iodine contamination, and contamination with adsorbed atmospheric xenon, all lead us to question the suitability of low-temperature data for use in the above relations

There are four stages of investigation that will provide checks on the correctness of the assertions made earlier in this article.

(1) *Applicability of Equation* (2). Do the irradiation and heating experiments (which we shall call for convenience 'correlation runs') give data that lie on a straight line, with a positive non-zero intercept, when ^{129}Xe/^{132}Xe is plotted against ^{128}Xe*/^{132}Xe ? Reynolds *et al.* (private communication) inform us that this is the case, although correlation runs have been made for only a small number of meteorites so far. This provides a check on our assertion that one may decompose the xenon-129 into ambient and indigenous components according to equation (2). Jeffery and Reynolds have kindly permitted us to reproduce a graph that makes use of the co-ordinate system suggested here and the data from their most recent correlation run on the meteorite Abee. It is shown as Fig. 2. All the high-temperature points ($\geqslant$ 900° C) show an excellent linear correlation, suggesting that in Abee we approach the ideal case of rapid cooling (hence a well-defined Δt) and a single dominant refractory iodine mineral. The data also suggest that a second iodine mineral is making some contribution to the points obtained at lower temperatures. The presence of two separate sources is indicated when one recognizes (as did Jeffery and Reynolds[14]) that the ratio ^{128}Xe*/^{132}Xe should reach a maximum at the temperature of maximum release of xenon from any given iodine-bearing mineral. By grouping the points of Fig. 2 into sequences 600°–1,000° and 1,100°–1,500° according to this rule, one observes that the former would yield a linear regression of smaller slope and larger intercept than the latter. The data are only suggestive however, and do not merit a formal computation. The lowest temperature points at 400° and 500° may be influenced by yet another release pattern, although they are those most subject to error due to iodine and atmospheric xenon contamination.

Let us return to considering the high-temperature correlation alone. In the case of Abee, the linear relationship that emerges from this type of plot is not surprising, considering the correlations obtained by Jeffrey and Reynolds[8]. The value of the intercept in Fig. 2 is 0·94 ± 0·16, indicating a ratio of xenon-129 to xenon-132 in the ambient gas at the time that the meteorite cooled that is not distinguishable from the terrestrial ratio of 0·983. Hence, in subtracting the terrestrial ^{129}Xe/^{132}Xe ratio, Jeffrey and Reynolds in effect subtracted the constant b from a set of linear relations of the form $y = ax + b$. In the case of some of the other meteorites, for which Reynolds and co-workers will shortly publish data, the correlations that emerge on using expression (2) were masked to some degree by the former type of analysis, since the ratio of ^{129}Xe/^{132}Xe in the ambient xenon is not always close to the terrestrial ratio.

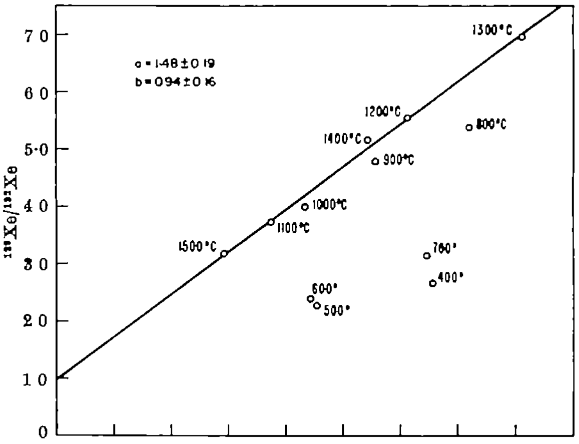

Fig. 2. Correlation of ^{129}Xe/^{132}Xe and ^{128}Xe*/^{132}Xe from Abee according to equation (4). The points for 900°–1,500° C were fitted by linear regression of ^{129}Xe/^{132}Xe on ^{128}Xe*/^{132}Xe

(2) *Evolution and source of ambient xenon.* Do the decay intervals obtained from equation (3) show a positive correlation with the parameter b of equation (2) ? This is necessary if we are to assume that the ambient xenon is a valid indicator of the 'age' of the gas that was reabsorbed in the meteorite on cooling, as expressed in its ^{129}Xe/^{132}Xe ratio. The results of Reynolds *et al.* suggest that this is also the case, although for several meteorites the data are only preliminary. It may eventually be possible to use the composition of the ambient xenon to define decay intervals independently of Δt's obtained from the parameter a.

The variation of b, the ratio of ^{129}Xe/^{132}Xe in the ambient xenon at the time of cooling, appears to be 30–40 per cent (based on 4 meteorites). This is easily seen to be too large a range to be explicable by free decay of iodine-129 in an undifferentiated homogeneous solar nebula. Adopting for the cosmic abundance of iodine-127 the value of 0·2 c.a.u. and for xenon-129 the value 1·05 c.a.u. (1 c.a.u. = 1 atom/10^6 silicon atoms; Suess and Urey[21]), a 40 per cent variation in b from one meteorite to another requires a ratio of ^{129}I/^{127}I at the time of formation of the meteorite with lowest b near 2·0. By way of contrast, three popular models for iodine synthesis yield considerably lower values for the ratio ^{129}I/^{127}I : $2 \times 10^{-4} \exp(-\Delta t''/\tau_{129})$ for solar nebula synthesis[22]; $5 \times 10^{-3} \exp -(\Delta t' + \Delta t'')/\tau_{129}$ for continuous galactic synthesis[23,24]; and $2 \times 10^{-1} \exp -(\Delta t' + \Delta t'')/\tau_{129}$ for a 'mixed' nucleosynthesis with a 10 per cent last-moment contribution[25]. Here $\Delta t'$ refers to the time-interval between the isolation of the solar nebula and the formation of the meteorite parent bodies, and $\Delta t''$ to the interval from formation of the parent bodies to the cooling of the meteoritic minerals with the lowest value of b. Thus, free decay in the undifferentiated solar nebula clearly cannot produce a 40 per cent modification in the ratio $^{129}\mathrm{Xe}/^{132}\mathrm{Xe}$. Such a modification can easily have been achieved in the postulated interior atmospheres however, for even the most xenon-rich meteorites (such as Murray) have iodine/xenon ratios 10^4 times higher than undifferentiated cosmic material.

(3) *Data from separated phases.* While we may rule out evolution of ambient xenon in an undifferentiated nebula, the question of whether the observed variation in b could have arisen during the dispersal of the solar nebula still remains to be studied. Fortunately, it is possible to recognize evolution in the closed environment of an interior atmosphere as opposed to that in the open environment of the solar nebula by the following test: Do correlation runs on separated phases of the same meteorite (which presumably cooled in the same environment) lead to a consistent value for the ratio $(^{129}\mathrm{Xe}/^{132}\mathrm{Xe})_e$, where $(^{129}\mathrm{Xe}/^{132}\mathrm{Xe})_e$ denotes the $^{129}\mathrm{Xe}/^{132}\mathrm{Xe}$ ratio that would eventually be reached in the ambient gas if all radiogenic xenon-129 were mixed with it? $(^{129}\mathrm{Xe}/^{132}\mathrm{Xe})_e$ is given by:

$$\left(\frac{^{129}\mathrm{Xe}}{^{132}\mathrm{Xe}}\right)_e = b + c\exp\left(-\Delta t/\tau_{129}\right) \qquad (4)$$

In the equation:

$$c = \left(\frac{^{129}\mathrm{I}}{^{127}\mathrm{I}}\right)_0 \left(\frac{^{127}\overline{\mathrm{I}}}{^{132}\overline{\mathrm{Xe}}}\right) \qquad (5)$$

where $(^{129}\mathrm{I}/^{127}\mathrm{I})_0$ is the ratio of radioactive to stable iodine at the beginning of the decay interval, and $(^{127}\overline{\mathrm{I}}/^{132}\overline{\mathrm{Xe}})$ is the average ratio of iodine-127 and xenon-132 in the entire mixing volume for the ambient xenon.

From equations (4) and (5) it is apparent that if b is plotted against $\exp(-\Delta t/\tau_{129})$ for three or more separated phases of the same meteorite, a linear relation should be defined. Its intercept is the parameter $(^{129}\mathrm{Xe}/^{132}\mathrm{Xe})_e$ that is characteristic of the environment of the meteorite, and the slope is $-(^{129}\mathrm{I}/^{127}\mathrm{I})_0(^{127}\overline{\mathrm{I}}/^{132}\overline{\mathrm{Xe}})$. Reynolds *et al.* have not yet obtained sufficient data on separated phases to study the adherence to equations (4) and (5), but it is evident that these relations do provide a powerful means of checking the validity of the assumption of evolution in a closed environment.

(4) *The environment parameter.* Let us assume that equations (4) and (5) will be found to be as much in accordance with future data on separated phases as equations (2) and (3) are with existing data for whole meteorites: then it will be possible to use the parameter $(^{129}\mathrm{Xe}/^{132}\mathrm{Xe})_e$ in a unique way. We have found that this parameter should characterize the mixing volume of the meteorite's environment (which may and probably does include a large fraction of the interior of the meteorite's parent body) rather than the meteorite itself. Hence it will be appropriate to call it the 'environment parameter'. Every meteorite formed in the same mixing volume should exhibit the same environment parameter, when correlation runs are performed on separated phases and interpreted according to equations (2), (3), (4) and (5). Conversely, meteorites formed in different environments—and by different we mean environments possessing appreciably different values for c—should be characterized by diverse values for the environment parameter.

The significance of the mixing volume is increased owing to the fact that it should represent at least the volume which participates in the common magmatic pool from which meteoritic minerals formed. If two meteorites were found to represent different mixing volumes, then it is very unlikely that they formed by differentiation from a common magmatic pool.

Thus, through studying the environment parameters of meteorites of different types it should be possible to learn whether it is correct to regard them as originating in the same parent object. And it may be possible in this way to gain new insight into the significance of the high- and low-iron groups of chondrites[26], the gallium–germanium groups of meteorites[27] and the clusters of cosmic ray ages[28,29].

Since c is determined by equation (4), and the ratio $(^{129}\mathrm{I}/^{127}\mathrm{I})_0$ is fixed by the nucleosynthetic model used, the ratio $(^{127}\overline{\mathrm{I}}/^{132}\overline{\mathrm{Xe}})$ would also be determined in such a study and could be used in a manner somewhat similar to that proposed for the environment parameter.

We thank Prof. John Reynolds, Mr. Craig Merrihue and Mr. Robert Pepin for several helpful discussions, and for making many experimental results available to us in advance of publication. We are particularly indebted to them for their permission to quote new results from the meteorite Abee, and to mention in a general way the preliminary results of other experiments that they will report in detail. Lacking this documentation, it would have been necessary to leave Parts 1 and 2 of the last section in as speculative a form as Parts 3 and 4. We are also grateful to Profs. V. Rama Murthy and Stanley L. Miller for their advice and helpful criticisms.

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EXISTENCE OF WEAK *R*-TYPE IONIZATION FRONTS

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THE theoretical work of Kahn[1] and Goldsworthy[2] on the gas dynamical effects of ionized H_{II} regions associated with the formation of *O* and *B* stars has enabled them to predict the formation of weak *R*-type ionization fronts in interstellar gas. Axford[3] has also examined the existence of weak *R*-type fronts and has discussed the anomaly introduced by the Chapman–Jouguet hypothesis which shows that they cannot exist.

In this article we give some evidence for the existence of weak *R*-type ionization fronts obtained during investigations of electromagnetically driven planar shock waves in the laboratory. Although we do not claim conclusive evidence, we think that the observed phenomenon is sufficiently interesting to be released before the publication of our main body of work elsewhere, and hope that it will help to resolve the anomaly studied by Axford.

Fig. 1 is a picture of streak (distance-time) and interference fringe photographs of the plasma motion superimposed on the same emulsion. Conditions under which this picture was taken are: 10-kV, 10-μF condenser used to drive the plane shock tube, which is filled with deuterium gas, at an ambient pressure of 4 mm mercury. Fringe shifts may be taken as directly proportional to the electron density in the plasma[4], and the dimensions of the shock tube[5] are such that one fringe shift represents an electron density increase of $0{\cdot}9 \times 10^{17}$ cm^{-3}. It will be seen that there are two distinct discontinuities in electron density demarcated by the fringe shifts, the second one coinciding with a visible luminous front. There is also a faint luminous front which coincides with the first discontinuity in electron density, but it is not possible to see it in Fig. 1 because self-light from the plasma has been deliberately attenuated to increase fringe definition[4]. A streak picture of the plasma taken without the superimposed interference fringes is shown in Fig. 2 to make this point clear. The interesting thing here is that the electron density behind the first front reaches an equilibrium, that is, the fringes pass through the first front, are displaced by a fixed amount and then remain parallel until they intersect the second front. At the second front they are further displaced until they attain a maximum shift in the direction of increasing electron density, after which they return to the undeflected position.

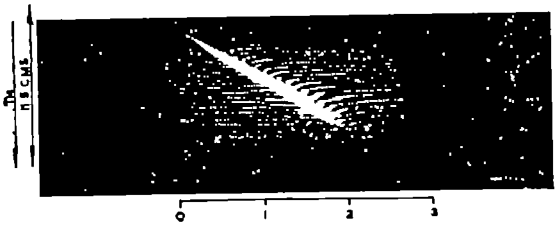

Fig. 1

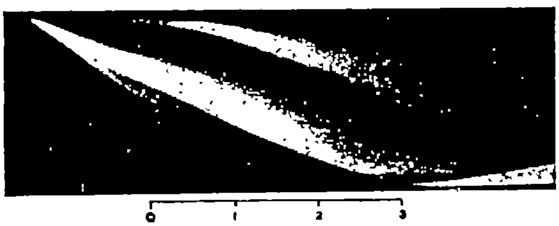

Fig. 2

Fig. 3 is an enlarged tracing of a typical fringe passing through the two luminous fronts and may be taken to be a graph of electron density with time at a fixed position in the shock tube. We now discuss possible explanations of the profile:

(1) Even though the electron density reaches equilibrium behind the first front it is difficult to see how the front can be contiguous with a classical strong shock discontinuity. Strong shock computations by Niblett[6] show that there should be $2{\cdot}2 \times 10^{16}$ free electrons cm^{-3} behind a shock front moving at 5×10^{6} cm sec^{-1} into deuterium at an ambient pressure of 4 mm mercury, and we have only 7×10^{16} cm^{-3} behind the first front.

(2) The Rosenbluth snowplough model[7] does not provide a satisfactory explanation even if we postulate a probability that a particle is swept up (Philips[8]), because it is concerned only with a thin sheath of ionized particles and we have here a relatively thick zone of ionized gas containing a second discontinuity of electron density.

(3) The flow pattern suggested by Goldsworthy and Axford of a weak *R*-type ionization front propagating ahead of a shock wave appears to fit the profile better. In their model the ionization is produced in the first front by ultra-violet radiation with quantum energies above the ionization potential. If the front advances with supersonic speed relative to the sound speed in the gas upstream and the ionized gas downstream of the front it is designated weak *R*-type. The weak *R*-type ionization front is followed by an 'isothermal' shock, and the ionizing radiation from the star is assumed to pass through the isothermal shock without absorption.

In applying this to the laboratory experiment the difficulty is to identify the source of radiation, which must, of course, be behind the second front, and since the ionization front moves at 5×10^{6} cm sec^{-1} it must provide about $3{\cdot}5 \times 10^{23}$ photons cm^{-2} sec^{-1} with energies above the Lyman limit. The emittance of ionizing photons at temperatures of 10^{6}, 10^{5} and 10^{4} °K were computed assuming the Planck distribution law. These are $1{\cdot}4 \times 10^{29}$, $1{\cdot}4 \times 10^{26}$ and $1{\cdot}5 \times 10^{20}$ cm^{-2} sec^{-1} respectively. Assuming that the radiation is bremsstrahlung from a slab of thickness 1 cm, we obtain much lower values of $1{\cdot}3 \times 10^{20}$, $3{\cdot}2 \times 10^{19}$ and $3{\cdot}2 \times 10^{14}$ ionizing photons cm^{-2} sec^{-1}. We therefore deduce that a black-body temperature between 10^{4} and 10^{5} °K

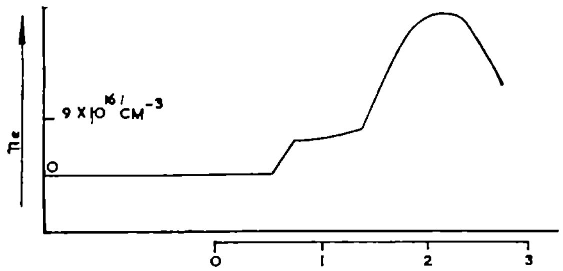

Fig. 3

would be an adequate source if we assume no loss mechanism.

There is some evidence to fix the radiation source at the current sheet because it has been observed that when the current has been short-circuited by a delayed discharge in the gas, which may contain impurities left behind at the wall, that the first luminous front starts to pull back into the second front (Fig. 2). Another possible source of radiation could be an impurity line radiator the frequency of which is close to the Lyman limit. Further evidence to support the existence of a weak R-type front is that the mean free path for photo-ionization ($\frac{1}{2}$ cm at 4 mm mercury at the Lyman limit) is of the same order as the front width.

Although more evidence must be obtained before the existence of a weak R-type system can be substantiated, the facts, so far as are known, in this experiment certainly indicate the existence of such a system.

We thank Dr. F. A. Goldsworthy for stimulating discussions.

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RESONANCE INTERACTIONS OF FLUORESCENT ORGANIC MOLECULES IN SOLUTION

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THE quantum efficiency of the characteristic fluorescence of aromatic molecules in solution decreases at high concentrations. This effect, known as concentration quenching, appears to be general, and is due to interactions between excited and unexcited molecules of the same kind. In some compounds the decrease in intensity of the normal structured emission band M, with increase in concentration c, is accompanied by the appearance of a broad structureless emission band D at longer wavelengths which increases in intensity with increase in c (ref. 1). M is characteristic of the excited unassociated (monomer) molecules: D is characteristic of excited dimers formed by the association of excited and unexcited molecules.

We may distinguish four types of concentration-dependent behaviour in solutions of fluorescent aromatic compounds.

(I) The appearance of the emission D at high c is accompanied by a new absorption band D_0 at longer wave-lengths than the normal (monomer) absorption band M_0. This behaviour, which is exhibited by perylene and anthranthrene, is attributed to the formation of fluorescent excited dimers which are also stable in the ground-state.

(II) The appearance of D is not accompanied by any changes in the absorption spectrum at high c. This behaviour is exhibited by pyrene[2] and 1:2-benzanthracene and by many of their derivatives[1] (Table 2). It is attributed to the formation of fluorescent excited dimers, known as excimers[3], which are not stable in the ground-state. The excimer emission D is accompanied by dissociation.

(III) Anthracene exhibits concentration quenching and a change in the absorption spectrum at short wave-lengths at high c following photo-excitation[4], but no emission D is observed[1]. This behaviour has been shown to be due to the formation of a non-fluorescent excited dimer (dianthracene) which is stable in the ground state.

(IV) In other compounds concentration quenching occurs, but there is no emission D and no change in the absorption spectrum. By analogy to I–III, this behaviour may be attributed to the transient formation of non-fluorescent dimers which are not stable in the ground state.

These four types of behaviour are summarized in Table 1.

Table 1

Type	Concentration quenching	Dimer emission	Dimer absorption
I	Yes	Yes	Yes
II	Yes	Yes	No
III	Yes	No	Yes
IV	Yes	No	No

Table 2 lists the properties of all the compounds which we have so far observed to exhibit excimer emission, that is, Type II behaviour. The wave-lengths in nm (mμ) of the first vibrational peak M_1 in the monomer emission spectrum (observed at low c to minimize self-absorption) and of the peak D_1 of the excimer emission spectrum, and the energy difference $\nu^1(M_1-D_1)$ in cm.$^{-1}$ between M_1 and D_1 are tabulated. The peak $M_1 = 341$ nm in 2,5 diphenyloxazole (PPO) is only observed at $c \leqslant 10^{-4}M$, and is absent from the spectrum reported by Weinreb[5].

The effects so far considered are due to interactions between identical molecular species, which are naturally in resonance. We wish now to report a new phenomenon, the resonance interaction between

Table 2. COMPOUNDS FORMING EXCIMERS

No.	Compound	M_1 (nm)	D_1 (nm.)	$\nu'(M_1-D_1)$ (cm.$^{-1}$)
1	1:2-benzanthracene	386	510	6,300
2	2′-methyl 1·2-benzanthracene	388	512	6,240
3	3′-methyl 1. 2-benzanthracene	387	507	6,120
4	4′-methyl 1: 2-benzanthracene	389	510	6,100
5	3-methyl 1 2-benzanthracene	389	504	5,870
6	4-methyl 1: 2-benzanthracene	386	507	6,180
7	5-methyl 1: 2-benzanthracene	386	510	6,300
8	6-methyl 1. 2-benzanthracene	387	508	6,150
9	7-methyl 1. 2-benzanthracene	389	504	5,870
10	8-methyl 1: 2-benzanthracene	387	510	6,230
11	9-methyl 1: 2-benzanthracene	396	504	5,410
12	10-methyl 1·2-benzanthracene	390	514	6,180
13	2′,4-dimethyl 1·2-benzanthracene	389	508	6,020
14	2′,6-dimethyl 1: 2-benzanthracene	388	511	6,200
15	Cholanthrene	389	510	6,100
16	Pyrene	372	482	6,180
17	1-methyl pyrene	374	484	6,080
18	3-methyl pyrene	376	488	6,110
19	4-methyl pyrene	376	490	6,190
20	1 : 2-benzpyrene	389	~467	~4,300
21	3 · 4-benzpyrene	403	514	5,360
22	1 · 2 : 4 : 5-dibenzpyrene	398	~488	~4,630
23	1 · 2 . 3 : 4-dibenzanthracene	377	486	5,950
24	1 : 12-benzperylene	408	~504	~4,670
25	2,5-diphenyloxazole (PPO)	341	445	6,850

two different molecular species A and B to form excited 'mixed dimers' $(AB)^*$ which may be either fluorescent or non-fluorescent.

We have compared the fluorescence spectra of solutions containing the following molar concentrations: (i) c of A, (ii) c of B, and (iii) c of A and c of B (prepared by mixing equal volumes of solutions containing $2c$ of A and $2c$ of B respectively). A and/or B are compounds which exhibit excimer emission. In general, if the first singlet π-excitation energy is S_1, and if $S_1(A) > S_1(B)$, non-radiative energy transfer occurs between A and B due to dipole-dipole inductive interaction[6] and the excitation energy becomes localized on B, the species with the lower value of S_1 (ref. 7). The magnitude of this non-radiative transfer and also of the corresponding radiative transfer process, depends on the degree of overlap of the emission spectrum of A and the absorption spectrum of B. In our experiments A and B are chosen as having very similar values of S_1 so that $S_1(A) - S_1(B) \leqslant 120$ cm.$^{-1}$. This minimizes the A–B spectral overlap, inhibits preferential energy transfer from A to B, and also establishes resonance between A and B.

Fig. 1 shows the observed spectra for A = pyrene, B = 1-methyl pyrene, at $c = 5 \times 10^{-3}M$. The scale of relative quantum intensity for the individual solutions of A and B is halved prior to plotting to allow for the fact that in the mixed solution half the incident light is absorbed by each of the solutes. If A and B did not interact, the resultant fluorescence spectrum of the mixture would be the sum $(A + B)$ of the spectra of the two components. The difference by a factor of 2 between the excimer components of the observed mixture spectrum Z and $(A + B)$ is therefore attributed to emission by 'mixed excimers' $(AB)^*$ formed by the diffusional collision and resonance interaction of A^* and B and of B^* and A.

Similar behaviour has been observed in the following pairs of compounds: 4-methyl pyrene and 1-methyl pyrene; 1:2-benzanthracene and 6-methyl 1:2-benzanthracene; 5-methyl 1:2-benzanthracene and 6-methyl 1:2-benzanthracene.

Fig. 2 shows the observed spectra for A = pyrene B = 3:4 benzphenanthrene, at $c = 5 \times 10^{-3}M$. 3:4 benzphenanthrene exhibits type IV behaviour (Tabl 1). In this case the relative intensity of the excime component of the mixture spectrum Z is only 0· that of the sum spectrum $(A + B)$. This is attribute to the formation of non-fluorescent 'mixed dimers' $(AB)^*$ by the resonance interaction of A^* and B and of B^* and A. Similar behaviour has been observed with A = pyrene, B = 6-methyl 1:2 benzanthracene, showing that even if both component

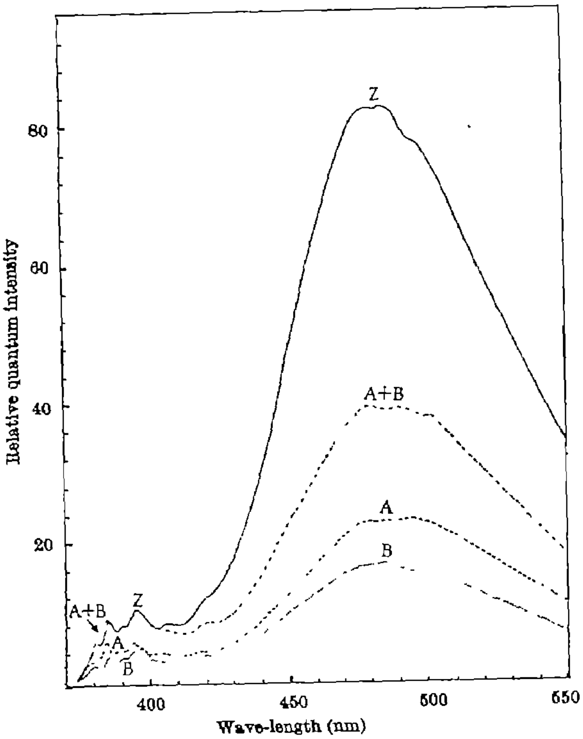

Fig. 1. Fluorescence spectra of solutions in cyclohexane. A, 5×10^{-3} M pyrene, B, 5×10^{-3} M 1-methyl pyrene; Z, 5×10^{-3} M pyrene and 5×10^{-3} M 1-methyl pyrene

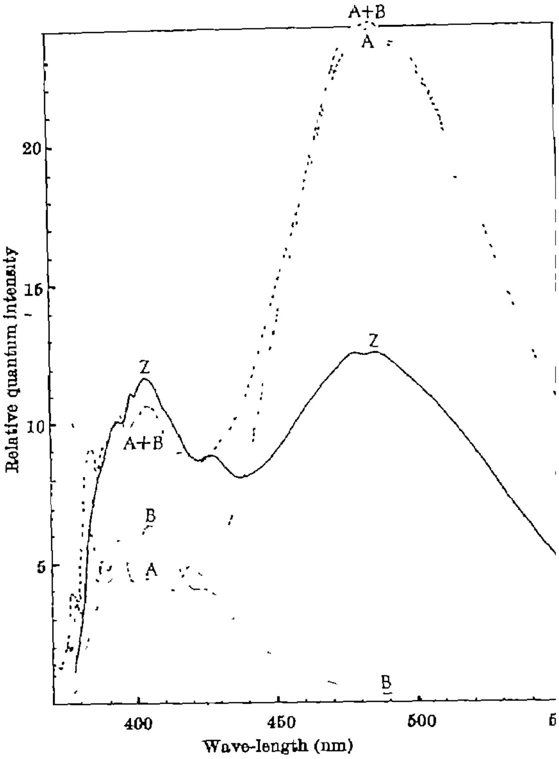

Fig. 2. Fluorescence spectra of solutions in cyclohexane. A, 5×10^{-3} M pyrene, B, 5×10^{-3} M 3 : 4-benzphenanthrene; Z, 5×10^{-3} M pyrene and 5×10^{-3} M 3 : 4-benzphenanthrene

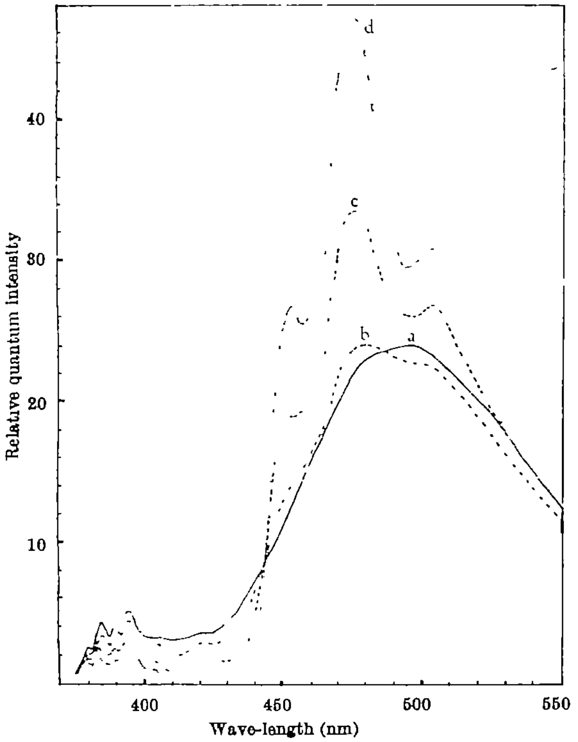

Fig. 3. Fluorescence spectra of solutions in benzene. *a*, $1\cdot1 \times 10^{-3}$ *M* pyrene, *b*, $1\cdot1 \times 10^{-3}$ *M* pyrene and $1\cdot1 \times 10^{-5}$ *M* perylene, *c*, $1\cdot1 \times 10^{-3}$ *M* pyrene and $5\cdot5 \times 10^{-5}$ *M* perylene; *d*, $1\cdot1 \times 10^{-3}$ *M* pyrene and $1\cdot1 \times 10^{-4}$ *M* perylene

form fluorescent excimers (Table 2), their 'mixed dimers' may be non-fluorescent if their molecular shapes differ. In this one case $S_1(A) - S_1(B) = 1040$ cm.$^{-1}$ but the energy transfer is sufficiently small to be neglected.

Hochstrasser[8] observed a band in the fluorescence spectrum of a solid solution of perylene in crystalline pyrene, intermediate in wave-length between the spectra of crystalline pyrene and crystalline perylene which he attributed to a 'mixed dimer' emission. Fig. 3 shows the effect of the addition of perylene on the fluorescence spectrum of a concentrated solution of pyrene in benzene. The strong emission band which appears in the 480-nm region is due to the perylene monomer emission *M*, excited preferentially by energy transfer from the pyrene.

It is possible that the similar band observed in the crystalline solution spectrum has a similar origin.

We may summarize the behaviour of the excited 'mixed dimers' so far observed in terms of the four types I-IV (Table 1).

(*a*) II + II→II (molecules of similar shape).
(*b*) II + II→IV (molecules of different shape).
(*c*) II + IV→IV

In addition, Furst and Kallmann[9] have observed cross concentration quenching between molecules of anthracene and 2-methyl anthracene. Anthracene is of type III and so probably is 2-methyl anthracene, so that we have:

(*d*) III + III (or IV)→IV (or III).

The possibility of stable 'mixed dimers' is an interesting one, and this and other possible types of 'mixed dimer' behaviour are at present being studied.

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NICOTINAMIDE 6-MERCAPTOPURINE DINUCLEOTIDE AND RELATED COMPOUNDS: POTENTIAL SOURCES OF 6-MERCAPTOPURINE NUCLEOTIDE IN CHEMOTHERAPY

By DR. M. R. ATKINSON, J. F. JACKSON, PROF. R. K. MORTON and A. W. MURRAY

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WE[1] have previously shown that an enzyme from liver nuclei catalyses the formation of dinucleotides from a number of compounds which resemble the natural substrates (nicotinamide mononucleotide and nicotinic acid mononucleotide, and adenosine triphosphate) thus:

$$\begin{array}{c}\text{substituted-}\\\text{pyridine}\\\text{mononucleotide}\end{array} + \begin{array}{c}\text{purine}\\\text{nucleoside}\\\text{5}'\text{-triphosphate}\end{array} \overset{\text{transferase}}{\rightleftharpoons} \begin{array}{c}\text{substituted-}\\\text{pyridine}\\\text{purine}\\\text{dinucleotide}\end{array} + \begin{array}{c}\text{pyro-}\\\text{phosphate}\end{array}$$

An impure sample of 6-mercaptopurine riboside 5′-triphosphate inhibited adenylyl transfer from adenosine triphosphate to nicotinamide mononucleotide; the reaction products contained traces of a new dinucleotide which had the properties expected of nicotinamide 6-mercaptopurine dinucleotide[1]. This and some related compounds are described here: they have potential value in cancer chemotherapy.

Nicotinamide 6-mercaptopurine dinucleotide. Nicotinamide 6-mercaptopurine dinucleotide has now been prepared from the two mononucleotides in a yield of 20–25 per cent by treatment with dicyclohexylcarbodiimide in aqueous pyridine, a method used by Todd *et al.*[2] for synthesis of nicotinamide adenine dinucleotide (coenzyme I). The compound was purified by ion-exchange chromatography on 'Dowex-2' (formate form) and electrophoresis on paper at *p*H 4·1 in 50 mM acetic acid–50 mM formic acid–50 mM triethylamine. It had the same electrophoretic mobility in this system as nicotinamide hypoxanthine dinucleotide. At *p*H 5, the absorption maxima were at 266 mμ (mε, 6·6) and at 321 mμ (mε, 23·4).

This new analogue of coenzyme I was reduced enzymatically with alcohol dehydrogenase at pH 9·5 in 0·3 M ethanol–0·1 M glycine. The maximum absorption of the reduced form was at 311 mμ (mϵ, 22·4); the increase in absorption at 340 mμ (Δmϵ, 5·9, based on phosphorus analysis) on reduction of the oxidized form was the same as that reported for coenzyme I (ref. 3). Nicotinamide mercaptopurine dinucleotide was also reduced with dihydrolipoamide and dihydrolipoamide dehydrogenase, and was hydrolysed to its constituent mononucleotides by nucleotide pyrophosphatase.

The dinucleotide obtained by chemical synthesis had the same electrophoretic mobility as the nicotinamide mercaptopurine dinucleotide obtained by the action of the nuclear transferase. In a coupled enzymatic assay with nicotinamide mononucleotide and the appropriate purine riboside triphosphate, together with the transferase, ethanol and alcohol dehydrogenase, formation of the reduced dinucleotide was measured at 340 mμ; the relative rates at pH 8 were: with 0·4 mM adenosine triphosphate, 100 units, with 0·4 mM mercaptopurine riboside 5′-triphosphate, 7 units, and with 0·4 mM hypoxanthosine triphosphate, less than 3 units. These results clearly confirm that 6-mercaptopurine riboside 5′-triphosphate is a substrate of the nuclear transferase[1].

6-Mercaptopurine riboside 5′-triphosphate. By a combination of paper electrophoresis and chromatography in isobutyric acid–ammonia, 6-mercaptopurine riboside 5′-triphosphate has now been purified. At pH 4·1, this compound has the same electrophoretic mobility as hypoxanthosine triphosphate. It is a substrate of hexokinase, and one equivalent of reduced nicotinamide adenine dinucleotide phosphate (reduced coenzyme II) was formed in an assay with the coupled reactions:

$$\begin{array}{c}\text{mercaptopurine}\\\text{riboside}\\\text{triphosphate}\end{array} + \text{glucose} \rightarrow \begin{array}{c}\text{mercaptopurine}\\\text{riboside}\\\text{diphosphate}\end{array} + \begin{array}{c}\text{glucose}\\\text{6-phosphate,}\end{array}$$

and

$$\begin{array}{c}\text{glucose}\\\text{6-phosphate}\end{array} + \begin{array}{c}\text{oxidised}\\\text{coenzyme II}\end{array} \rightarrow \begin{array}{c}\text{6-phospho-}\\\text{gluconic acid}\end{array} + \begin{array}{c}\text{reduced}\\\text{coenzyme II,}\end{array}$$

using hexokinase and glucose 6-phosphate dehydrogenase.

In confirmation of the previous finding with impure material[1], purified 6-mercaptopurine riboside 5′-triphosphate is a potent inhibitor of adenylyl transfer to nicotinamide mononucleotide catalysed by the nuclear adenylyl transferase. In the coupled assay with the transferase and alcohol dehydrogenase at pH 8, K_m for adenosine triphosphate was $7{\cdot}4 \times 10^{-5}$ M and K_i for 6-mercaptopurine riboside 5′-triphosphate was 5×10^{-5} M.

Formylaminoimidazolethioamide nucleotide. During the preparation of crystalline pyridinium 6-mercaptopurine riboside 5′-monophosphate, it was found that this nucleotide was partly converted into a new nucleotide which had 25 per cent greater anionic mobility on electrophoresis at pH 4·1. The new nucleotide was isolated by paper electrophoresis; it had maximum absorption at 280 mμ, was hydrolysed to a nucleoside by 5′-nucleotidase, and was converted rapidly into 6-mercaptopurine nucleotide in 0·15 N sodium hydroxide and more slowly at pH 9. This compound is very probably the nucleotide of formylaminoimidazolethioamide, an analogue of the natural precursor (the nucleotide of formylaminoimidazolecarboxamide) which forms hypoxanthosine 5′-monophosphate in the cell[4].

Lethal synthesis in malignant cells. Recent investigations indicate that 6-mercaptopurine nucleotide and its metabolic products are responsible for the effects of 6-mercaptopurine in chemotherapy[1]; resistance to the drug appears to be due to the failure of entry into the tumour cell, or to decreased synthesis of the nucleotide after uptake of the free base[5]. The inhibition of the nuclear adenylyl transferase by 6-mercaptopurine riboside 5′-triphosphate may largely account for the selective action of 6-mercaptopurine against certain tumours[1]. The ease with which derivatives of 6-mercaptopurine are modified by oxidative and hydrolytic enzymes, and by enzymes which catalyse group transfer reactions, suggests that certain derivatives of 6-mercaptopurine mononucleotide, such as dinucleotides, polynucleotides, diesters and nucleoside 3′-5′-cyclic phosphates, may be converted into active antimetabolites within the cell by lethal enzymatic reactions. The ready conversion of derivatives of aminoimidazolethioamide into 6-mercaptopurine derivatives as found by Shaw and Butler[6], and especially the apparent conversion of formylaminoimidazolethioamide nucleotide into 6-mercaptopurine nucleotide as described here, suggests that this class of compound may be a source of 6-mercaptopurine nucleotide in cells resistant to 6-mercaptopurine. These compounds are now being studied, along with analogues of nicotinamide nucleotides[7].

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ESTIMATION OF MOLECULAR WEIGHTS OF PROTEINS BY GEL FILTRATION

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GEL filtration is a fractionation method which utilizes the 'reversed' sieving properties of a bed of porous gel granules. During passage through the bed, solute molecules which are small enough to penetrate the porous gel are retarded relative to larger molecules unable to enter the pores. Its application to large molecules was demonstrated by Lathe and Ruthven[1], who achieved some separation of polypeptides and proteins on columns of starch grains. They also proposed the method as a means of molecular weight estimation, and reported interesting results in this connexion; but their starch columns did not effect enough separation between different proteins for the idea to be developed further. The at present available 'Sephadex' gel filtration media have the same disadvantage. However, agar gels[2-4] and synthetic polymer gels[5,6] are effective for separating a variety of macromolecules, so they provide a means of testing Lathe and Ruthven's suggestion.

For this purpose it is desirable to know the molecular weight range over which a gel functions optimally. In practice, each gel separates solute molecules of a wide range of molecular weights, indicating that the pore size in the gel is far from uniform. However, the inverse relationship between concentration of gel-forming substance and mean pore diameter[3,7] suggests that gels of different concentration will give optimal separation in different molecular weight ranges, the more dilute gels being better for larger molecules. This effect has now been studied with agar gels, using proteins as solute molecules, and the gel filtration technique applied to the estimation of protein molecular weights.

Preparation of agar gels and columns. Gels were prepared by heating mixtures of agar (standard grade; Davis Gelatine, Ltd., Warwick) and water at 90° with stirring until the water was all absorbed, then autoclaving the swollen agar at 15 lb./sq. in. pressure until it had liquefied completely. Autoclaving for 45–60 min was required for gels containing 9 and 12 per cent by weight of agar, but 20–30 min was sufficient for less-concentrated ones. The gels were allowed to cool at room temperature for at least 12 h before being disintegrated in a rotating-blade homogenizer ('Atomix'; M.S.E., Ltd.) run at full speed, water being added as required. The temperature was kept below 30°. Slurries of granules prepared in this way were easily pushed through standard sieves for grading according to size with the aid of a broad flat spatula. The treatment needed to give a good yield of granules of the required size varied with the gel, but was soon determined by experiment. The sieved granules were thoroughly washed with water, the washings being decanted. Prepared in this way, granules have been stored under water in the cold for several months without noticeable change.

To facilitate the correlation of results, all experiments were performed with gel columns measuring 2·5 × 50 cm. These were prepared by pouring aqueous suspensions of gel granules into vertical glass chromatographic columns, and allowing the water to flow through under gravity, until beds 53–55 cm long were obtained. When a small pressure was applied these columns shrank to about 50 cm long, and when composed of 100–200 mesh granules had flow-rates of 10–30 ml./h except in the case of 2·5 per cent agar, where 60–100 mesh granules afforded a maximum flow-rate of only about 5 ml./h. Experiments with 9 per cent agar showed that proteins were eluted from columns of 100–200 mesh granules as rather sharper bands than from 60–100 mesh granules (cf. Flodin[8]), but changes in flow-rate in the above range had no effect on the separations.

Washing and equilibration of the columns with buffer solutions reduced contamination of the effluent to the following: optical density, about 0·02 at 260 and 280 mμ, about 0·05 at 215 mμ (1 cm cell); carbohydrate, 1–3 μg/ml. (estimated as hexose). These values were further reduced by use or prolonged washing.

Table 1. MOLECULAR WEIGHTS AND DIFFUSION COEFFICIENTS OF PROTEINS USED AS STANDARDS IN GEL FILTRATION EXPERIMENTS

Protein*	Molecular weight†	Diffusion coefficient† cm²/sec × 10⁷
α-Lactalbumin	16000	10·6
β-Lactoglobulin	36000	7·8
Serum albumin	70000	5·9
γ-Globulins (human)	160000	c. 4·5
Thyroglobulin	670000	2·5

* All the proteins except γ-globulins are of bovine origin.
† Data are taken from recent literature.

Effect of agar concentration on separation of proteins. Separation between the compounds listed in Table 1 was attempted with columns of gel ranging from 2½ to 12 per cent in agar concentration, equilibrated with 0·04 M potassium phosphate buffer pH 7·0 containing 0·12 M potassium chloride. The tops of the columns were very susceptible to disturbance, so that the samples, dissolved in buffer (2 ml.), were applied by layering under buffer. Cytochrome *c* (2 mg) was regularly included to provide a visual check for even flow and a guide to the progress of a separation. Indian ink was unsuitable for either purpose because it was partially adsorbed on the gel. Hog gastric mucoid behaved as though completely excluded from all the gels except the 2½ per cent one, and even in this case the eluted peak had a sharp leading edge, indicating exclusion of some of the larger molecules. Hog gastric mucoid, cytochrome *c*, and the colourless proteins were estimated spectrophotometrically at 215, 412 and 280 mμ respectively. In each experiment the column effluent was collected in 3 ml. fractions, beginning when the sample had just entered the column, and the precise volume collected at the emergence of the maximum of each solute peak ('peak volume') was derived from a plot of the optical density of the fractions comprising the peak. To facilitate comparisons, the peak volume of hog gastric mucoid, which varied a little with different gels,

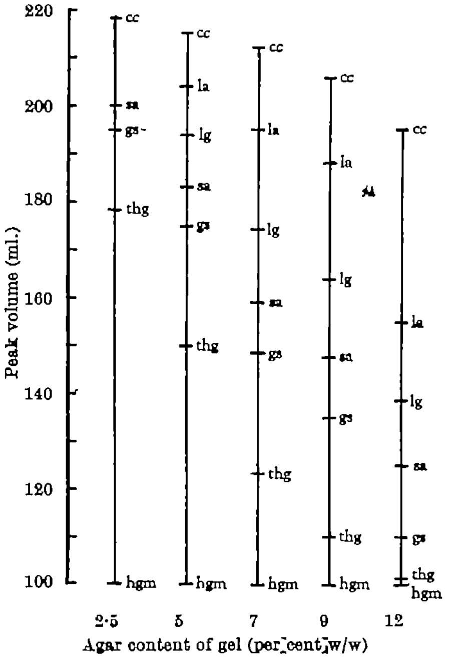

Fig. 1. Effect of agar content of gels on protein separation by gel filtration. See text for experimental details; cytochrome *c* is retarded during passage through the columns by ion-exchange effects. Abbreviations: cc, cytochrome *c*; la, α-lactalbumin, lg, β-lactoglobulin; sa, bovine serum albumin; gs, human γ-globulins; thg, bovine thyroglobulin; hgm, hog gastric mucoid

is taken as constant, and the other figures corrected accordingly. Fig. 1 shows the results of these experiments. Their reproducibility is demonstrated by the fact that separation between the peak volumes of serum albumin and α-lactalbumin varied only between 38 ml. and 42 ml. in several dozen runs with columns composed of the same preparation of 9 per cent agar gel granules.

As anticipated, decrease in the agar content of the gels shifted their effective range, the ability to separate the smaller protein molecules giving way to an ability to distinguish between larger ones. Two typical separations are illustrated in Fig. 2. Peak-widths for compounds retarded during passage through the columns were about the same in all experiments, the 'spreading' in 5 per cent agar mentioned by Polson[8] not being observed. The recovery of compounds in Fig. 1 was 85–100 per cent with quantities in the range 2–20 mg, but lower for cytochrome *c* on freshly prepared gels.

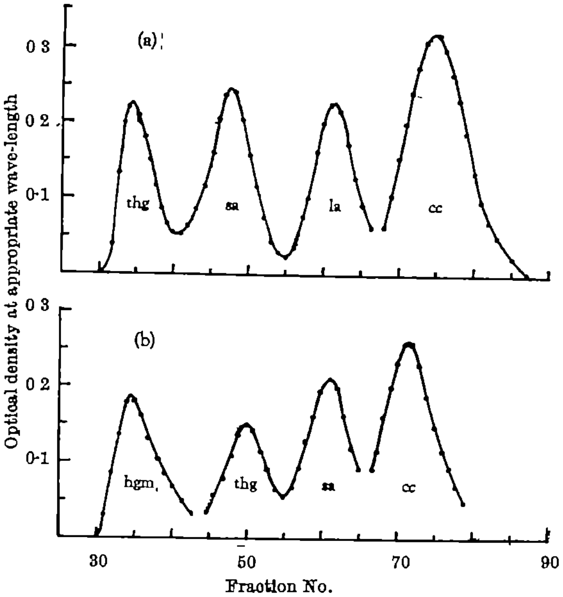

Fig. 2. Separation of proteins by agar-gel filtration. *a*, On a column of 9 per cent agar equilibrated with 0·04 M *tris* *p*H 7·5 + 0·12 M potassium chloride. Cytochrome *c* is retarded by ion-exchange effects. *b*, On a column of 5 per cent agar, equilibrated with 0·04 M phosphate *p*H 7·0 + 0·12 M potassium chloride. Interaction between agar and cytochrome *c* is less than in *tris* buffer. Abbreviations : see Fig. 1

In further experiments, a change from phosphate to *tris*(hydroxymethyl)aminomethane (*tris*) buffers in the range *p*H 7–8, containing various amounts of potassium chloride up to 0·36 M, was found to have no effect on separations between the proteins of Table 1, all of which have isoelectric points below *p*H 7. However, passage of the basic protein cytochrome *c* through the columns was accelerated, relative to the other proteins, by increasing salt concentrations, so demonstrating the cation-exchange properties of agar[9]. The retardation of cytochrome *c* by ion-exchange was apparently eliminated by addition of 0·24 M potassium chloride to the eluting buffers (0·04 M *tris* or potassium phosphate *p*H 7·5), since further addition of salt had no effect on its movement relative to the other proteins, and its behaviour then corresponded to that expected for a protein of molecular weight 13,000. Ion-exchange effects may be avoided by using columns of agarose[9], the neutral polysaccharide constituent of agar, but as both starch[1] and 'Sephadex'[10-12] also appear to possess ion-exchange properties, these effects may be difficult to avoid completely.

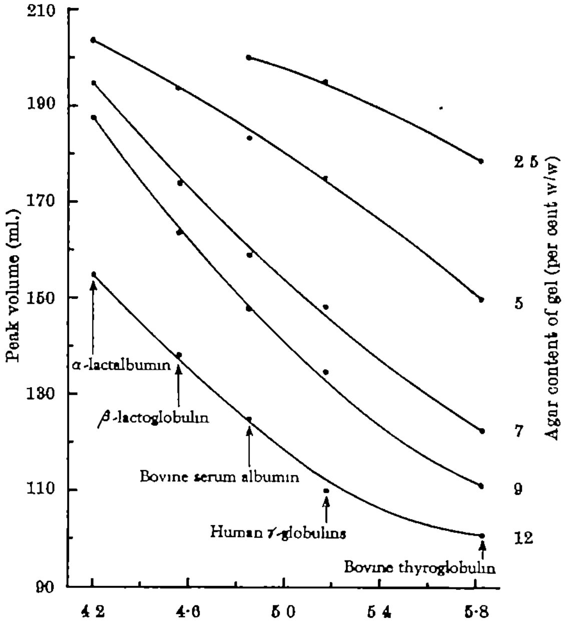

Fig. 3. Separation of 'standard' proteins. Plots of peak volumes against log molecular weights of proteins for gels of different agar content

Molecular weight estimation. A plot of peak volume against log molecular weight of the proteins listed in Table 1 gives, with the results for each gel, a smooth curve which approaches linearity in the molecular weight range where separation efficiency is optimal (Fig. 3). Granath and Flodin[13] observed a similar relationship in the results of gel filtration experiments with dextrans. Fig. 3 provides a means of estimating molecular weights of proteins from their gel filtration behaviour, and experiments to test its value were performed with 9 per cent agar columns.

Column runs were carried out, as already described, in the cold. Each sample consisted of crude or purified enzyme, cytochrome *c*, and sufficient of one or two of the 'standard' proteins (Table 1) for their peaks in the column effluent to be clearly distinguished from the protein in the enzyme preparation. Enzymatic activities in the column fractions were assayed by the appropriate methods[14]; hæmoglobin was estimated spectrophotometrically at 410 mμ. The peak volumes for standard proteins and enzymes were read from the elution diagrams. The width of enzyme and standard protein peaks were about the same. Refinements in technique such as described by Hjertén and Mosbach[9] could result in some reduction of peak-width, but may have little effect on the precision with which the separation between maxima can be measured. To detect interaction with the agar, each enzyme was run in the presence of two different salt concentrations (buffer plus 0·12 and 0·24 M potassium chloride). The behaviour of the alkaline phosphatases was most influenced by salt concentration, but slight effects also occurred with hæmoglobin and malic and isocitric dehydrogenases. However, as with cytochrome *c*, increase in salt concentration above 0·24 M had no further effect.

Of the enzymes used in these experiments, rennin and milk alkaline phosphatase (generously provided by Dr. G. C. Cheeseman and Dr. R. L. J. Lyster, respectively) were highly purified, the mammary tissue glucose 6-phosphate dehydrogenase was in a crude particle-free tissue extract, and the remainder were commercial partially purified samples. The hæmoglobin was a crystalline sample.

Results of the molecular weight estimations are collected in Table 2. Agreement with recent values determined by the usual physical methods is generally good. The gel filtration result for rennin favours the lower, more recent, literature value[16], and agrees well with a molecular weight of about 32,000 calculated by Dr. G. C. Cheeseman from amino-acid analyses (personal communication). Bovine hæmoglobin was applied to the columns as a 0·06 per cent w/v solution, and during the runs this concentration decreased. The value obtained for its molecular weight indicates dissociation into half-molecules under these conditions, a result agreeing with Gutfreund's observations[22] on very dilute hæmoglobin solutions. The separation between bovine serum albumin and rabbit hæmoglobin on agar columns, reported by Polson[2], may well be due to dissociation of the latter protein, resulting in a considerable molecular weight difference between the two proteins, rather than, as suggested, to the small difference between their diffusion coefficients.

Table 2. PROTEIN MOLECULAR WEIGHTS ESTIMATED BY GEL FILTRATION

Protein (source)	Molecular weight: Estimate from gel filtration behaviour*	Molecular weight: Literature value(s)	Reference
Rennin (calf stomach)	30,000	40,000 34,400	15 16
Hæmoglobin (ox blood)	32,000	68,000	see text
Malic dehydrogenase (pig heart)	40,000	c. 50,000	17
Hexokinase (yeast)	47,000	45,000	18
Isocitric dehydrogenase (pig heart)	57,000 33,000†	60,000	19
Glucose 6-phosphate dehydrogenase (guinea pig mammary gland tissue)	74,000	—	—
Glucose 6-phosphate dehydrogenase (yeast)	89,000	—	—
Alkaline phosphatase (calf intestine)	92,000	100,000	20
Alkaline phosphatase (milk)	105,000	—	—
Alcohol dehydrogenase (yeast)	120,000	150,000	21

* Possible error due to uncertainty in values of peak volumes ± 6 per cent.

† ±10 per cent.

The isocitric dehydrogenase preparation (obtained from Boehringer and Soehne) apparently contained unequal amounts of two enzymatically active constituents, the minor one having roughly half the molecular weight of the other; but since the two components were not completely separated from each other, the precise estimation of peak volume for the minor component was not possible. The result for hexokinase confirms Schachman's value[18], which was measured at pH 8. The hexokinase gel filtration experiment and the enzyme assays were performed at pH 7·5, and all the activity was associated with the component of molecular weight 47,000. The results indicate that this is the size of the enzymatically active hexokinase molecule, while the value of 96,600, measured at pH 5·5 and so often quoted in the literature, represents a dimer. Molecular weight data for glucose 6-phosphate dehydrogenase have not been published, although the enzyme has recently been highly purified from yeast[23] and from cow mammary tissue[24]. Gel filtration indicates that the molecular weight of both this enzyme and alkaline phosphatase varies with the source.

Recovery of stable enzymes in the column effluents was 90–100 per cent, except in experiments with egg-white lysozyme, when none was detected. In such cases, where agar is unsuitable, polyacrylamide or another synthetic gel may be a satisfactory filtration medium.

The quantity of protein needed for molecular weight estimation by gel filtration depends on the specificity and sensitivity of methods for its detection, so that, particularly with enzymes, an extremely small amount is sufficient, and it is possible to estimate molecular weights in very dilute solution. In addition, purification of enzymes may well be unnecessary. Martin and Ames's ultracentrifugal method[25] has similar advantages but requires more elaborate equipment than does the gel filtration method, without necessarily having greater accuracy. Both methods have the disadvantage that, when applied to impure proteins, interaction between solute macromolecules is potentially a source of error. Further experiments should show the frequency of this occurrence.

Both the size and shape of molecules in solution probably influence their behaviour in gel filtration, so this method for estimating molecular weights has to be used with caution in relation to molecules that differ significantly in shape from those used as molecular weight standards. Judging from the results in this paper, the proteins listed in Table 1 are suitable for comparison with enzymes, most of which are believed to be globular proteins with a frictional ratio of about 1·15. However, the validity of the method for very large protein molecules has yet to be assessed.

Diffusion coefficients for the enzymes in Table 2 were also estimated from their gel filtration behaviour, using a graph of peak volume against diffusion coefficient of the standard proteins (Table 1) for interpretation. The values so obtained were on the average 11 per cent lower than the corresponding literature values. This disparity is about twice the average deviation from literature values for similarly estimated molecular weights. The conclusion is that, for enzymes at least, the method described here is probably of more value for estimating molecular weights than diffusion coefficients.

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DEVELOPMENT OF CHROMOPHORES

Development of Chromophores in the Carbonyl-Amino System

THE main pathways in non-enzymatic browning appear to commence with carbonyl/amino group reactions, and αβ unsaturated carbonyls seem to be concerned in the development of the initial chromophores as opposed to the later production of larger brown polymers. We have mentioned[1] the isolation from glucose/glycine systems of a number of non-volatile, water-soluble carbonyl compounds, some of them of an αβ unsaturated nature, and at the time the work was progressing it was reported[2-4] that several unsaturated carbonyl compounds of unknown structure had been detected in stored foodstuffs. It was decided to compare the potentialities of saturated and unsaturated carbonyl compounds as chromophore developers, particularly in the presence of amino compounds, since these are also present in foods and since we had found that the carbonyl compounds isolated from the glucose/glycine system browned more readily on incubation with glycine than with glucose.

At the same time we were examining the effect of sulphites on the aldose/glycine system, and used the reactions of citral[5] with sulphites to interpret our results[6].

Citral is an αβ unsaturated carbonyl compound with a second non-conjugated double bond, and so should show the normal αβ unsaturated carbonyl reactions with amino groups in the absence of sulphite. In the presence of the same quantities of sulphite one might expect the initial reactions leading to chromophore development to be less hindered in the case of citral than in carbonyl compounds with only the αβ site available for co-sulphonation.

Preliminary comparative experiments showed that on incubation with glycine, αβ unsaturated carbonyl compounds developed chromophores faster than did saturated carbonyls and that the rate of chromophore development with citral decreased as the more sulphite (1–3 moles) was added to the system.

In this connexion it is worth mentioning that in our extensive experiments on the development of chromophores in sugar/amino systems it has appeared that for rapid initiation of colour development, a rapid development of unsaturation must occur, and agents or physical treatment which produce such unsaturation lead to rapid chromophore development. The presence of nitrogen functions leads to the early development of unsaturation and when unsaturated carbonyl compounds are present in the system the browning reaction is very much faster in the presence of a nitrogen function than in its absence.

Owing to the availability at the time, the first experiments were limited to a few representative carbonyl compounds. Citral (76 mg) was incubated with glycine (2 gm) in 2 : 1 methanol/water (30 ml.) at 50° C in the presence and absence of 1 equivalent of sodium metabisulphite. After 20 min the unsulphited sample was browning and within 24 h a brown polymer had separated. This polymer rapidly darkened owing to further polymerization. The sulphited citral showed no chromophore development after 2 months incubation. A comparison of glycine incubations with saturated and unsaturated aldehydes, namely, crotonaldehyde, butyraldehyde, 2 ethyl-*n*-hexanal, 2 ethyl hexene-2-al under similar incubation conditions (crotonaldehyde and 2 ethyl hexene-2-al were used at two concentrations) showed that initial chromophore development occurred earlier with the αβ unsaturated aldehydes, and that 2 ethyl hexene-2-al took much longer to discolour than crotonaldehyde or citral. None of the other mixtures developed any colour nor did a number of saturated ketones apart from *cyclo*pentanone. None of the sulphited aldehyde or ketone series developed any colour except crotonaldehyde, which developed a faint colour after 7 days incubation. It was noted that the presence of an ethyl substituent on the α-carbon atom greatly diminished the tendency to promote browning. In further studies 0·1 m.mole of the carbonyl compound was incubated in a screw-cap bottle with 0·6 m.mole of glucose and 1·3 m.moles of glycine in 1·0 ml. water and 2·0 ml. ethanol at 50° C. The compounds investigated in this experiment were butyraldehyde and crotonaldehyde; 2 ethyl hexanal and 2 ethyl-2-hexenal; caproic aldehyde and 2-hexenal. The tubes were examined visually and the shade and intensity of the colour was estimated. Table 1 summarizes the results.

It is clear that αβ unsaturated straight-chain aldehydes greatly accelerated the initial development of chromophores in this system as well, and that this effect was not shown by the unsaturated aldehyde with an ethyl substituent on the α-carbon atom. The saturated aldehydes appeared to have a slight retarding effect on colour development in the glucose/glycine series.

In similar glucose/glycine reaction systems we found that the major losses of sulphite were not mainly due to conversion to sulphate, and that chromophore development only took place when the carbonyl-inactivating sulphite had been removed to some other site where it was more strongly bound. The αβ unsaturated carbonyl compounds examined are themselves not coloured, and the product of a combination between a single amino-group and a straight-chain mono unsaturated aldehyde will still be colourless, as postulated[6] on bathochromic considerations. It seems clear that the initial chromophore development with carbonyl or potential carbonyl compounds may proceed in one of two ways.

The first is the possible development of an ene-dione or diene-al type due to unsaturation being produced

Table 1 COLOUR DEVELOPMENT IN GLUCOSE/GLYCINE MIXTURES WITH ADDED ALDEHYDE*

	3 hours	20 hours	4 days	8 days	18 days
Butyraldehyde	slight pink	vv pale straw	vv pale straw	straw	brown
Crotonaldehyde	v. light brown	golden brown	red brown	deep red brown	red brown
2 ethyl hexanal	—	—	—	straw	brown
2 ethyl-2-hexenal	—	—	v. pale straw	straw	brown
Caproic aldehyde	—	—	—	straw	brown
2 hexenal	pale straw	golden yellow	light brown	brown	dark brown
Glucose/glycine control	—	—	pale straw	yellow	light brown

* 0·1 m.mole of aldehyde incubated with 100 mg glycine and 100 mg glucose in 1 ml. water + 2 ml. ethanol at 50° C.

in the hydroxylic sugar chain followed by cyclization or aldolization. This may explain the initial light yellow coloration often observed; with ensuing polymerization of the unsaturated and carbonyl compounds, more absorptive chromophores will be produced. In the presence of a nitrogen function the reactive carbonyl compounds which develop, often themselves lightly coloured, will combine with the nitrogen function to give more absorptive derivatives, probably cyclic in nature. Evidence exists for the presence of cyclic nitrogen units in water-soluble browning polymers from aldose/glycine systems[7,8] and they apparently form in drying oils[9].

The second is that the initial combination of the nitrogen function with the potential carbonyl group leads, itself, to the development in the sugar chain of unsaturation which is at first conjugated with the C–N link and which may then be followed by: (1) cyclization to give pyrrolic or pyridinic ring compounds which may be self-coloured or which are capable of polymerization; or (2) the conjugated unsaturated carbonyl compound splitting off and engaging in (*a*) cyclization and self-polymerization to develop chromophores or (*b*) reaction with further nitrogen functions to give probably cyclic chromophores which are capable of further polymerization to darker compounds.

There are indications in our work for all these types of reaction since chromophore development can occur under a variety of conditions (for example, *p*H and availability of nitrogen). Many of the reactants (that is, the carbonyl compounds isolated from a glucose/glycine system) give a slow development of chromophores in the absence of a nitrogen function, but a rapid development in its presence.

Where small carbonyl compounds appear to cause browning it would seem that the chromophore development must occur as a result of combinations of the type mentioned at some stage.

In a previous publication[6] we found that heavy sulphiting prevented the appearance of conjugated unsaturated carbonyl compounds in the system and prevented chromophore development. From theoretical considerations one would expect the sulphonyl group to attach to the β-carbon atom and in this position it is clearly a block to αβ unsaturated carbonyl-*N* type chromophore development. The development of αβ unsaturated carbonyls in many foods is thus probably a first step in chromophore development.

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Sulphites and Carbonyl-Amino Chromophore Development

The development of carbonyl compounds in systems undergoing non-enzymatic browning has been reported by many workers. In the course of our investigations in this field we came to the conclusion that conjugated unsaturated carbonyl compounds were important intermediates in the development of chromophores in some pathways to melanoidin formation, and that sulphites might form very stable addition compounds at some unsaturated centres[1,6,10].

It is known that αβ unsaturated carbonyl compounds can react with sulphites both at the double bond and at the carbonyl group, but Dodge[5] seemed to be of the opinion that the αβ sulphonyl group could be readily hydrolysed, at least under mild alkaline conditions, and suggested that it might readily migrate.

We have confirmed that sulphite addition to olefinic bonds can occur under mild experimental conditions, but have found that the addition of sodium metabisulphite at the olefinic bond of crotonaldehyde is not reversible under the acidic-hydrolytic conditions of the Monier–Williams determination[11] of sulphur dioxide.

The preceding communication describes the chromophoric development occurring with a number of saturated and unsaturated carbonyl compounds. A similar group of carbonyl compounds comprising crotonaldehyde, mesityl oxide, 2 ethyl-2-hexenal and their saturated analogues were converted into bisulphite compounds. The normal carbonyl bisulphite compounds were prepared by dropwise addition of the carbonyl compound to an excess of an ice-cold saturated solution of sodium bisulphite. The crystalline bisulphite compounds were recrystallized rapidly from aqueous ethanol. The 'abnormal' sulphonated bisulphite compounds of the unsaturated carbonyls, in which addition had occurred both at the olefinic and at the carbonyl groupings, were prepared by stirring and warming the unsaturated carbonyls with an excess of sodium bisulphite solution for 30 min at 60° C. The bisulphite compounds were obtained on cooling as a mass of waxy crystals, and were recrystallized with difficulty from aqueous ethanol.

Consideration of possible reaction mechanisms leads to the conclusion that one of the sulphonic acid groups will be attached to the β-carbon atom, and this conclusion, which had been reached much earlier[12]. is supported by the recent work of Finch[13] on bisulphite addition to acrolein. Unsaturated fatty acids readily give sulphonates; when the double bond is αβ, sulphonic acids are always produced, thus crotonic acid gives β sulphobutyric[14] acid. In the unsaturated ketone series it has been found that most unsaturated ketones will add sodium bisulphite[15–18] in contrast to their saturated analogues.

Confirmation that the olefinic double bond is not readily attacked by bisulphite in the cold but is attacked at 60° C is provided by the fact that only the bisulphite compounds prepared in the cold from unsaturated carbonyl compounds retained the property of rapidly decolorizing bromine water. An investigation was carried out into the hydrolysis of the bisulphite compounds of crotonaldehyde (which analysed for approximately one and two molar additions of sodium bisulphite respectively) under the conditions of the Monier-Williams determination of sulphur dioxide content. The normal aldehyde bisulphite compound gave 90–92 per cent of the calculated quantity of sulphur dioxide. The 'abnormal' bisulphite compound (C_4H_6O, $2NaHSO_3.H_2O$) released only 45 per cent of the calculated amount of sulphur dioxide, showing that one molecule of $NaHSO_3$ was not released on acid hydrolysis. Under the same conditions glucose hydroxy sulphonate (D-glycero-D-ido-hexahydroxy hexyl sulphonate) gave about 97 per cent of the theoretical yield of sulphur dioxide. Mangan and Doak[19] have reported that for foodstuffs the Monier-Williams method often gave inexplicable results, and Williams[20] queried its value as a criterion of the protective powers of sulphite. The former authors suggested that sulphur dioxide was lost by an unidentified reaction which did not require oxygen and yet was responsible for the apparent loss of sulphur dioxide. Hägglund *et al.*[21]

found that heated sugar solutions contained substances which bound sulphite in a non-recoverable manner, and we have shown that in the glucose/glycine systems the appearance of conjugated unsaturated carbonyl compounds is delayed and that the level of these substances can be depressed by heavily sulphiting the system, when the sulphite itself became largely irrecoverable by the Monier-Williams method.

In view of the ease with which the olefinic bond of unsaturated carbonyl compounds is attacked by sulphite it is likely that this mode of combination can account for some of the sulphite loss (as determined by the Monier-Williams method) observed on storing sulphited foodstuffs at temperate and tropical temperatures.

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EFFECT OF DEHYDROEPIANDROSTERONE AND Δ^4-ANDROSTENEDIONE ON THE REPRODUCTIVE ORGANS OF FEMALE RATS: PRODUCTION OF CYSTIC CHANGES IN THE OVARY

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RECENT work has shown that the polycystic ovaries of Stein–Leventhal syndrome contain and secrete abnormal quantities of androgens such as dehydroepiandrosterone (DHA) and Δ^4-androstenedione[1-3]. These are relatively weak androgens, and when administered intravenously to rats[4] or perfused through the liver of dogs[5] are partly converted to testosterone. Mahesh and Greenblatt[6] have administered orally the foregoing two steroids to normal women and found elevated levels of plasma testosterone. These observations may serve to explain the frequent occurrence of hirsutism in the polycystic ovary syndrome.

The other manifestations of this syndrome are absent or infrequent ovulation, amenorrhœa, sterility, and microcystic changes in the ovary. Although various theories have been proposed to explain the pathogenesis of this syndrome, considerable gaps in our knowledge remain. Many steroids are known to influence the physiology of reproduction in variable degrees[7]. However, no attention has hitherto been paid to the role of the aforementioned androgens in the pathogenesis of the polycystic ovary syndrome. This is especially pertinent because testosterone has been shown to cause anovulation and cystic changes in the ovary in mice[8] and rats[9] if given in early neonatal life. Therefore it seemed of interest to study the effect of DHA and Δ^4-androstenedione on the reproductive organs of young female rats, and to ascertain whether cystic changes in the ovary can be induced.

Forty-two female Sprague–Dawley rats, 22–23 days old and weighing 42·3 ± 3·2 g were divided into 7 groups of 6 each. One group was kept as normal controls. Six other groups were treated with graded doses (0·1, 0·3, 1, 3, 10, and 30 mg/kg body-weight) of DHA in 0·2 c.c. of corn oil given subcutaneously for 36 days. Another batch of 42 female rats of the same age and weighing 43·9 ± 2·8 g were similarly divided and treated with identical graded doses of Δ^4-androstenedione. The control rats received the injections of 0·2 c.c. of oil alone.

The animals were kept under uniform husbandry conditions and were killed on the day following the last injection. They were anæsthetized with intraperitoneal injections of 'Nembutal', and the blood was drawn from the abdominal aorta. The ovaries, uteri, adrenals and pituitaries were dissected out carefully and weighed on a precision balance. The plasma was processed for steroid fractionation studies and the pituitaries for the determination of their luteinizing (LH) and follicle-stimulating hormone (FSH) contents. The other organs were fixed in 10 per cent formalin for histological studies.

The results of the studies with DHA and Δ^4-androstenedione are presented in Tables 1 and 2 respectively. The greater gain in the body-weight of the animals in the steroid-treated groups is probably due to the anabolic action of the test compounds. In DHA-treated animals the relative weight of the ovary showed a progressive decrease with doses 0·3 mg and greater, whereas such a decline in ovarian weight was noted with only 10- and 30-mg doses of Δ^4-androstenedione. This is caused presumably by inhibition of pituitary gonadotropin secretion. It may be assumed that by the same action these steroids will also be able to inhibit ovulation. However, it is well to mention the fact that Nelson and Patanelli[10] pointed out it is probably not essential that complete or even marked inhibition of gonado-

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Table 1. EFFECT OF GRADED DOSES OF DEHYDROEPIANDROSTERONE ON THE WEIGHT OF THE BODY, OVARY, UTERUS AND ADRENALS OF YOUNG RATS

Treatment	Mean body-wt. (gm)		Mean ovary wt. (mg) with S E		Mean uterus wt. (mg) with S.E.		Mean adrenal wt. (mg) with S.E.	
	Initial	Final	Absolute	Relative	Absolute	Relative	Absolute	Relative
Control	42·0	162·3	46·9 ± 4·5	28·9 ± 2·3	211·0 ± 39·7	130·0 ± 21·8	40·3 ± 8·0	24·8 ± 1·6
DHA 0·1 mg/kg	42·0	159·8	44·6 ± 7·0	27·9 ± 4·7	190·8 ± 17·4	119·4 ± 16·7	49·0 ± 4·8	30·7 ± 4·0
DHA 0·3 mg/kg	42·1	186·5	45·0 ± 3·3	24·6 ± 1·5	184·0 ± 14	98·7 ± 8	43·8 ± 1·8	23·5 ± 1
DHA 1 mg/kg	42·0	185·7	44·7 ± 2·5	24·1 ± 1·49	273·6 ± 64·5	147·4 ± 34	44·5 ± 1·7	24·0 ± 1·2
DHA 3 mg/kg	42·0	186·2	40·7 ± 3·9	21·9 ± 1·82*	186·8 ± 13·2	100·3 ± 6·2	43·2 ± 1·8	23·2 ± 0·7
DHA 10 mg/kg	44·0	185·3	33·1 ± 2·2*	17·9 ± 1·3*	218·1 ± 19·6	117·7 ± 18·0	41·8 ± 1·7	22·9 ± 0·46
DHA 30 mg/kg	42·3	197·6	30·1 ± 4·3*	15·2 ± 2·3*	360·8 ± 18·7*	182·6 ± 12·6	36·8 ± 2·4	18·6 ± 0·88*

* Difference from the respective controls is statistically significant.

Table 2. EFFECT OF GRADED DOSES OF Δ^4-ANDROSTENEDIONE ON THE WEIGHT OF BODY, OVARY, UTERUS, AND ADRENALS OF YOUNG RATS

Treatment	Mean body-wt. (gm)		Mean ovary wt. (mg) with S.E.		Mean uterus wt. (mg) with S.E.		Mean adrenal wt. (mg) with S.E.	
	Initial	Final	Absolute	Relative	Absolute	Relative	Absolute	Relative
Control	43·8	173·7	45·7 ± 3·9	26·3 ± 1·7	197·8 ± 25·6	113·9 ± 13·5	46·2 ± 2·4	26·6 ± 1·3
Δ^4-androstenedione (0·1 mg/kg)	44·2	193·7	57·2 ± 4·8	29·4 ± 2·2	221·9 ± 25·5	114·6 ± 12·8	46·6 ± 1·7	24·1 ± 0·82
Δ^4-androstenedione (0·3 mg/kg)	43·6	191·8	49·3 ± 2·5	25·7 ± 1·5	225·2 ± 14·9	117·4 ± 8·6	44·4 ± 2·6	23·1 ± 1·6
Δ^4-androstenedione (1 mg/kg)	43·9	192·8	47·0 ± 3·6	24·4 ± 1·7	277·3 ± 20·5*	143·8 ± 10·4	50·8 ± 2·6	26·3 ± 1·5
Δ^4-androstenedione (3 mg/kg)	43·9	195·2	49·5 ± 2·9	25·4 ± 1·2	403·0 ± 62·4*	206·5 ± 31·2*	48·2 ± 2·4	24·7 ± 1·1
Δ^4-androstenedione (10 mg/kg)	43·8	203·3	24·3 ± 4·7*	11·9 ± 2·1*	337·8 ± 57·9*	166·2 ± 27·4	43·6 ± 1·7	21·4 ± 0·85*
Δ^4-androstenedione (30 mg/kg)	43·8	186·7	27·1 ± 1·8*	14·5 ± 1·1*	454·2 ± 28·3*	243·6 ± 14·1*	39·9 ± 1·5	21·3 ± 0·48*

* Difference from the respective controls is statistically significant.

tropin be produced by the various steroids to prevent ovulation. It is required only that the FSH/LH ratio be disturbed to a degree that follicular maturation and rupture do not progress normally. On this basis it may be postulated that such inhibition of ovulation will be possible by DHA and Δ^4-androstenedione, which have been shown to be converted into testosterone in the body.

At the dosage of 10 and 30 mg, both the compounds produced cystic changes in the ovaries of most of the animals and at 3-mg dose only a few ovaries showed such change. They were very easily noted under a magnifying glass and were filled or distended with clear fluid. None of the control animals showed any cysts in its ovaries on macroscopic examination. This change may be due to a direct effect of the steroids on the ovary or to modification of gonadotropin action or to a change in the sensitivity of the ovary or to a combination of two or more of these factors.

At the higher dosage-levels these steroids increased the weight of the uterus. Such action of DHA was manifested only with the 30-mg dose, whereas the uterotrophic effect of Δ^4-androstenedione was overt with 1 mg and more. It is worthy of note that there was an increase in the absolute and relative weights of the uterus in spite of a marked decrease in the ovarian weight when larger doses of steroids were administered. Some of the uteri were distended with fluid in their lumen. It is not known whether the uterotrophic action of these steroids is due to a direct effect or to their conversion into testosterone or œstrogen.

A significant decrease in the relative weights of the adrenals was noted at 30-mg dose-level of both the steroids and also at 10-mg dose of Δ^4-androstenedione. This may be caused by inhibition of secretion of adrenocorticotrophic hormone from the pituitary.

Based on the recent observations[2,3] and on the findings of the present study, a tentative hypothesis for the pathogenesis of the polycystic ovary syndrome of Stein–Leventhal may be suggested. In such ovaries, due to the enzymatic abnormalities, œstrogen biosynthesis is partially blocked. As a result there occurs a relative lack of œstrogen in the body which probably causes increased secretion of pituitary gonadotropin, primarily FSH. At this stage the ovaries may become enlarged by the stimulation of excess gonadotropin. In an attempt to produce physiological amounts of œstrogen such ovaries produce large quantities of androgens like DHA and Δ^4-androstenedione. Now the high level of circulating abnormal androgens may act on the pituitary and suppress the already elevated gonadotropin secretion to some extent. But the more important effect probably is that they may modify the FSH/LH ratio in such a way as to inhibit ovulation and thus expose the ovaries to a continuous and a preponderance of FSH stimulation. Added to these, the androgens probably modify the response of the ovaries to gonadotropin, leading to polycystic changes in the ovary.

It is not known whether there is any enzymatic defect in the ovarian hormone synthesis in patients with adrenogenital syndrome. However, in the light of the observations of the present study, the occurrence of polycystic ovaries in patients with adrenogenital syndrome may probably be explained on the basis of high level of adrenal androgens circulating in the blood.

Investigations in animals as well as in human beings are in progress for further elucidation of the problem. The results will confirm, extend or modify the above hypothesis.

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LACTOSE FERMENTATION BY *SHIGELLA SONNEI*

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IN 1919 Thjelta[1] reported that *Shigella sonnei* colonies turned red on lactose agar after 48 h of incubation. Nelson[2] reported that this organism produced acid without gas in laxtose broth by the third day and sometimes not until the seventh day. Sears and Schoolnik[3] found that slow lactose-fermenting *S. sonnei* fermented lactose rapidly after being transferred several times in lactose broth. Hershey and Bronfenbrenner[4] stated that it was possible to induce rapid lactose fermentation by various manipulations. According to Glynn and Starkey[5] the lactose-positive papillæ of *S. sonnei* did not breed true. They found that each time the secondary papillæ were replated, the colonies started as a lactose-negative colony and formed the lactose positive papillæ only on continued incubation. Neter[6] felt that the appearance of these papillæ on prolonged incubation was due to fermentation of lactose at a later time rather than to the slow utilization of the carbohydrate. It has been reported that *S. sonnei* can be 'trained' to ferment lactose more rapidly by cultivation in lactose media[3]. Cook *et al.*[7] believed that the speed of lactose fermentation depended on the proportion of lactose-fermenting mutants present. The percentage of mutant cells is dependent on rate of mutation, relative growth-rates of normal and mutant cells, and possible inhibitory effect of normal cells on growth or adaptation of the mutant. Late fermentation may be due to the time interval necessary for the mutants to establish themselves in culture in sufficient numbers to produce the detectable effects. Mundt and Hitchner[8] were not able to increase or decrease the rate of lactose fermentation by various manipulations. Kacoyanis and Baker[9] reported that one-third of a culture of *S. sonnei* was lactose-positive after holding for 10 days in lactose broth. The lactose-fermenting mutants were stable and retained their ability to ferment lactose within 48 h even after 30 sub-cultures in media not containing lactose.

The ability of *S. sonnei* to ferment lactose is of diagnostic importance. Since this organism ferments lactose slowly (several days to several weeks) we thought that reliable methods for accelerating this diagnostic reaction would be of clinical importance. This investigation deals with the study of various factors which affect the lactose-fermenting ability of *S. sonnei*.

Three strains of *S. sonnei* used in this work were obtained from Commander T. M. Floyd, Naval Medical Center, Bethesda, Maryland. Three strains of *S. sonnei* were received from Dr. Maxwell Finland, Boston City Hospital, Boston, Massachusetts. One strain was obtained from Dr. Arthur Abrams, Walter Reed Army Hospital, Washington, D.C., and a strain was received from Dr. H. M. Gezon, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania. *Shigella dysenteriae* 1, *S. boydii* 1, *S. flexneri* 2*a*, and strain 01 of the Alkalescens-Dispar group (Edwards and Ewing[10]) were supplied us by Dr. W. H. Ewing, Laboratory Branch, Communicable Disease Center, Atlanta, Georgia. The culture of *Escherichia coli* used in this work was purchased from the American Type Culture Collection, Washington, D.C. The organisms were grown on nutrient broth (Difco), nutrient agar (Difco), and the chemically defined medium of Erlandson and Mackey[11]. Lactose fermentation broth was prepared by adding 5 g/1,000 ml. of lactose in nutrient broth and 0·1 per cent bromthymol blue (Baltimore Biological Laboratory, Baltimore, Maryland) as the *p*H indicator. This indicator was blue at a *p*H of 7·6 or higher and turned yellow at a *p*H of 6·0 or lower. A 1 per cent concentration of fructose, mannose, glycerol, maltose, and lactose was used in place of glucose in the chemically defined medium during certain phases of this work. The media were prepared in distilled, de-ionized water ('Crystalab Deeminizer', Crystal Research Laboratories, Inc., Hartford, Connecticut), adjusted to *p*H of 7·0, and autoclaved at 15 lb. of pressure for 15 min. The sugars were sterilized by Seitz filtration and added aseptically to the sterile media. Eosin methylene blue (EMB) agar (Difco) plates were used to determine the number of lactose-fermenting colonies.

Stock cultures of the organisms kept as reference cultures were grown for 24 h on nutrient agar slants and then retained in the frozen state (−20° C). The cultures were checked periodically for purity by biochemical, serological and morphological methods. The size of the inocula was determined prior to inoculation into lactose fermentation tubes. This was done by making an appropriate number of serial

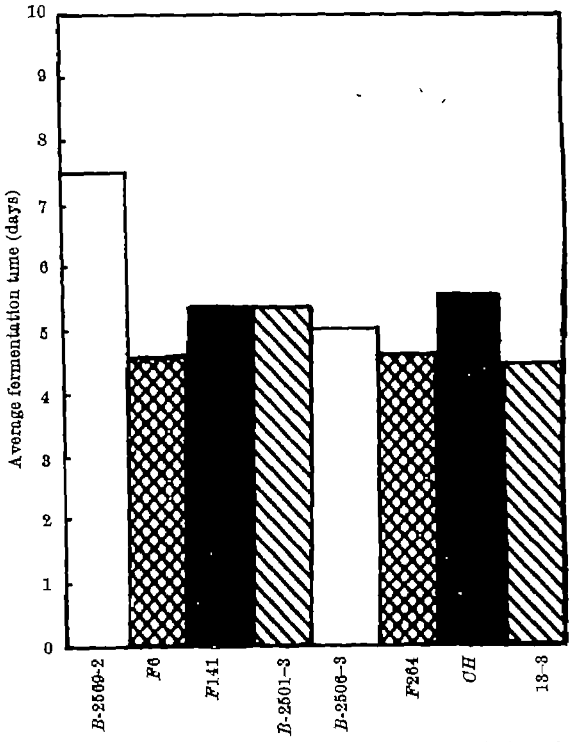

Fig. 1. Average lactose fermentation time for 8 strains of *S. sonnei*

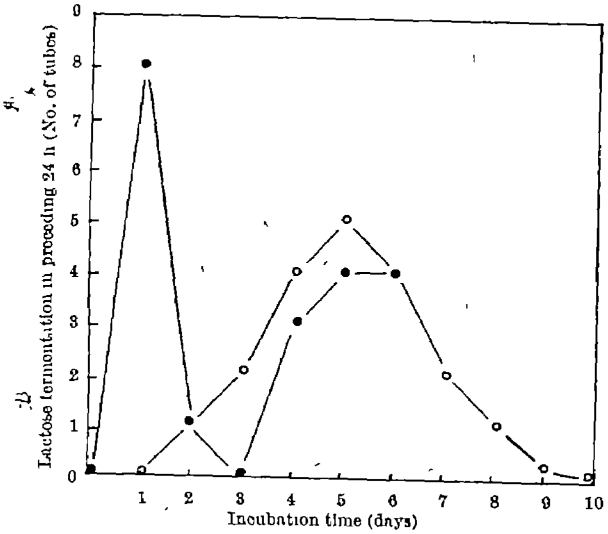

Fig. 2 A comparison of the lactose fermentation time of *S. sonnei* grown in 0·5 per cent lactose (●) with a composite curve of 8 strains of *S. sonnei* not grown in lactose (○) (uninduced)

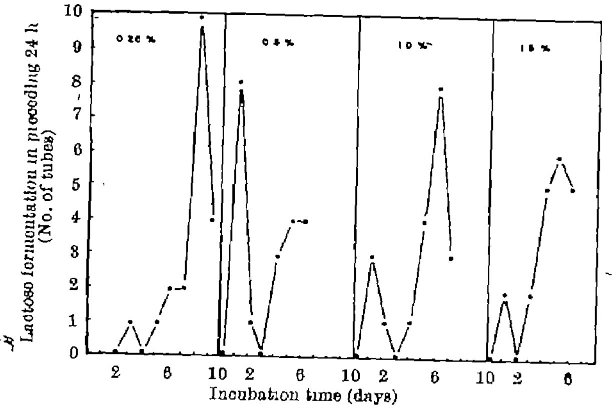

Fig. 3. A comparison of lactose fermentation times of *S. sonnei* grown in various concentrations of lactose

dilutions and streaking 0·1 ml. of the final dilution over a nutrient agar plate and streaking with a bent glass-rod 'hockey stick'. The plates were inverted and incubated for 24 h at 37° C and the colonies were counted with the aid of the Quebec darkfield colony counter (Spencer). Cell suspensions were similarly grown on EMB agar to determine the percentage of lactose-fermenting colonies. The plates were daily for 7 days.

In order to determine the lactose fermentation time, the lactose fermentation broth was dispensed into test tubes (16 × 100 mm); 5 ml. of the broth were placed into each test tube. Each experiment was performed with 20 tubes. The cells used for inocula were transferred daily for 5 days and suspended in sterile distilled water to yield a count of 1·0–1·5 × 10^8 cells per ml. The fermentation tubes were inoculated with 0·1 ml. of this cell suspension and examined visually daily for acid production. The appearance of the yellow colour indicative of acid production was sometimes so gradual that it became necessary to compare the tubes with a definitely positive reaction produced by a 24-h culture of *E. coli*. The number of tubes that became positive each day was recorded, and tabulated. Admittedly there was a degree of subjectivity in our method of recording lactose fermentation. Later we read the tubes in the Bausch and Lomb 'Spectronic 20' spectrophotometer as an aid to supplement our visual observations. The tubes were held until they had all produced the acid reaction. During certain phases of our work the volume of the lactose fermentation broth was varied.

We also investigated the effect of ultra-violet light on the ability of *S. sonnei* to ferment lactose. A General Electric Co. 15-W 'germicidal' lamp (*G15T8*) with an approximate wave-length of 2,537 Å was used. The calculated dose was 17·2 ergs/sec/mm². The cells were irradiated for 1, 5, and 10 min. Immediately after irradiation aluminium foil was placed over the dishes to minimize the possibilities of photoreactivation. The irradiated cells were grown for 3 days (transferred daily) and then assayed for their lactose-fermenting abilities. Unirradiated cells, similarly treated, served as controls. Various inducers were also studied to determine their activities in accelerating lactose fermentation times.

The average lactose fermentation times of the 8 strains of *S. sonnei* tested are shown in Fig. 1. The data are averages obtained from 20 tubes. Except for one strain (*B*–2569–2) the other strains produced an acid reaction in lactose broth in 4½–5½ days. *E. coli* fermented lactose in less than 24 h. None of the other species of *Shigella* fermented lactose during the 20 days these tubes were held for observation. There was no appreciable change in the lactose fermentation times when the volume of the lactose fermentation broth was increased from 5 to 10 ml. On EMB agar there were no detectable pink colonies of *S. sonnei* after 24 h of incubation for any of the 8 strains tested. However, after 48–72 h of incubation lactose-positive colonies appeared on the EMB agar. The percentage of positive colonies ranged from 11 to 57 per cent (of all the visible colonies) after 48 h and from 44 to 81 per cent after 72 h of incubation. The results are given in Table 1. It is interesting to note that there was a considerable variation in the percentage of lactose-positive colonies whereas there was only a slight variation from strain to strain when the lactose-fermenting ability was tested in broth.

S. sonnei was grown in 0·5 per cent lactose to determine its effects on the lactose fermentation times. As can be seen in Fig. 2 there was a marked acceleration in the time necessary for the fermentation of lactose. The largest number of lactose-positive tubes appeared on the first day and the average fermentation time was 3·3 days compared with 5·3 days for organisms not grown on lactose (a composite

Table 1. PERCENTAGE OF LACTOSE-POSITIVE COLONIES OF *Shigella* ON EOSIN METHYLENE BLUE AGAR

Organism and strain number	Percentage of colonies that were lactose-positive following various incubation periods		
	24 h	48 h	72 h
E. coli	100	—	—
S. flexneri	0	0	0
S. dysenteriae	0	0	0
S. boydii	0	0	0
S. sonnei, *B*–2506–3	0	11	62
S. sonnei, *B*–2569–2	0	24	44
S. sonnei, *B*–2501–3	0	57	81
S. sonnei, *F*–6	0	36	45
S. sonnei, *F*–141	0	20	52
S. sonnei, *F*–264	0	27	78
S. sonnei, *CH*	0	39	80
S. sonnei, 13–8	0	47	72

Table 2. AVERAGE LACTOSE FERMENTATION TIMES OF *S. sonnei*, STRAIN *B*–2506–3, GROWN IN VARIOUS CONCENTRATIONS OF GALACTOSE

Concentration of galactose (per cent)	Average No. of days for lactose fermentation*
0	5 1
0 1	7·7
0 25	7 9
0 5	7·9
1·0	7·6

* Obtained by averaging the results in 20 tubes

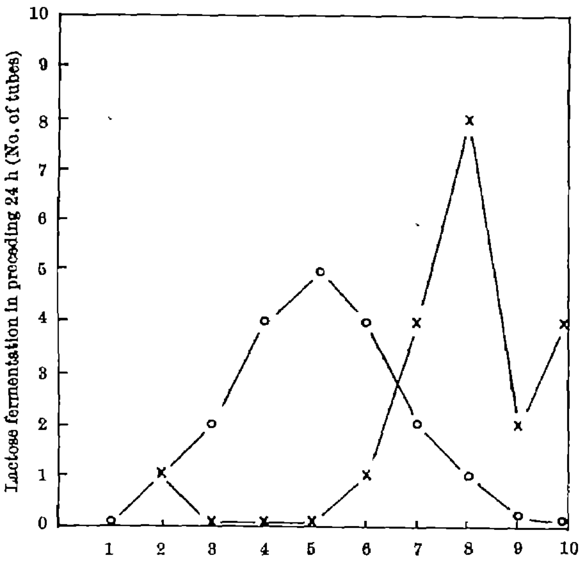

Fig. 4. A comparison of the lactose fermentation times of *S. sonnei* grown in 0·5 per cent galactose (×) with a composite curve of 8 strains not grown in galactose (○)

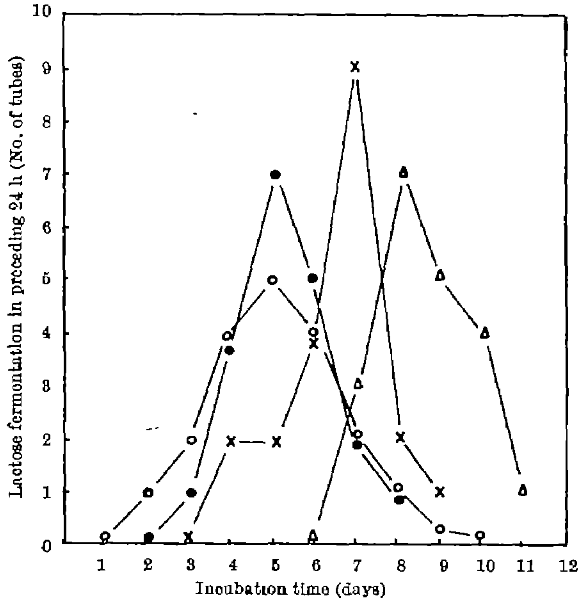

Fig. 5. The average lactose fermentation times of *S. sonnei* irradiated with ultra-violet light for 1 min (×), 5 min (●), and 10 min (Δ), compared with unirradiated (○) controls

of the 8 strains). Other concentrations of lactose were tested as inducers of lactose fermentation. The data are summarized in Fig. 3. Growth of *S. sonnei* in 0·25 per cent produced the slowest lactose fermentation time (average of 7·5 days); growth in 1·0 and 1·5 per cent lactose resulted in average fermentation times of 4·9 and 4·4 days, respectively. The shortest lactose fermentation time was produced by growth in 0·5 per cent lactose. There appears to be an optimal concentration of lactose which accelerates the lactose fermentation of the cells.

S. sonnei was grown in the presence of galactose to discover if galactose could stimulate the lactose fermentation of this organism. The results are summarized in Table 2. The time necessary for lactose fermentation did not vary much when the organisms were grown in 0·1–1·0 per cent galactose. As a matter of fact the lactose fermentation times following growth in galactose were larger than the organisms grown in the absence of galactose. This suggests that growth in galactose produced an inhibition in the ability of the *Shigella* to ferment lactose. Figure 4 shows the relationship between the lactose fermentation time of organisms grown in the presence of galactose compared with organisms grown in the absence of galactose. The average lactose fermentation time of *S. sonnei* following growth in 0·5 per cent α-melibiose was 7·3 days. Melibiose also produced a slight inhibition of the ability of this organism to ferment lactose.

Fig. 5 shows the effect of ultra-violet irradiation on the ability of *S. sonnei* to produce lactose fermentation. The lactose fermentation times of cells irradiated for 1 and 10 min were increased whereas the lactose fermentation time of cells irradiated for 5 min was similar to the unirradiated controls. After 1 min of irradiation the average fermentation time was 6·5 days; after 10 min of irradiation the average fermentation time was increased to 8·7 days. Unirradiated cells and cells irradiated for 5 min produced average fermentation times of 5·3 days. An adequate explanation is not available for the inhibition in fermentation produced by 1 min irradiation when 5 min of irradiation had essentially no inhibitory effect.

The fermentation of lactose by *S. sonnei* is an extremely variable property. Glynn and Starkey[5] reported that it took a broth culture 2–32 days to ferment lactose. On the other hand, utilizing large numbers of tubes, we have found that the average lactose fermentation times are rather consistent. It seems that the number of fermentation tubes used in a given experiment is an important variable. Variations in the average fermentation times between strains of *S. sonnei* appeared to be only slight. Glynn and Starkey[5] suggested that the time required for lactose positive colonies to appear on lactose agar could be roughly correlated with the time required for the organism to ferment lactose in a broth medium. We have found that lactose-positive colonies appeared on EMB agar within 2–3 days whereas the average lactose fermentation time was about 5 days. We feel that it is unreliable to determine lactose fermentation in *S. sonnei* by the inoculation of one or several tubes since variations in individual tubes are great even when the tubes have been inoculated from the same original culture. We have found that growth of *S. sonnei* in lactose increase the rapidity of lactose fermentation by this organism. This is in agreement with many other workers[12,13].

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ZONE PRECIPITATION

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N preparative and analytical biochemistry precipitation and crystallization have been largely uperseded by more sophisticated procedures such as ounter-current extraction, zone electrophoresis and arious kinds of chromatography. Solubility is a owerful 'separation parameter' that deserves to be tilized also when efficient fractionation is attempted. t is the purpose of this article to describe a column rocedure based on precipitation-dissolution proesses.

Suppose a precipitating agent moves in a sorbent ed with a slower speed than the substances to be recipitated. If adsorption or molecular sieving do ot affect the latter, solubility would be the only actor to cause their differential migration. Such onditions prevail, for example, when proteins move n beds of particulate, highly cross-linked dextran[1] n which the concentration of ammonium sulphate gradually increases from the top downwards.

If a protein is filtered through such a column it moves faster than the ammonium sulphate, and a region is reached where precipitation occurs. Clearly the critical concentration of ammonium sulphate for formation of a precipitate depends on the nature and concentration of the protein. When the particles have grown to a certain size their migration ceases. As the solvent passes the precipitate will soon be back in an unsaturated environment. Dissolution starts and the substance moves again. These events are presumably repeated to yield a continuous migration of a solute-precipitate containing zone. When a protein mixture is passed through the bed the components will precipitate in different parts of the column according to their relative solubilities. The zones thus formed move through the column and the substances appear separated in the eluate. The principle is schematically illustrated in Fig. 1.

The separation efficiency may be reduced by the fact that the crystals or particles have to grow large until they are entrapped in the bed. The choice of the gel in the present investigation has been made on the assumption that a limited penetrability of the proteins may be advantageous, since nuclei will then be formed in the gel phase where extensive growth is impossible.

The term 'zone precipitation' is tentatively suggested for the method, which is not limited to gel filtration only.

Some exploratory experiments have been made, two of which are described here. They were performed under primitive conditions since adequate instruments were not available either for the production of a continuous linear gradient or for convenient recording of the ammonium sulphate concentration in the effluent. Gradients covering intervals of 25–35 per cent saturated ammonium sulphate solution seem to give the best results. Extraction has been made with decreasing ammonium sulphate, but this act does not appear to have affected the fractionaion to any extent.

(1) Human serum was fractionated on a 'Sephadex G200' column as described by Flodin and Killander[2]. A fraction corresponding to the substances present in peak 3 and the rear part of peak 2 was concentrated to one-fourth of the original volume of the serum.

A chromatographic column tube (3·5 × 40 cm) closed at the bottom with a porous plate[3] was filled with a suspension of the dextran gel 'Sephadex G100' (140–200 mesh, obtainable from Pharmacia, Uppsala, Sweden) in a solution of 85 per cent saturated ammonium sulphate containing 0·5 M tris-hydroxy-methylamino-methane and adjusted to pH 8·8. The 'Sephadex' particles were allowed to settle. A bed 33 cm high was obtained. The solution above the sediment was removed. Five millilitre portions of ammonium sulphate–buffer solution were washed into the bed in a sequential manner to produce a stepwise gradient from 85 to 55 per cent saturated ammonium sulphate solution. Three millilitres of the serum fraction were washed into the column followed by ammonium sulphate–buffer solution. The ammonium sulphate concentration was continuously lowered from 55 to about 20 per cent saturated ammonium sulphate solution.

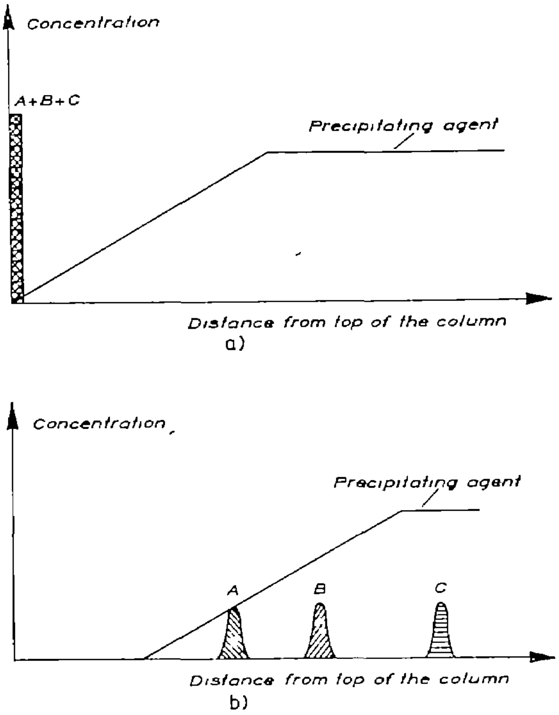

Fig. 1. The principle of zone precipitation exemplified for three substances A, B and C of different solubilities. a, Starting conditions; b, in the first phase of washing the substances A, B, C all move faster than the gradient substance. After reaching the gradient they display different behaviour C moves independent of the gradient, A and B are retarded to different extents

The eluate was collected in 3·5-ml. fractions, the ultra-violet absorption of which were measured at 280 mμ. Fig. 2 shows the material distribution. The last peak contained material precipitated on top of the column and later dissolved in the eluent. Fractions were pooled as indicated in the diagram, concentrated and subjected to electrophoresis on paper and in polyacrylamide. The results of the paper electrophoresis experiments are shown in Fig. 3. Peak 1 contained albumin, ferritin and some α_1-globulin. In peak 2, α_1, α_2, β and fast-globulin were found to be present together with small amounts of albumin. Peak 3 contained two globulin components which were clearly separated by paper electrophoresis. Only traces of faster components were found in this fraction.

(2) α-Hydroxysteroid dehydrogenase of *Pseudomonas testosteroni*[5] was purified by ammonium sulphate precipitation followed by gel filtration on 'Sephadex *G*100' (ref. 6). The activity was collected and concentrated. Three millilitres of concentrated solution were applied to a column prepared as described in experiment 1. Elution was also performed in the same manner. The enzyme activity was determined as previously described. The distribution of ultra-violet absorbing material and activity is shown in Fig. 4; it strikingly resembles a good elution chromatogram in the sharpness of the enzyme zone.

Instead of a stepwise gradient as used in these experiments, continuous smooth change in concentration of the ammonium sulphate should be preferred, particularly in cases where zone precipitation is used to test homogeneity of protein preparations.

Capacity, optimal rate of development and bead size are largely unknown factors. In a series of experiments on a certain column it was found that similar distribution patterns were obtained for loads of 0·5–3 ml. serum; 5 ml. was too high a load.

Clogging of the beds has never been observed as a result of precipitation within a column, but a heavy precipitate formed at the top may cause distortion of the zones.

Large bed volume changes seriously affect the packing and the flow rate. The swelling of 'Sephadex *G*100' and '*G*200' are very slightly affected by the ammonium sulphate but a volume expansion as great as 20–25 per cent has been noticed occasionally for 'Sephadex *G*25'.

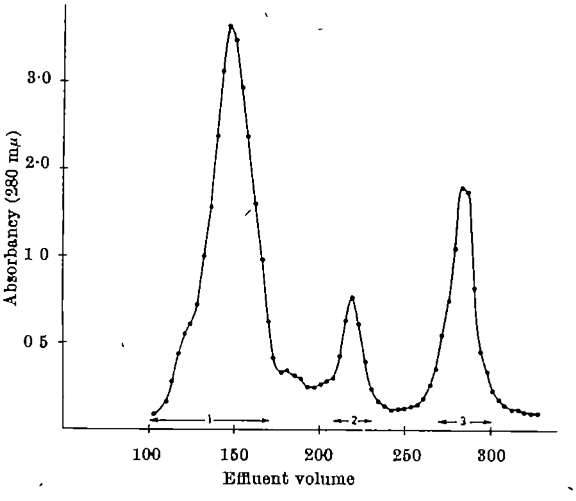

Fig 2 Zone precipitation diagram of a serum fraction. A step gradient of ammonium sulphate from 85 to 55 per cent saturation was used

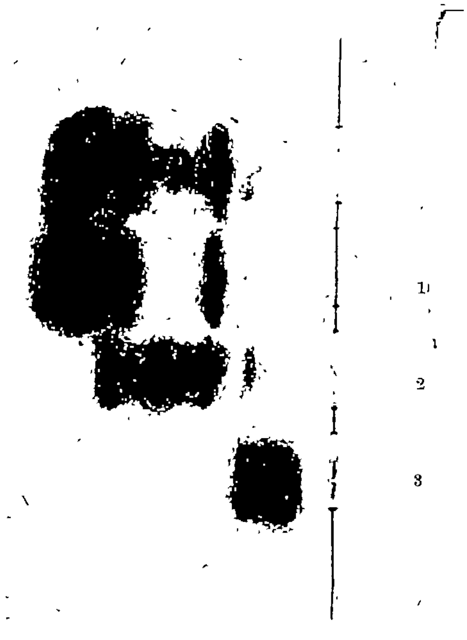

Fig. 3. Paper electropherograms of starting material and Fractions 1, 2 and 3 referred to in Fig. 2 Buffer according to Aronsson-Gronwall (ref. 4), pH 8·9; Whatman No. 1 paper; 5–6 m.amp, 225 V; 24 h. Paper stained with bromophenol blue

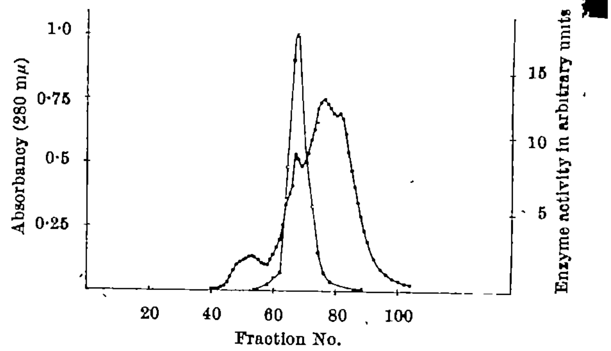

Fig. 4. Zone precipitating diagram of a α-hydroxysteroid dehydrogenase preparation. Gradient, 85–55 per cent saturation of ammonium sulphate

Beside its value as a purification method, zone precipitation may also be of great help in finding suitable conditions for crystal formation. This may be a very important application in view of the increasing importance of X-ray analysis in elucidation of protein structures.

In this presentation of the zone precipitation method its usefulness for protein fractionation may have been overemphasized. Very likely it will be rewarding to explore its potentialities in other fields.

This investigation has been financially supported by a grant from the Swedish Natural Science Research Council.

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A CHALLENGE TO THE CONCEPT OF SELECTION BY MALARIA IN GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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IN considerations of polymorphisms in man, a pre-eminent role has frequently been invoked for infectious diseases, and in this regard malaria has featured prominently. Increased resistance to *Plasmodium falciparum* infection was shown by Allison[1] in 1954 to be conveyed by the sickle-cell trait, and it has been concluded[2] on the basis of cumulative evidence from many investigators that *falciparum* malaria appears to act as a selective pressure favouring the heterozygote carriers, with consequent high frequency for the sickle-cell gene in areas holoendemic for this form of malaria.

Fewer critical studies have been performed for thalassæmia, although the geographical distribution of the trait follows that of *Pl. falciparum*[3]. Ceppellini[4] has shown, however, that there is a much higher incidence of thalassæmia in malarious regions of Sardinia than in non-malarious regions of the island, and similar demography has been observed in New Guinea (Curtain, Kidson, Gorman and Gajdusek, unpublished work). Similarly, the geographical distribution pattern of glucose-6-phosphate dehydrogenase (G6PD) deficiency has suggested a relationship to malaria[5,6]. Investigations in Africa[5,7], Melanesia[8,9] and Sardinia[10] have indicated a pattern of varying frequency of the trait which is roughly proportional to varying malarial incidence within a given country. Of equal interest is the apparent absence of the trait in Japanese[6], Eskimos[6] and Australian Aborigines[11], all people occupying non-malarious regions, suggesting that the gene responsible either has never existed in these populations or has failed to survive.

The overall distribution pattern represented by the world map for these genetic conditions—sickle-cell, thalassæmia and G6PD deficiency—has a similarity to the map for *falciparum* malaria, as stressed by Motulsky[6]. It could be argued, however, that very many other parasitic diseases, in addition to variable degrees of nutritional inadequacy, are seen in this same tropical belt, and may be concerned, likewise, as selective pressures influencing the frequency of one or more of these traits. The temptation to implicate malaria alone has been strengthened by consideration of the effects of these particular mutant conditions on the physiology of the human erythrocyte—especially understandable in the case of G6PD deficiency[12]—which provides the essential biochemical environment for malarial parasite life and reproduction. The demonstration by Allison and Clyde[13] that *Pl. falciparum* parasite-rates and densities were lower in young African children with G6PD deficiency than in children with normal enzyme-levels has appeared to lend further emphasis to the probability that G6PD deficiency conveys increased resistance to *falciparum* malaria, making the trait common in malarious areas.

Examination of the world distribution in detail, however, shows some important exceptions to the apparent rule of correlation of high malarial endemicity with high incidence of G6PD deficiency. Thus, both Malays[14] and Indonesians (Lie-Injo, L.E., personal communication) possess the gene in low frequency although in surrounding populations in some regions of Thailand[15], the Philippines[6] and coastal New Guinea[8] higher frequencies occur. Both Malaya and coastal Indonesia are highly malarious areas and the Malay peoples have been resident there for about 4,000 years, a time-period sufficient for the gene to build up to higher levels under selective pressure from *falciparum* malaria. Armenians are another population who have inhabited a malarious area for a long time period yet have a very low incidence of G6PD deficiency[16].

In a further series of investigations of G6PD deficiency in the Melanesian populations of New Guinea and New Britain[18] it has become evident that a direct correlation of incidence of the trait with endemicity of *falciparum* malaria represents too superficial an appraisal of the question of selection pressures, and that in fact malaria may not even be the major factor involved in this process. New Guinea presents in many ways a rather unique situation for examining human genetic patterns in relation to selective forces, containing as it does several hundred linguistic groups living under very similar environmental conditions, groups which have maintained their separate identity for long periods of time, while possibly coming in the distant past from a common source[17]. Population groups are available for study from sea-level to altitudes above 5,000 ft. All coastal areas of New Guinea and New Britain are holoendemic for malaria—predominantly *falciparum* and *vivax*—while gradations of malarial endemicity are present from the coast to the highlands, the latter possessing little or none of the disease except in occasional epidemic form. Early surveys of this enzyme deficiency in Melanesians[8,9,18], based on examination of randomly collected blood samples sent by air by various field workers in New Guinea to our laboratory in Melbourne, had revealed a general trend showing a very low frequency (< 0·01) of G6PD deficiency in the highlands and a moderately high frequency (> 0·10) in coastal regions. These findings appeared to follow the pattern observed in Africa[5,7] and later noted in Sardinia[10].

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The more recent field examination[19], however, has enabled a detailed investigation of the distribution pattern of the trait among various linguistic groups, with results which throw strong doubt on the theory of malarial selection. Three areas were examined: (1) the Gazelle Peninsula, New Britain, less than 500 ft. altitude; (2) the Sepik River district, New Guinea, less than 500 ft. altitude; (3) the Markham River valley, near Lae on the east coast of New Guinea, from sea-level to the Eastern Highlands, with an altitude of more than 5,000 ft. G6PD activity of red blood cells was estimated semi-quantitatively in the field by a rapid modification[20] of the dye decoloration test[21] and confirmed by quantitative assay[22] on blood samples sent by air to the laboratory in Melbourne.

In Table 1 are shown the results for the gene frequency in males in linguistic groups of the Gazelle Peninsula in New Britain and the Sepik River district of New Guinea. The difference between the Tolai linguistic group and the Sulka and Baining groups in the Gazelle Peninsula is striking, the latter having a much higher incidence than the Tolai ($P < 0{\cdot}01$). A similar, though less striking, difference is seen between the Abelam and Sause groups in the Sepik River district ($P < 0{\cdot}01$). In each area the various linguistic groups examined live under similar conditions of nutrition and of parasitic infestation, although those in the Gazelle Peninsula inhabit a much more fertile region and probably enjoy a more adequate diet than those in the Sepik River district. *Falciparum* malaria is holoendemic in both regions. Thus, we find the phenomenon of culturally distinct population groups living in close proximity, both possessing the mutant gene for G6PD deficiency, yet exhibiting extreme differences in frequency of that gene under conditions where it might be expected that malaria would exert an equal selection pressure for G6PD deficiency on both groups. It is possible, of course, that the gene has spread only recently to the Tolai and Sause; but it is significant that among the latter, at least, the gene is scattered among many villages and the incidence does not reflect a single family breeding group. This argues against random drift, and suggests that the gene has been present in these population groups for some time but either has not reached high proportions due to favourable selective pressure or has been kept at low levels by adverse forces.

Table 1. GENE FREQUENCIES FOR G6PD DEFICIENCY IN THE GAZELLE PENINSULA AND THE SEPIK RIVER DISTRICT

Linguistic group	No. examined (males)	Gene frequency (males)
Gazelle Peninsula New Britain		
Tolai	122	0·008
Sulka	102	0·150
Baining	108	0·120
Sepik River District New Guinea		
Sause	324	0·009
Abelam	49	0·080

Findings in the Markham River valley and the Eastern Highlands of New Guinea are summarized in Fig. 1. The apparent gradation in incidence of G6PD deficiency with changing altitude, from high incidence on the coast to low incidence in the highlands, would seem to be readily explained by a parallel influence of malaria, chronic malaria being less frequent among the Mumeng than in the Wampur and Bukawa groups, and rare in the highlands peoples. However, it is equally possible that this pattern may in fact be the result of spread from a large gene pool on the coast near the river mouth, with dilution and diminution of the gene frequency along the valley towards the highlands, and evidence accumulating from studies of other genetic parameters (Curtain, Gorman and Kidson, unpublished) suggests that this may indeed be so.

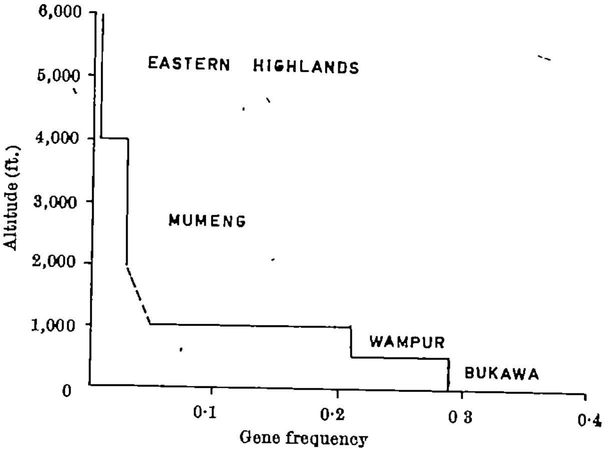

Fig. 1. Variation of gene frequency for G6PD deficiency with altitude in linguistic groups in the Markham River valley and in the Eastern Highlands

The gene frequencies for G6PD deficiency in several linguistic groups studied in the Eastern Highlands are shown in Table 2, to demonstrate their distribution. While the overall incidence is low, the frequency in some linguistic groups such as the Auyana may reach or exceed that in some coastal groups.

Table 2. GENE FREQUENCIES FOR G6PD DEFICIENCY IN THE EASTERN HIGHLANDS

Linguistic group	No. examined (males)	Gene frequency (males)
Tairora	88	0·011
Gadsup	89	0·000
Auyana	40	0·050
Kukukuku	116	0·000
Fore	39	0·000

Comparison of the demography in New Guinea and New Britain of G6PD deficiency and thalassæmia has revealed a dissociation between the distribution patterns of these two genetic traits. Thus, while G6PD deficiency shows the above erratic frequencies in the linguistic groups examined, thalassæmia shows a consistent incidence of 4–7 per cent in all groups in coastal regions, rapidly diminishing as altitude rises above 1,000 ft. (Curtain, Kidson, Gorman and Gajdusek, unpublished). This dissociation strongly suggests that different selective forces operate for the two conditions.

Assessment of parasite-rates and densities in the populations investigated here have proved completely unreliable because of the spasmodic administration of antimalarial drugs at uncertain time intervals. Wilson[23] has pointed out that Allison and Clyde's[13] finding of differences in parasite densities between African children with low enzyme-levels and those with normal levels does not provide evidence of protection from the lethal effects of *falciparum* malaria. Moreover, the recent report of Kruatachue and Harinasuta[15] suggests that no significant difference in *Pl. falciparum* parasite densities occurs between affected and normal children under 3 years of age in Thailand and that affected children in this age group may have higher densities of the non-lethal *Pl. vivax*. It is doubtful, therefore, whether satisfactory evidence is available from this source to assist in the evaluation of the part played by *falciparum* malaria in selection for this gene.

It is possible that selection factors may act rather differently in different population groups. That G6PD deficiency may in fact represent a common end-product of multiple underlying genetic mechanisms was first suggested by Marks and Gross[24], who pointed out the quantitative differences between affected Negroes and affected Caucasians, and was strengthened by the subsequent findings of different tissue distribution of enzyme deficiency in these two groups[25,26]. Primary deficiency of an activator of G6PD was recently reported in Sephardic Jews in Israel by Rimon *et al.*[27]; we have found heterogeneity of response to the activation reaction in Melanesians[28], suggesting that multiple mutations may be involved, and have shown that deficiency of an activator is not the primary defect in enzyme-deficient Negroes[29]. It may well be that selection pressures operate somewhat differently in these differing groups, even though the final result, deficiency of the enzyme, is common to all.

Tempting as it is to correlate the distribution and frequency of *falciparum* malaria with the frequency of a genetic trait such as G6PD deficiency, such a concept must face the contrary evidence of low incidence of the deficiency in large groups such as Malays, Indonesians and Armenians and small groups such as the Tolai and Sause in Melanesia, all of which inhabit holoendemic malarious areas. Although similar conditions of parasitic infestation pertain, it is of course remotely possible that restricted cultural contact between the Melanesian linguistic groups could result in limited epidemics of certain virus diseases, reported in some cases as initiating acute hæmolysis in enzyme-deficient subjects[20], thereby causing differential loss of the mutant gene by death of carriers in affected groups. However, the influence of social factors themselves, as emphasized by Lehmann[31] for sickle-cell disease, must not be forgotten in considerations of G6PD deficiency.

Vastly differing marriage customs are known in many regions in New Guinea, some tending to lead to marked inbreeding, others to the contrary; doubtless similar variation exists in other regions such as Africa. Then, too, population pressures have frequently led to small groups being pushed inland from coastal precincts into highland areas, a fact which may explain genetic gradients from coast to highlands in many parts of the world. Geographically limited pockets of rare syndromes such as Kuru bear exotic witness to the not infrequent phenomenon of inbreeding in New Guinea. It is possible that a complex interaction of malaria, viral infections, dietary habits and social customs is involved in producing the present pattern of distribution of G6PD deficiency, with social customs playing a much more dominant part than has been attributed to them in previous considerations of genetic polymorphisms in man.

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EFFECT OF THYMIDINE ANALOGUES ON REPRODUCTIVE MORPHOGENESIS IN *ARABIDOPSIS THALIANA* (L.) HEYNH.

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RECENT reports have indicated that the inductive dark period processes required for the initiation of floral morphogenesis in short-day plants can be affected by modifications of nucleic acid metabolism. Flowering has been promoted by added RNA nucleosides[1], and inhibited by 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FUDR)[2,3]. In micro-organisms 5-bromodeoxyuridine (BUDR) and 5-iododeoxyuridine (IUDR) have been shown to replace thymidine in the biosynthesis of DNA. As a consequence of such incorporation, mutational changes may occur in subsequent DNA replication[4]. IUDR incorporated into mammalian chromosomes has enhanced radiosensitivity[5]. More recently, the incorporation of

Table 1. EFFECT OF GIBBERELLIC ACID (GA), 5-IODODEOXYURIDINE (IUDR), AND THYMIDINE (Thy.), ON GROWTH

Treatment	No. rosette leaves	No. cauline leaves	Mean stem-height (mm)
Exp. 1: 33 days after sowing	$\bar{x}$ $s_{\bar{x}}$	$\bar{x}$ $s_{\bar{x}}$	
Control	11·3 ± 0·2	0	0
GA	9·6 ± 0·2	3·23 ± 0 3	15·8
IUDR	14·9 ± 0·2	0	0
IUDR + GA	10 7 ± 0 5	4·0 ± 0 5	20·5
Expt. 2: 26 days after sowing			
Control	9·4 ± 0 1	0	0
GA	7·8 ± 0 1	2 7 ± 0 1	30
Thy.	10·4 ± 0·1	0·	0
GA + Thy.	8·8 ± 0·1	1·6 ± 0 1	19
IUDR	11·5 ± 0·1	0	2
GA + IUDR	8·0 ± 0 1	3 6 ± 0 1	37
Thy. + IUDR	10·3 ± 0 1	0	2
GA + IUDR + Thy.	8 1 ± 0 2	2 8 ± 0 1	26

tritium labelled IUDR into the DNA of chromosomes of *Vicia faba* root tip cells has been demonstrated[6]. These results suggest that incorporation of IUDR and other thymidine analogues into apical meristem cells of higher plants may result in both mutagenic and morphogenetic changes.

Arabidopsis thaliana (L.) Heynh., a facultative long-day plant, has been shown to respond to gibberellic acid (GA), under short day, by accelerated flowering[7].

The experiments reported here show that the thymidine analogue IUDR also promotes flowering in *Arabidopsis thaliana* after application to the vegetative meristem, and acts in an additive manner to enhance the effect of GA. Thymidine was found to have no stimulatory effect on flowering and did not significantly decrease the stimulation caused by IUDR and GA. FUDR at the same concentration completely suppressed growth and caused bleaching and death. However, both these effects were partially reversed by thymidine.

The plants used were derived from a single plant of a race 'Estland', and were cultured aseptically on mineral agar in test tubes. In the first experiment cultures were placed at 6° C for 30 h prior to transfer to a constant 25° C tube temperature, 800 ft.-candles light intensity, and 12-h photoperiod. In the second experiment the 6° C seed treatment was omitted without decreasing germination, though flowering dates of the controls differed. In both experiments drops of sterile 'Tween 20' (0·1 per cent in half-strength mineral solution) were applied to plant axils to facilitate penetration of the treatment solutions; the drops were shaken off before treatment chemicals were applied.

Experiment 1. Treatment solutions were sterilized in an autoclave. The syringe and 20-gauge needles used permitted application of 50 µl. of each solution to provide the following treatments: (*a*) control ('Tween 20'); (*b*) 5 × 10^{-4} M IUDR; (*c*) 2·10 × 10^{-4} M GA; (*d*) IUDR + GA applied consecutively. There was an average of 15 plants per treatment.

Experiment 2. Treatment solutions were sterilized only by 'Millipore' filtration. Treatments were applied aseptically using 1 c.c. syringes and 24-gauge needles, which enabled accurate placement of 20-µl. drops in the region of the growing apex. Treatments were applied consecutively to provide all possible combinations of the following main treatments: (*a*) control; (*b*) thymidine 5 × 10^{-4} M; (*c*) GA 2·10 × 10^{-4} M; (*d*) IUDR 5 × 10^{-4} M; (*e*) FUDR 5 × 10^{-4} M. There were ten plants per treatment combination.

The treatments were applied to 13-day-old plants, which were uniformly at the rosette stage with the third leaf pair not fully expanded. A plant was scored as flowering when the first flower opened. Six days after treatment with FUDR plants became bleached and showed complete growth inhibition. Eventually all FUDR-treated plants died. Delay of bleaching and different degrees of recovery were observed in treatments with thymidine plus FUDR. In these treatments 80 per cent of the plants were alive after 56 days, though renewed growth was either chlorotic or distorted. In 25 per cent of the plants flowering occurred and seed was set. In the absence of thymidine, FUDR caused 100 per cent eventual death in other treatment combinations.

Some distortion of new leaves produced after IUDR application occurred in several plants, but most were normal and the major effect was an acceleration of growth and production of pro-cauline leaves prior to stem elongation (Table 1). In both experiments IUDR and GA, either alone or in combination, induced earlier commencement of reproductive development and earlier flowering (Figs. 1 and 2). These effects were highly significant and did not interact. Thymidine did not reverse the IUDR effect on flowering time. The effect of IUDR on flowering time was less than that of GA and could be distinguished from GA additionally by a highly significant reduction in the mean stem-height at flowering. This effect on stem-height was significantly reversed by thymidine but not significantly by GA (Table 2) The observed effects are magnified by growth from primary events occurring in the apical meristem. The absence of interaction between GA and IUDR or thymidine indicates that the hormone and nucleoside sites of action are different and non-competitive.

Table 2 EFFECT OF GIBBERELLIC ACID (GA), THYMIDINE (Thy.) AND 5-IODODEOXYURIDINE (IUDR) ON FLOWERING TIME AND PLANT HEIGHT AT FLOWERING IN *Arabidopsis thaliana* (L.) HEYNH

Treatment	No. of plants	Days to flower	Height at flowering
Experiment 1		$\bar{x}$ $s_{\bar{x}}$	$\bar{x}$ $s_{\bar{x}}$
Control	14	50 4 ± 0 6	83 7 ± 2 6
GA	16	41·4 ± 0 1	85 1 ± 3 0
IUDR	15	44·6 ± 0 4	67·4 ± 3 2
GA + IUDR	18	39 0 ± 0 6	73 5 ± 3 8
Experiment 2			
Control	10	44 0 ± 0 8	94 5 ± 4 8
GA	10	35 4 ± 0 6	95·7 ± 3 5
Thy.	10	43·3 ± 0 7	91·5 ± 2·6
GA + Thy.	10	36 6 ± 0 6	82 0 ± 4·0
IUDR	10	39·3 ± 0·6	66 3 ± 4·4
GA + IUDR	10	32 7 ± 0·5	70 9 ± 3·7
Thy. + IUDR	10	39 9 ± 0 6	73·6 ± 2 5
GA + Thy. + IUDR	10	34 3 ± 0 5	69 7 ± 3·1

Contrast	Mean squares for main effects and interactions* Days to flower	Height at flowering
GA	831 57†	217·9
IUDR	259 78†	3003 0†
IUDR × GA	46·58	84 0
Error (59 D.F.)	23 69	190·5
GA	945 31†	72 2
Thy.	9 11	140·4
IUDR	214·51†	8652 8†
GA × Thy.	10 50	460 8
GA × IUDR	12·01	101·2
Thy. × IUDR	3 61	649 8‡
GA × Thy. × IUDR‡	1 01	6 0
Error (72 D F.)	4·32	134 9

* Mean squares of 2 × 2 analysis weighted for disproportionate sub-class numbers. † Highly significant (*P* 0·001). ‡ Significant (*P* 0 05).

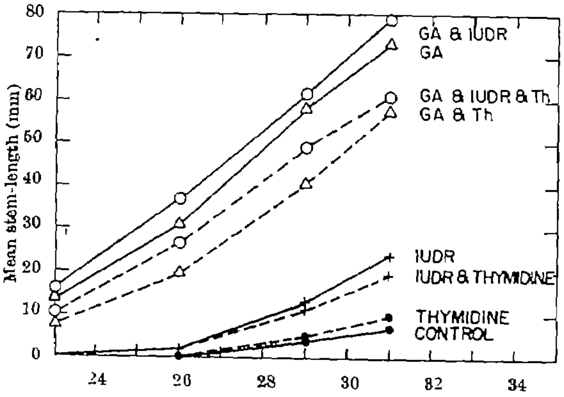

Fig. 1. Effect of GA, IUDR and thymidine on stem-elongation

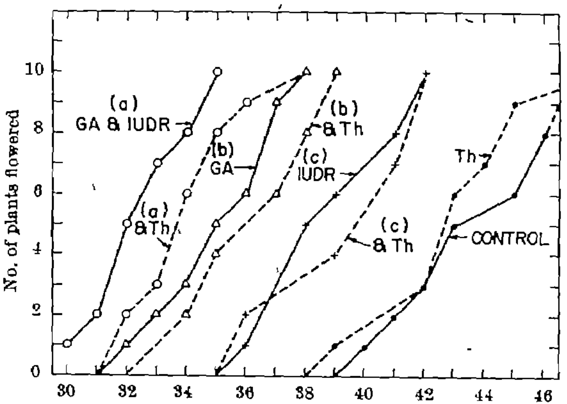

Fig. 2. Effect of GA, IUDR and thymidine on flowering-time in *Arabidopsis thaliana* (L.) Heynh.

Thymidine reversal of FUDR inhibition occurs at the free nucleotide-level, since FUDR acts primarily by blocking the formation of thymidylic acid from uridylic acid by inhibiting the methylating enzyme thymidylate synthetase[3,8]. IUDR, however, has not been shown to bind this enzyme but does replace thymidine in the synthesis of DNA and is effectively incorporated even in the presence of normal cellular levels of thymidine into bacterial, mammalian and plant root meristem DNA. Since there is little turnover of formed DNA, then if the site of action on morphogenesis of IUDR is at the DNA polymer-level, rather than at the free nucleotide-level, interaction with thymidine would tend to be weak; for once IUDR DNA is formed, free thymidine can no longer be an effective competitor. At the free nucleotide-level, however, strong interaction should result from the presence of added equi-molar amounts of IUDR and thymidine. The fact that a weak interaction was observed, and was significant for the specific IUDR effect on height at flowering, indicates that the IUDR effect is due to the analogue molecule, and is consonant with a DNA site of action.

An interpretation of these preliminary results might be based on the concept that a precocious induction of floral morphogenesis occurs following temporary impairment of the active nuclei of the vegetative meristem. This could result in the release of normally relatively quiescent nuclei to form the reproductive meristem. Other nucleoside analogues, particularly BUDR, may act similarly to IUDR.

Note added in proof. In subsequent experiments now reported[9], BUDR has caused the same morphogenetic effect as IUDR.

I thank Hoffman LaRoche Inc. for their gift of FUDR. This work was carried out under the auspices of the U.S. Atomic Energy Commission.

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SYNTHETIC DIET FOR SILKWORM RAISING

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PREVIOUSLY, some of us[1,2] have succeeded in rearing the whole larval stage of not only the domestic silkworm (*Bombyx mori*) but also wild silkworms (*Philosamia cynthia ricini*[3], *Antheraea pernyi*[4], etc.) on an artificial food which consists mainly of the dry leaf powder of plants such as mulberry, castor-oil plant or oak. We have since tried replacing the leaf powder with some simple nutrients to make a synthetic diet for each silkworm. This article is concerned with the preparation of a synthetic diet which makes possible the rearing of the silkworm, *Bombyx mori*.

We found that the silkworm ate continuously a diet composed of 1 g sucrose, 6·5 g cellulose powder, 50 mg β-sitosterol and 11 ml. of water, solidified with 1·5 g potato starch. In preparing the synthetic diet, some nutrients necessary for growth of the larvæ were added in place of some parts of cellulose powder, which occupies a great portion of the basal diet. These were a mixture of amino-acids, vitamins or mineral substances (Tables 1–3), on the basis of the amount in the mulberry leaves, determined by the microbiological method, and the others. The alcohol extracts of mulberry leaves added, when necessary, to the diet to stimulate feeding was prepared by extracting dry mulberry leaves with hot absolute methyl alcohol for 5 h and by removing the alcohol by reducing pressure. A mixture of all the vitamins, except for ergosterol and β-carotene, was stored in a refrigerator in solution. A certain amount of ergosterol, β-sitosterol, alcohol extracts of mulberry leaves and β-carotene were respectively dissolved in ether, and were present in a certain amount of the cellulose powder added. The ether was completely removed first.

The synthetic diet was prepared as follows. A beaker containing the sucrose, the amino-acid mixture, the vitamin mixture, except for the ergosterol and the β-carotene, and water was heated, and the hot solution was poured into a beaker containing the potato starch, the cellulose powder, each cellulose powder containing the β-sitosterol, the ergosterol, the β-carotene and the alcohol extract of mulberry leaves (when necessary) and mineral substance mixture. The contents were well stirred, formed as a pie, steamed for 3 min and stored in a refrigerator. The newly moulted fifth instar larvæ, hybrids between Nichi 122 × Nichi 124 and Shi 115 × Shi 124, were reared, according to the procedure described in a previous paper[1], on a synthetic diet in a box at a temperature of 25° C and at 73 per cent relative humidity until they began spinning their cocoons. The diet was cut in slices 0·1–1 mm thick. These were fed to the silkworms during the fifth stage twice daily at 12-h intervals. The composition of synthetic diets prepared is shown in Table 4, and the growth of the silkworm reared on the diet in Table 5. Five silkworms were tested for each experiment.

The silkworms scarcely ate diet 20 on the first day, but did so continuously after the second day. The body-weight of the silkworms reared on the diet increased gradually after two days, and was about doubled by the eighth day. Among five silkworms tested, two became mature larvæ at the eleventh day, and afterwards spun their silk thread, but the amount was only 21 mg per larva. Three silkworms died after maturation; a larva at the tenth day and the two at the eleventh day. In the larva which died at maturity the pair of silk-glands were well developed and were equal in size to the silk-glands of the silkworm at the fifth day of the fifth stage reared on fresh mulberry leaves. The silkworms scarcely ate diet 15, which differs from diet 20 in content of sucrose and β-sitosterol. The silkworms reared on diet 10, containing alcohol extract of mulberry leaves the concentration of which corresponds to 2 per cent for the diet, and 4·4 per cent for its dry matter, did not eat so much for the first day, but fed comparatively well after becoming familiar with the diet. The body-weight of the silkworms reared on diet 10 increased four times in body-weight. All the five silkworms tested became matured larvæ, and spun their cocoons. The weight of the cocoon fibres was 83 mg (av.), and the value was about 70 per cent of the weight of the cocoon fibres produced by the silk worms reared on a diet[2] which consisted mainly of the dry mulberry leaf powder.

Table 1. MIXTURE OF AMINO-ACIDS (L-form)

Amino-acids	Amount (g)
Alanine	1·0
Arginine	1·0
Aspartic acid	2·1
Cystine	1·0
Glutamic acid	2·3
Glycine	1·2
Histidine-HCl	0·7
isoLeucine	1·2
Leucine	1·8
Lysine-HCl	1·5
Methionine	0·8
Phenylalanine	1·1
Serine	1·2
Proline	1·1
Threonine	1·1
Tryptophan	0·8
Tyrosine	0·7
Valine	1·4
Total	21·0

Table 2. MIXTURE OF VITAMINS

Vitamins	Amount (mg)
p-Aminobenzoic acid	2·0
Ascorbic acid	400·0
Biotin	0·1
β-Carotene	20·0
Choline chloride	550·0
Ergosterol	400·0
Folic acid	0·4
Inositol	100·0
Nicotinic acid	40·0
Calcium pantothenate	80·0
Pyridoxin hydrochloride	4.0
Riboflavin	15·0
Thiamin	4·0
Total	1,565·5 g

Table 3. MIXTURE OF MINERAL SUBSTANCES

Mineral substances	Amount (g)
$NaH_2PO_4 \cdot H_2O$	1·067
$MgSO_4$	2·360
$CaCO_3$	4·590
K_2SO_4	4·518
NaCl	0·813
Total	13·848

Table 4. COMPOSITION OF SYNTHETIC DIETS

Component	Diet No. 20	Diet No. 15	Diet No. 10
Potato starch	1·5 g	1·5 g	1·5 g
Sucrose	1·5 g	1·0 g	1·0 ,,
Amino-acid mixture	1·0 g	1·0 g	1·0 ,,
Vitamin mixture	16·0 mg	16·0 mg	16·0 mg
Mineral substance mixture	0·8 g	0·8 g	0·3 g
Cellulose powder	4·6 g	5·2 g	4·8 g
β-Sitosterol	100·0 mg	50·0 mg	50·0 mg
Alcohol extract of mulberry leaves			0·4 g
Water	11·0 ml.	11·0 ml.	11·0 ml.

Table 5. GROWTH OF THE SILKWORMS REARED ON SYNTHETIC DIETS (G PER LARVA)

Fifth Stage	Diet No. 20	Diet No. 15	Diet No. 10
1st day	0·64	0·64	0·64
2nd ,,	0·64	0·63	0·77
3rd ,,	0·76	0·66	0·94
4th ,,	0·83		1·18
5th ,,	0·98		1·54
6th ,,	1·09		1·86
7th ,,	1·14		2·08
8th ,,	1·20		2·17
9th ,,	1·15		2·40
10th ,,	0·86		2·23
11th ,,	1·15		

Generally speaking, it is necessary, in rearing silkworm larvæ on artificial food, to take care in the choice of their races, because the acceptance by silkworm larvæ of the diet varies remarkably with race. A diet, such as 10, containing small amounts of the alcohol extracts of mulberry leaves, gives a satisfactory result in comparison with a diet such as 20, which contains no alcohol extract. This fact seems to suggest to us that there are some important substances which stimulate the feeding of silkworms in the alcohol extracts of mulberry leaves in addition to β-sitosterol[5,6] and sucrose. It will be clear, judging from an experimental result, that silkworms reared on a diet from which some amino-acids or some vitamins had been removed (diet 10) died within three or four days. In this article it is shown that it is possible to rear silkworms on synthetic diets. At present an investigation to improve the composition of the synthetic diet prepared is being carried out. Recently we also succeeded in preparing a synthetic diet which consists of agar-agar, sucrose, amino-acids, mineral substances, vitamins, cellulose powder, β-sitosterol and water for the silkworm raising.

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LETTERS TO THE EDITOR

GEOPHYSICS

Nuclear Explosions and Normal Mode Oscillations of the Earth

The article in *Nature*[1] under the title "Recent Nuclear Explosions and Earth Tides" suggesting that nuclear explosions can excite the normal mode oscillations of the Earth needs careful reconsideration.

The free oscillations of the Earth, though theoretically predicted even in the nineteenth century, have been experimentally observed only on two occasions. The first observation was by Benioff *et al.*[2] when they reported fifty-seven minute waves on strain seismograph records after the Kamchatka earthquake of November 4, 1952. The great Chilean earthquake of May 22, 1960, also caused very noticeable oscillations and several of the spheroidal and toroidal modes of the Earth were detected and identified from gravity meter and strain seismograph data. Ness, Harrison and Slichter[3] used a very high resolution La Coste–Romberg gravity meter with the smallest measurable signal of the order of 0·023 microgal (r.m.s. noise figure) to record the Chilean earthquake and detected several of the spheroidal modes (only these are observable on gravity meters) in the period range of 3–55 min. Many other observational and theoretical discussions on the subject have appeared and a comprehensive bibliography is given at the end of the masterly discussion on toroidal oscillations of the Earth by MacDonald and Ness[4].

The energy demand to excite normal mode oscillations of the Earth is enormous and hence only very large earthquakes cause noticeable effects and even then very sensitive instruments are required to detect them. The Kamchatka earthquake had a magnitude of 8·25 and the Chilean earthquake was even bigger with a magnitude of 8·75. These magnitudes correspond to an energy release of the order of 10^{27} ergs. The largest nuclear bomb exploded so far communicated energy of the order of 10^{20} ergs (magnitude of equivalent earthquake, 5·5–5·75) to the ground. It will indeed be an 'earth-shaking' effect if this small amount of energy (relatively speaking) can excite free oscillations of the Earth.

One should not lose sight of the fact that gravity meters and vertical seismographs are very sensitive to barometric fluctuations, and whether the Askania gravity meter referred to in the note in *Nature*[1] could be the exception is a point worth investigating.

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WHILE reporting the effects of nuclear explosions on the gravimeter installed in our laboratory, it was mentioned that possibly sometimes the free oscillations of the Earth could also be observed. It is obviously necessary that an adequate amount of energy will have to be communicated to the Earth for this to occur and in order to identify these oscillations the records have to be harmonically analysed. This is now being done.

The instrument is pressure-tight and shows a change of less than 0·05 mgal. for pressure variations corresponding to a difference in height of 500 metres. The observed changes in g are much greater than this and hence may not be attributed to the pressure fluctuations.

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Annual Change in the Declination ('Variation') in Western Scandinavia and the North Sea

THE annual change (secular variation) for about 10 years (1941–52) in the declination was remarkably constant, namely + 8′ (E.), for Norway, Denmark, Sweden and also for the North Sea (Lerwick, Shetland). However, starting in 1952, there has been a gradual decrease in the annual change, and for the period 1952–60 it was about + 5′ (dropping from 8′ to 4′). Fig. 1 gives the secular change for three magnetic observatories, Lerwick (Shetland), Rude Skov (Denmark) and Tromsø (Norway). For Rude Skov it may be seen that there is a gradual decrease, disregarding scatter, from 11′ to 4′ during the period 1927–60, apart from the temporary stagnation mentioned here. The same tendency may be shown for

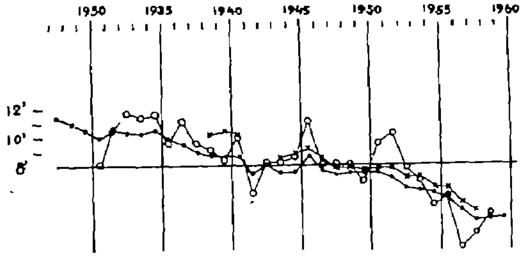

Fig. 1. Annual secular variation in declination for the observatories in Lerwick (Shetland) (×), Rude Skov (Denmark) (●) and Tromsø (Norway) (○)

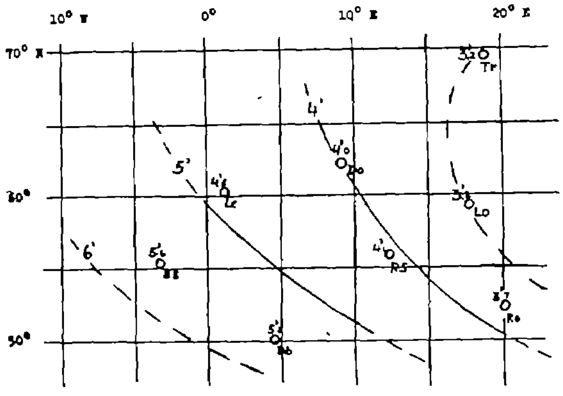

Fig. 2. Secular change in declination 1957/58 for eight north European observatories

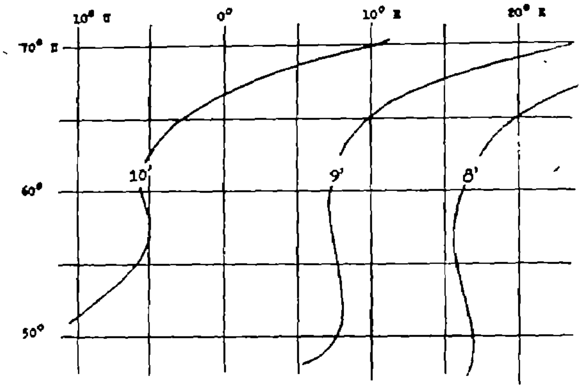

Fig. 3. Secular variation in declination epoke 1942.5 for north Europe (after ref. 1). The curves represent smoothed values

other north European observatories. The rough sketch in Fig. 2 shows the secular variation in declination for 1957/58 for eight north European observatories. Comparing Fig. 2 with Fig. 3, which gives the isopors (lines of equal annual secular variation) for epoke 1942.5[1], it can be seen that the pattern of isopors is still moving westward.

We are directing attention to the change in the secular variation in the declination because we have found that many sea maps west and south of Norway from various publishers have not brought the secular variation up to date, but operate still with the old value of + 8′.

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PHYSICS

A Characteristic of Electron Emission Curves

DURING an investigation of the breakdown mechanism in vacuum, the pre-breakdown currents arising from copper and steel electrodes under alternating voltages were measured. The general form of the apparatus has already been described[1]. The total current, capacitive plus emission current, was measured by noting the voltage across a resistor of known value in the earth connexion of the low-voltage electrode. Once emission started, certain peaks of the voltage across the resistor increased in magnitude. The value of the emission current was found by subtracting the estimated capacitive current from the total current obtained from these peaks.

The electrodes were polished with fine emery cloth and washed with ether. Thus the surface of the electrodes was rather rough and heavily oxidized. Measurements were made over a gap-range of 0·5 mm to 2 mm and a 'stress'-range of 2×10^{5} to $4{\cdot}3 \times 10^{5}$ V/cm, where stress is taken simply as applied voltage divided by electrode separation.

The exact mechanism of electron emission from such surfaces at room temperatures and at these stresses is not clearly understood. Some investigators support either a Schottky type emission or a Fowler–Nordheim type emission, or modifications of these[2–5].

Table 1. ESTIMATED CONSTANTS FROM FOWLER–NORDHEIM PLOT FOR 1-MM COPPER ELECTRODE GAP

Stress range (V/cm)	Estimated work function (eV)	Estimated emission area (cm²)
2×10^{5}–3×10^{5}	0·12	$2{\cdot}9 \times 10^{-8}$
3×10^{5}–5×10^{5}	0·03	$8{\cdot}3 \times 10^{-11}$

When the present results were plotted in either Schottky or Fowler–Nordheim form straight-line relationships were obtained, but in each case, as shown in Figs. 1 and 2, a sharp bend occurred in the curves. The bends arose in a roughly constant stress region between $2{\cdot}3 \times 10^{5}$ V/cm and 3×10^{5} V/cm independent of material. The magnitude of the currents was, however, strongly dependent on material. Table 1 shows the estimated work functions and emitting areas, for copper electrodes and a 1-mm gap, based on a Fowler–Nordheim plot, assuming no intensification of the field at the surface.

Nicholas[6] and Watts[7] have investigated a similar effect in the characteristics of emission from electrodes in gases. Critical stresses reported by Nicholas were $2{\cdot}5 \times 10^{5}$ V/cm for a nickel cathode and $2{\cdot}2 \times 10^{6}$

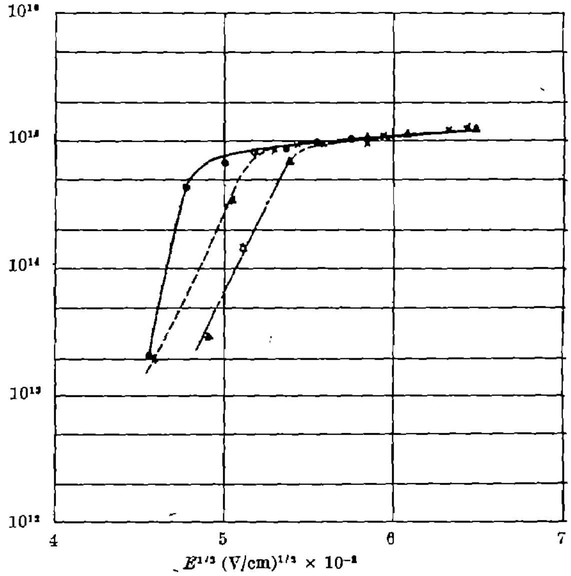

Fig. 1. Emission currents from copper electrodes Schottky plot ——, 2-mm gap, – – –, 1-mm gap, —·—, 0·5-mm gap

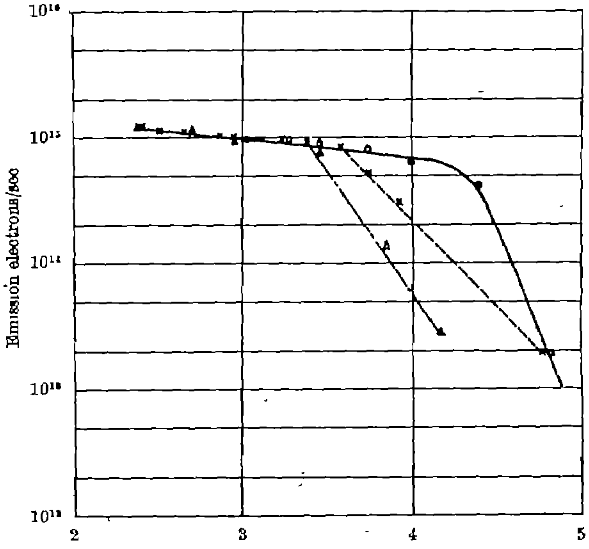

Fig. 2 Emission currents from copper electrodes. Fowler–Nordheim plot. ○, 2-mm gap; ×, 1-mm. gap; △, 0·5-mm gap

V/cm for a gold cathode; temperature dependence of the emission has also been investigated[8].

I thank the director of research of C. A. Parsons and Co., Ltd., for permission to publish this communication and Prof. F. Llewellyn-Jones, University of Wales, for permission to make use of results obtained in his laboratories.

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Measurement of Relaxation Times of Macromolecules by the Kerr Effect

Information on macromolecules may conveniently be found by application of the Kerr electro-optic effect. When an electric field in the form of a pulse of rectangular shape is applied to a solution the molecules will attempt to line up to a state of minimum potential energy during the rise period of the pulse. Once lined up they will remain stationary in alignment when the field amplitude is constant and then become disorientated by Brownian motion when the field ceases. If the orientation is sufficient the solution will exhibit birefringence, but since large molecules will take time to come into alignment and to return to a state of random array the rise and decay of the birefringence will lag behind that of the applied field. In general, therefore, a gradual increase of birefringence occurs, which may or may not reach a steady value according to the duration of the pulse, followed by a decay. Observations of the rise can give information on dipole moments and the steady value can be related to the Kerr constant and to molecular anisotropy. The decay for a solution of k types of molecules occurs according to the equation[1]:

$$\Delta n = \sum_{1}^{k} \delta n_i e^{-t/\tau_i}$$

in which Δn is the birefringence at any time t, δn_i is the maximum birefringence shown by molecules of type i and τ_i is a relaxation time which is a measure of the time the molecule takes to depart from its position of minimum potential energy. If it be assumed that the effect of the field is to produce rotation of the entire molecule then the polar or non-polar nature of the molecule is made manifest by the symmetry or asymmetry of the birefringence rise and fall, and τ_i can be related[1] to the rotary diffusion constant D_i by the equation $\tau_i = 1/6D_i$. Values of relaxation times and diffusion constants obtained in this laboratory are reported here.

The experimental arrangement used is as follows: a collimated beam of light is linearly polarized by a polarizing prism P, traverses the cell C containing the solution, a quarter wave plate Q and then passes through an analyser A on to a photon-multiplier PM. P and A are crossed, and the direction of the field is at 45° to the vibration direction of P and A. The azimuth of Q is zero with respect to the vibration direction of P. The cell is of metal lined with 'Teflon' and contains two parallel stainless steel electrodes centrally placed at a distance 0·25 cm apart. The length of path between the electrodes is 2 cm. Rectangular pulses of duration 1·7 μsec with a rise and decay-time of 2×10^{-8} and 3×10^{-8} sec respectively, and having amplitudes variable up to 8 kV, are applied to the electrodes. The pulses are generated by a hydrogen thyratron and delay line assembly. When the liquid becomes birefringent light passes through A on to PM and the photon-multiplier output is displayed on a cathode ray oscillograph able to show fast rise times. To increase the sensitivity the linear detection method described by Badoz[2] has been used. For this purpose a second Kerr cell filled with nitro-benzene to which a constant potential is applied is inserted between P and C. The apparatus is suitable for investigation of molecules having relaxation times of the order of 5×10^{-8} sec and greater.

The shape of the applied electrical pulse is shown in Fig. 1*a*; it is inverted simply because it was easier to obtain a good photographic trace this way. Representative traces obtained with birefringent liquids are given in Figs. 1*b*, 1*c* and 1*d*. Traces obtained with glycine, which has a relaxation time of about 10^{-11} sec (ref. 3), were almost identical with that of Fig. 1*a*, which showed that the decay inherent in the apparatus was negligible compared with that shown by the solutions examined. Measurements taken off the traces using a double traverse microscope enabled graphs of log Δn against t to be plotted. When excessive noise

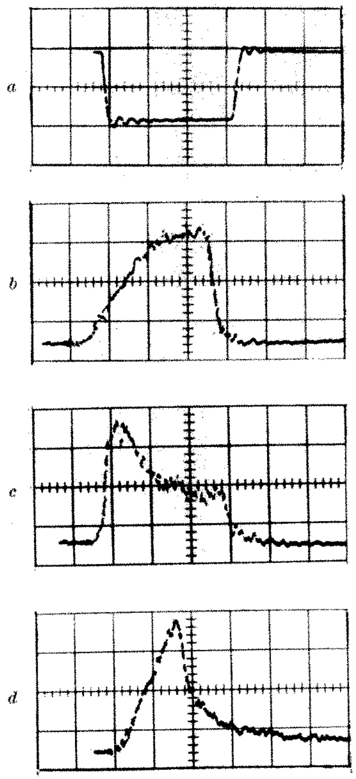

Fig. 1. (*a*) Applied electrical pulse 6 kV (shown inverted). Photomultiplier output pulses for (*b*) bovine γ-globulin, (*c*) β-lactoglobulin, (*d*) carboxymethylcellulose. Each division of time-scale represents 0·5 μsec in (*a*), (*b*) and (*c*) and 1·0 μsec in (*d*)

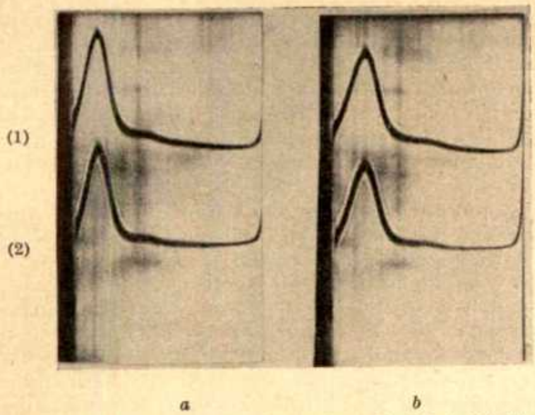

Fig. 2. Schlieren ultra-centrifuge patterns for β-lactoglobulin at 290,000*g* after (*a*) 26 and (*b*) 34 min. Schlieren angle, 55°. Concentrations (1) 1 per cent; (2) 0·89 per cent by weight. Temp., 23° C.

was present smooth curves were drawn through the traces to average out the noise before taking measurements. From the graphs relaxation times were found. The method quickly enables reliable results to be obtained and these are given in Table 1 for a number of substances. For most solutions two relaxation times were found which may be due to the presence of two components. Velocity sedimentation runs using a Spinco model *E* analytical ultra-centrifuge were made to check this. An example is shown in Fig. 2 for β-lactoglobulin in which the presence of two components is indicated.

Table 1. RELAXATION TIMES τ AND ROTARY DIFFUSION CONSTANTS D

	Solute	Concentration (per cent wt.) and temperature (° C.)	τ (10^{-8} sec)	D (10^{3} sec^{-1})
1	Carboxymethyl-cellulose	0·02 (23°)	146; 24	114; 694
2	β-Lactoglobulin (cows' milk)	1·0 (22°)	35; 7·6	476; 2,193
3	Ribonucleic acid (sodium salt, yeast)	1·00 (25°)	< 10	> 1,667
4	Ribonucleic acid (sodium salt, yeast)	1·00 (25°)	45; 16·5	370; 1,010
5	Bovine plasma albumin	1·0 (23°)	12·2	1,366
6	Bovine-γ-globulin (fraction II)	1·0 (23°)	21·5	775
7	Sodium alginate	0·02 (20°)	86; 32	194; 521
8	Polymethacrylic acid (sodium salt)	0·02 (23°)	66; 22	253; 758
9	Sodium polyphosphate	— (20°)	40; 100; 284	417; 167; 58·7
10	Deoxyribonucleic acid (calf thymus)	0·01 (25°)	3,460; 20,000	4·82; 0·833

Water was the solvent in all cases except No. 4, which was in 50 per cent water/glycerol. Concentrations of 2 and 6 were taken before centrifuging at 3,000*g* for 15 min. Nos. 1–4 were supplied by Light and Co., Nos. 5 and 6 by Armour Pharmaceutical Co. No. 7 was a commercial sample of 'Manucol' obtained from Alginate Industries. Nos. 8–10 were supplied by Dr. K. A. Stacey, Experimental Radiopathology Research Unit, Hammersmith Hospital. Nos. 5 and 6 appeared slightly disperse.

The method has also been used to investigate the influence of *p*H, of variations in ionic strength, concentration and temperature and the effects of different solvents with the view of obtaining information on mechanisms responsible for dielectric polarization in macromolecular solutions[4]. Studies of the effect of ribonuclease enzyme on ribonucleic acid (RNA) have also been performed and preliminary results indicate that the action of the enzyme in breaking down the RNA can be accurately and quickly followed by this method. It is hoped that detailed results will be published later.

One of us (P. I.) thanks the Department of Scientific and Industrial Research for the award of a postgraduate research studentship. The centrifuge used was purchased by a D.S.I.R. grant for special researches.

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CRYSTALLOGRAPHY

Sucrose Crystallization

FURTHER investigations of the crystallization of sucrose have continued to reveal items of general crystallographic interest[1].

The effect on crystal form of an increasing degree of supersaturation was dealt with in the last report of this series. Spherulitic and radialitic nucleation and growth were recorded as characteristic of supersaturations of 3·0 and greater. Both nucleation incidence and growth-rate appear to peak at about 2·5 supersaturations, thereafter falling off, until at really high supersaturation the near amorphous system may take years to crystallize. Evidence now justifies recording that at intermediately high supersaturation a new type of nucleation and growth-form is developed. Instead of the normally large number of component ray crystals produced at supersaturations 3·0 and 4·0, at this much higher concentration the crystal system produced appears to be relatively simple, being composed of several rays, or, even of one crystal only, as revealed by rotation under the polarized light microscope. Growth is very slow and the contours of the crystals are generally somewhat curved[2] as compared with normal rectilineal contours of monocrystals grown in the region of 1·1 supersaturations. Dendritic growth may also develop if given sufficient time, and this again appears different in style when compared with that produced at the lower range of supersaturation.

I have found that hopper crystals frequently provide an excellent natural record of habit modification during growth. A ¼-in. layer of pure sucrose

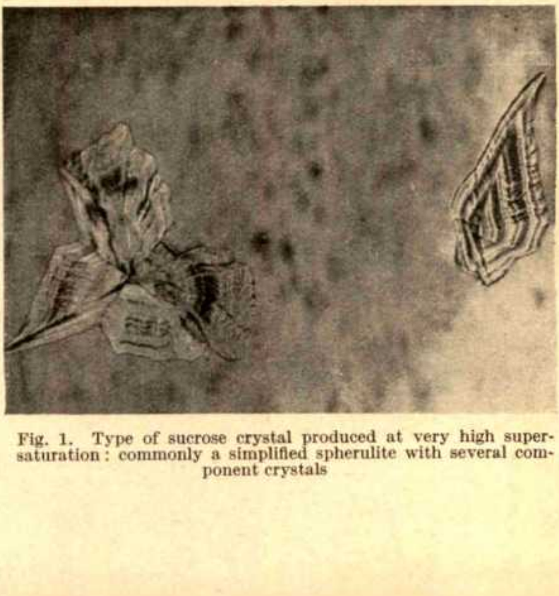

Fig. 1. Type of sucrose crystal produced at very high supersaturation: commonly a simplified spherulite with several component crystals

solution in a covered Petri dish, stored for months in a normal air-conditioned room, will slowly crystallize to dryness, some hopper crystals frequently being present. Examples of the development of needle to rectilineal, also of triangular to hexagonal contours have been so recorded, and one micrograph records the initial development followed by the elimination of a subsidiary face during the growth of such a hopper crystal.

The sucrose crystal exhibits many different forms of layer growth[3]. We now know that inclusions abound in sucrose crystals, of dimension from molecular to macroscopic. As a result, there is so intense a distribution of faults in the development of the lattice as to generate some form of layer growth continuously. It seemed to me probable that long-continued uniform growth might be expected to favour the perpetuation of growth spirals. This regular growth may easily be achieved by means of a humidator with vapour pressure control, as described in my previous report. Such a system was therefore set up, using as seed several small clean cleaved sections from large Kandi crystals. This was permitted to develop without disturbance for four months in a constant temperature room. At the end of this period the humidator was opened up and the crystals, which had grown to an average size of one inch in length, were seen to have developed in most cases, one large spiral covering the whole 100 face. Under suitable lighting conditions, this spiral could easily be seen with the unaided human eye.

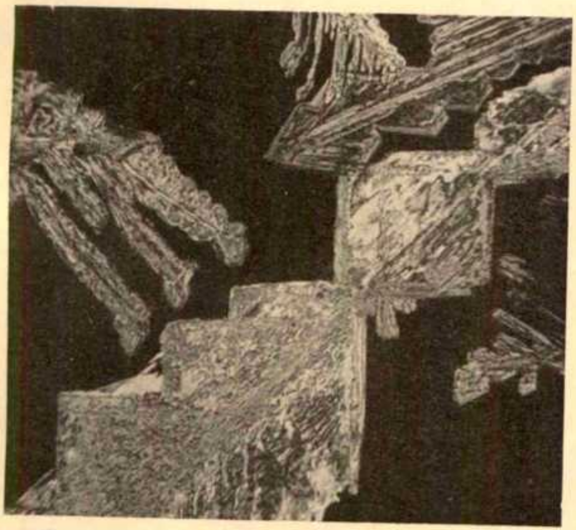

Fig. 2. Dendritic growth under same conditions as Fig. 1

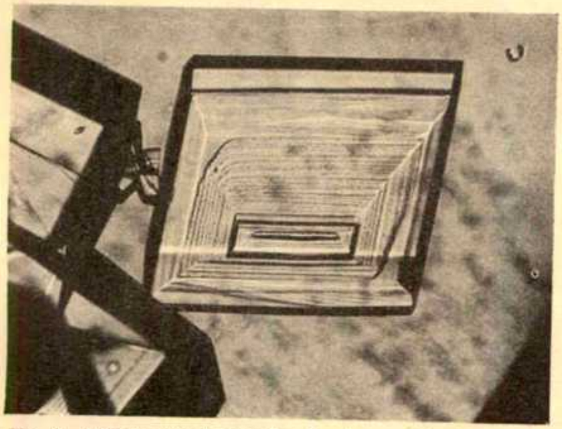

Fig. 3. Hopper crystal recording development from needle to rectilineal contour

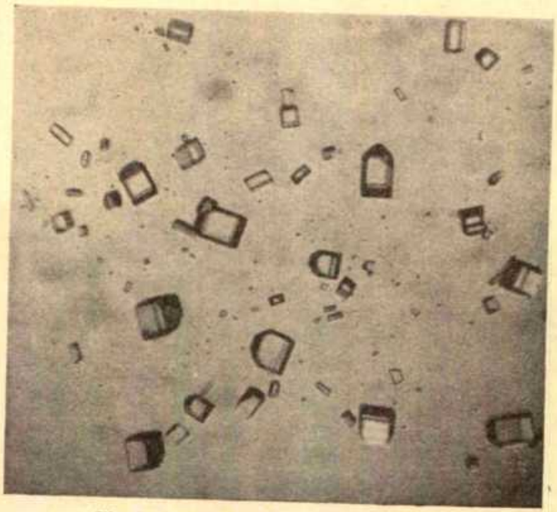

Fig. 4. Result of differential rate of growth

In previous published reports[4] I have given evidence of the differential growth-rate of sucrose crystals growing freely in the same system. This would be a logical and predictable result consequent on: (*a*) the increasing solubility of micro-crystals with decreasing size, also of (*b*) the varied efficiency of the various growth mechanisms. Cumulative evidence appears to indicate that the former is significant up to a crystal length of 10μ or thereabouts. This may be demonstrated prettily by introducing fondant seed crystals of up to two or three microns length in solutions of very low supersaturation, when some, mainly the larger crystals, will grow with increasing speed, while others remain static in size. In an accurately temperature-controlled system one should be able to assess the solubility at various sizes within the range of size visible under the microscope. This has been recorded much more informatively by means of time-lapse cine-micrography, but the final stage of one such growth run is shown in Fig. 4.

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CHEMISTRY

Fluorescence of Some Substituted Benzenes

Some mono- and di-substituted benzenes fluoresce in aqueous solution. Phenol, for example, fluoresces in the ultra-violet and the salicylate ion in the visible region of the spectrum. Williams[1] pointed out that the fluorescent mono-substituted benzenes were those containing the classical *ortho-para*-directing groups. We have now examined this phenomenon more closely and also determined the quantum efficiency of fluorescence using a commercial Aminco-Bowman spectrophotofluorimeter.

The compounds examined were dissolved in water using a little ethanol to assist solution where necessary, and measurements of fluorescence were made at a concentration of 1 μg/ml. Corrected data were obtained as follows. The activation and fluorescence wave-length scales were corrected with a 'Pen Ray' mercury lamp as recommended by the manufacturers of the instrument[2]. The activation system of the fluorimeter, which consists of a high-pressure Osram 150-W xenon lamp, diffraction grating, and mirrors was calibrated from 230–420 mμ by Parker's potassium ferric oxalate actinometer method[3]. The fluorescence system, consisting of diffraction grating, mirrors, and an RCA *IP* 28 photomultiplier tube, was calibrated between 250 and 420 mμ by reflecting light from the calibrated activation system into the fluorescence system by means of a Hilger–Watt front-silvered, optically flat mirror (1 cm²) placed at 45° in the cell compartment and measuring the photomultiplier response to this light every 10 mμ on the fluorescence monochromator. Between 380 and 600 mμ the fluorescence system was calibrated using a modification of White's method[4] which involves using a lamp of known colour temperature. Quantum efficiencies (Q.E.) were calculated using the comparative method of Parker and Rees[5]. The calibration was checked against compounds of known quantum efficiency using quinine bisulphate in 0·1 N sulphuric acid (1 μg/ml.) as standard (Q.E. 55 per cent[6]). The following efficiencies were found: eosin in 0·1 N sodium hydroxide, 18·3 per cent (cf. Parker and Rees[5], who give 23 per cent), fluorescein in 0·1 N sodium hydroxide, 85 per cent (72 per cent[5]), rhodamine *B* in ethanol, 66·8 per cent (69 per cent[5]), and anthracene in ethanol, 36 per cent (28 per cent[5]).

The results for some mono-substituted benzenes are shown in Table 1, and it is clear that under our conditions only one type of mono-substituted benzene fluoresces. In this type of compound the substituent group contains an unshared pair of electrons on the atom adjacent to the ring and the electronic mechanism is $\overset{\curvearrowright}{Ph\longrightarrow R}$, the inductive effect being negative ($-I$) and the mesomeric effect being positive ($+M$). However, not all compounds of this type showed fluorescence, for chlorobenzene, bromobenzene and acetanilide appeared to be non-fluorescent. This suggests that the occurrence of fluorescence depends on mesomerism and the consequent conjugation of the group with the benzene ring. In the fluorescent compounds, for example, phenol, aniline and fluorobenzene, the mesomeric effect is known to be more dominant than in a compound such as chlorobenzene (for example, ref. 7). The dominance of the mesomeric effect is reflected in the value of the extinction coefficient (ε_{max}) of the benzenoid absorption band of these compounds[8] (Table 1).

Table 1 also shows that mono-substituted benzenes of other electronic configurations are non-fluorescent. Those containing *meta*-directing groups and where *M* is negative are non-fluorescent, as are also those where the electronic configuration is influenced by the inductive effect only. The phenoxide ion has the configuration $+I$ $+M$, but is non-fluorescent. It is possible that in this case the π-electrons, which are responsible for fluorescence, are too strongly localized to allow fluorescence.

Table 1 also shows that the quantum efficiency of the fluorescent compounds is low and is less than 10 per cent. The most efficient compound is *N,N*-dimethylaniline (Q.E. 9·7 per cent) and the least is fluorobenzene (Q.E. 0·7 per cent). The quantum efficiencies quoted can be roughly correlated with the known strength of the mesomeric effect.

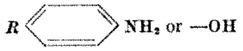
Table 1. FLUORESCENCE OF MONO-SUBSTITUTED BENZENES, C_6H_5R
Concentration used was 1 μg/ml. in water
Nitrogen was bubbled through the solution for 15 min before fluorescence was measured

R	Benzenoid absorption $\lambda_{max.}$ (mμ)	$\varepsilon_{max.}$	Excitation max (mμ) obs. (Electronic configuration, $-I + M$)	Excitation max (mμ) corr.	Fluorescence max (mμ) obs.	Fluorescence max (mμ) corr.	Quantum efficiency (%)
(Quinine)							(55·0)[6]
OH	270	1450	282	270	330	330	3·2
OCH_3	269	1480	279	270	302	303	3·4
NH_2	280	1430	290	280	345	350	2·5
$NHCH_3$	288	1259	290		360		
$N(CH_3)_2$	291	1259	297	286	363	365	9·7
F	259	516	265	257	285	289	0·7
Cl	263	190	Non-fluorescent				
Br	261	190					
I	no benzenoid band (ref. 9)						
$NHCOCH_3$	no benzenoid band						

R	Non-fluorescent compounds: Electronic configuration	*R*	Electronic configuration
COOH NO_2 SO_2NH_2 CHO AsO_3H_2	$-I$ $-M$	$\overset{+}{N}H_3$ $\overset{+}{N}(CH_3)_3$	$-I$
		CH_3	$+I$
COO^-	$+I$ $-M$	O^-	$+I$ $+M$

In Table 2 a list is given of the fluorescence of some *p*-substituted anilines and phenols. Referring to quantum efficiencies, the *p*-F group considerably enhances the fluorescence of aniline, but has little effect on that of phenol. The *p*-chloro group diminishes the fluorescence of both aniline and phenol, whereas *p*-bromoaniline and *p*-bromophenol are non-fluorescent. The weak *m*-directing groups, SO_2NH_2 and SO_3^-, increase the fluorescence of aniline, while the strongly *m*-directing NO_2 group abolishes the fluorescence. *p*-Methyl and *p*-methoxy groups both increase the fluorescence of aniline and phenol. *p*-Aminoacetanilide shows a very weak fluorescence. It is probable that the fluorescence of di-substituted benzenes depends on the resultant effect of both groups (which may oppose or enhance each other)

Table 2. FLUORESCENCE OF *p*-DISUBSTITUTED BENZENES IN WATER
(Concentration 1 μg/ml.; solution de-oxygenated as in Table 1)



R	Absorption (mμ) λmax.	Excitation max. (mμ) obs.	Excitation max. (mμ) corr.	Fluorescence max. (mμ) obs.	Fluorescence max. (mμ) corr.	Quantum efficiency (%)
		Anilines				
(H)						(2·5)
F	287·5	300	289	360	362	12·3
SO_3^-	249	270	254	350	352	5·0
NH_2SO_2*	258	278	259	350	352	4·2
OCH_3†	296	310	297·5	370	375	4·2
CH_3†	286	300	289	355	357	2·8
Cl	290	300	290·5	360	362	1·7
$NHCOCH_3$	259	300	290	350	352	0·018
Br	290	Non-fluorescent				
NO_2	381					
		Phenols				
(H)						(3·2)
CH_3	279	287	278·5	312	313	8·8
OCH_3	288	298	289·5	329	328	5·9
F	277·5	288	276	315	315	3·0
Cl	279·5	292	280	317	317	0·89
Br	280	Non-fluorescent				
NO_2	317·5					

* Sulphanilamide undergoes rapid photo-decomposition.

† The fluorescence of *p*-anisidine and *p*-toluidine were markedly affected by temperature. At 8° C., the fluorescence intensity of these two compounds is about twice that at 28°.

on the extent of delocalization of π-electrons of the benzene ring. The overall qualitative picture suggests that the more delocalization there is, the more fluorescent the compound is likely to be. On these grounds we have been able to predict whether a *p*-substituted benzene would fluoresce. Thus one could predict that a *p*-substituted aniline or phenol containing a weak *m*-directing group such as SO_3^-, SO_2NH_2, and AsO_3H_2 and its ionized forms would fluoresce; in fact, the fluorescence of *p*-arsanilic acid increases with each step in the ionization of the AsO_3H_2 group. Bromo- and nitro-groups abolish, whereas chloro- and acetamido-groups diminish, the fluorescence of aniline and phenol. Fluoro, methyl and methoxy groups usually enhance or do not alter, rather than diminish, the fluorescence. Other compounds have been studied, but the results will be reported elsewhere.

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Paper Chromatography of 2-Halogenated Carboxylic Acids : *N*,*N*-Dimethyl-*p*-phenylazoaniline as an Acid-Base Indicator Reagent

IN work directed toward the separation of several volatile 2-halogenated carboxylic acids by paper chromatography, it was found desirable to develop these acids, not, as is usually the case, in the form of their salts[1], but as the free acids. The so-called 'swamp acid' technique[2] used for this purpose simply involves the use of a relatively large quantity of some acid as part of the solvent system, usually acetic or formic. This acid is necessary to repress ionization of the unknown acid, which would otherwise give rise to 'tails' or 'ghosts'. However, it soon became clear in working with the 2-halogenated acids that removal of the 'swamp acid' preparatory to spraying with the more common acid-base indicators also removed the volatile unknown acids. Therefore, some scheme had to be found for locating the unknowns in the presence of the 'swamp acid'.

It was found that water solutions of these 2-halogenated acids had a lower *p*H than a solution of acetic (or formic) acid in water. In addition, it was found that the dye, *N*, *N*-dimethyl-*p*-phenylazoaniline, changes from yellow to red at a *p*H of 2·9. This change is just right so as to indicate these acids and not acetic or formic. Therefore, if this indicator is used it is unnecessary to remove the 'swamp acid' since its presence does not interfere with the detection of the unknown acids.

It has been established that the following acids can be detected with this indicator in the presence of acetic acid: chloroacetic, trichloroacetic, 2-chloropropionic, 2,2-dichloropropionic, 2,2,3-trichloropropionic, 2-chlorobutyric, 2,2-dichlorobutyric, 2,3-dichlorobutyric, and 2-bromoacetic acids. The lower limit of detection in the case of 2-bromoacetic acid was 3 μg.

The procedure for detection of unknown acids using this spray reagent is as follows: After development of the chromatogram, the acids were located by spraying (without drying) with a 0·1 per cent solution of *N*,*N*-dimethyl-*p*-phenylazoaniline in 90–95 per cent isopropyl alcohol. The location of the spots is shown by a red-purple colour, stable for long periods of time, on a bright yellow background. If the spot does not appear immediately on spraying, the paper can be carefully dried in a stream of cold air until the spot shows up.

This technique, that is, spraying the indicator solution on top of the 'swamp acid' to give a satisfactory definition of the volatile acid in question, should be of quite general application whenever there is a measurable *p*H difference between the 'swamp acid' and the unknown acid, and the proper indicator can be found to take advantage of this difference.

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An Application of Paper Electrophoresis to the Examination of Completeness of Reaction and Purification in the Synthesis of Radioactive Compounds

THE usefulness of [(carboxymethylimino)*bis*(ethylenenitrilo)]tetraacetic acid (DTPA) in correcting iron chlorosis induced by lime in plants has been well documented[1,2]. DTPA labelled with carbon-14 was required to facilitate studies concerning the metabolism of the compound in plants; accordingly, the synthesis was undertaken. The method used involved an alkylation of diethylenetriamine (DET) with bromoacetic acid. In preliminary work, it became evident that the usual methods available for determining chelating materials, such as titration with calcium ion in the presence of oxalate or sprays with a nickel-dimethylglyoxime reagent, would not be satisfactory for following the course of the reaction. These reagents will neither indicate the distribution nor detect all the compounds arising as a result of incomplete alkylation. Paper electrophoresis was found to be a very powerful tool for the delineation of all the compounds formed in the reaction.

Bromoacetic-2-^{14}C acid (0·296 mmole, specific activity 1·69 mc./mmole) in benzene (1·6 ml.) in a 3-ml. test tube at 5° was treated with 96 μl. of 6·28 N sodium hydroxide (0·60 mmole). The addition was made in three portions with stirring. DET (6·18 mg, 0·06 mmole) in water (35 μl.) was then added with stirring to this cold solution. The reaction mixture was allowed to stand at room temperature for 4·5 h with occasional stirring. At the end of this time, one drop of an ethanolic solution of 'Eriochrome Black *T*' indicator (*Color Index* No. 203) was added and the reaction mixture was made slightly acid with 0·80 N hydrochloric acid (0·20 ml.). This indicator and its use in 'Versene'(R)–metal titrations are described[3]. The aqueous phase of the acidified reaction mixture was separated from the benzene and made up to 5·00 ml. with water washings.

One µl. of the diluted reaction mixture was submitted to paper electrophoresis on Whatman No. 3 paper. The conditions of this and all subsequent electrophoresis runs were as follows: electrolyte, 0·025 M phthalate buffer, *p*H 5·9 (ref. 4); ambient temperature; 13 V/cm; time, 4 h. The apparatus used was of the closed strip type as described by Block *et al.*[5]. Autoradiographs were prepared using 'No-screen' X-ray film. The delineated spots were quantitized by counting with an end-window Geiger tube.

The apparent electrophoretic mobilities of the compounds resulting from the reaction of DET with bromoacetate are shown in Table 1. Under the conditions of the experiment, the separation of all the components was remarkably clear cut.

Table 1. APPARENT ELECTROPHORETIC MOBILITY OF COMPOUNDS RESULTING FROM THE REACTION OF DIETHYLENE-TRIAMINE WITH BROMOACETATE

Compound*	Mobility, cm (±)
DTPA	12 5–16·0 (+)
DTTA	10·8 (+)
DTTriA	0
DTDA	3·6–6·8 (—)
DTMA	10·0 (—)
Glycolate	5·2–8·2 (+)
Bromoacetate	0 5–1·3 (—)

* DTTA, diethylenetriaminetetraacetate; DTTriA, triacetate; DTDA, diacetate; DTMA, monoacetate.

The quantitative distribution of radioactive products formed in the first reaction is given in Table 2.

Table 2. QUANTITATIVE DISTRIBUTION OF RADIOACTIVE COMPOUNDS RESULTING FROM THE REACTION OF DIETHYLENETRIAMINE WITH BROMOACETATE

	First reaction			Second reaction	
Compound*	Per cent of total activity on electrophoretogram	Per cent No. of CO_2H	mmoles present	Per cent of total activity on electrophoretogram	mmoles present
DTPA	18·0	3 6	0·011	76·0	0·051
DTTA	2·4	0 6	0 002	12·7	0·009
DTTriA	5·0	1·7	0·005	0	0
DTDA	6·5	3·3	0·010	0	0
DTMA	10·8	10·8	0·032	0	0
Glycolate	4·0	4·0	0·012	11·3	0·038
Bromoacetate	53·3	53·3	0·160	trace	trace

*See Table 1.

Because of the incomplete conversion of reactants to DTPA, a second reaction was carried out using inactive bromoacetate (0·6 mmole) and the crude products from the first reaction; this was allowed to stand at room temperature for 16 hr. The unreacted bromoacetic acid was removed by ether extraction. After electrophoresis, the quantitative distribution of products was found to be as shown in Table 2.

DTPA-^{14}C was obtained in 96 per cent purity (determined by electrophoresis) by chromatography on 'Dowex-50' using water for elution. The specific activity was 4·8 mc./mmole.

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Self-sustained Discharge Detector for Gas Chromatography

IN a recent communication to *Nature*, Drs. Evrard, Thevelin and Joossens[1] described their experience with a self-sustained discharge detector for chromatographic analysis of permanent gases. Argon containing 200 p.p.m. of ethylene was used as carrier gas, a mixture similar to that described elsewhere by Willis[2] for detecting atmospheric gases with an ionization detector containing a radioactive source. In both these methods, permanent gases quench the current flowing in the detector.

In their summary of the literature concerning the use of non-radioactive, electric discharge detectors, Evrard *et al.* state the belief that a concentration of organic vapour must be present if a self-sustained discharge is to be excited in argon. This is contrary to our experience.

In work reported by us previously[3], a stable, self-sustained discharge was excited in both argon and helium without the intentional addition of any contaminants. As would be expected, the voltage across an argon discharge was higher than the 500 V reported by Evrard *et al.* for the argon–ethylene mixture. The voltage varied between 1,600 and 1,900 V, depending on cell geometry, temperature and pressure, and fell to minimum levels of 500 V in the presence of organic vapour. The sensitivity of this discharge to organic vapours suggested its use as a detector for gas-liquid chromatography and it was so used with both polyester columns and silicone rubber columns at high temperatures and with silicone columns at room temperature, all with good effect. An analysis of fatty acid esters using a self-sustained argon discharge detector is shown in Fig. 1.

The argon used in these experiments was sufficiently free of organic contaminants to give a very low baseline current when used as carrier gas with a hydrogen flame ionization detector. While it may be argued that the gas-liquid column, even at ambient temperature, may provide a low concentration of organic vapours, the same argument cannot apply when well-conditioned gas-solid columns are used.

We have reported the sensitive detection of atmospheric gases in a self-sustained helium discharge[4]. Addition of small concentrations of each of the atmospheric gases caused an increase in the conductivity of this discharge. Wiseman, using a slightly different experimental approach, stressed the importance of purifying the helium prior to using it, but otherwise essentially confirmed our results[5].

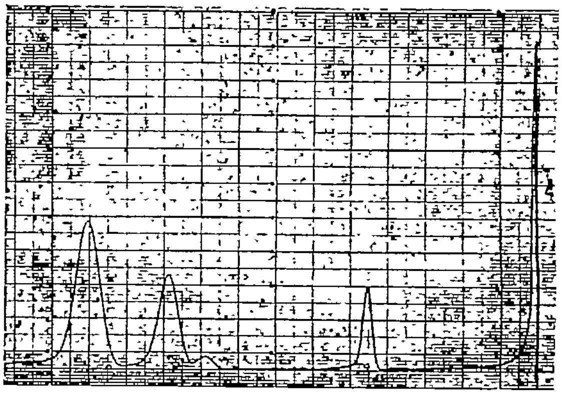

Fig. 1. Analysis of a mixture of methyl esters detected by an argon discharge. Total sample, 3 µg

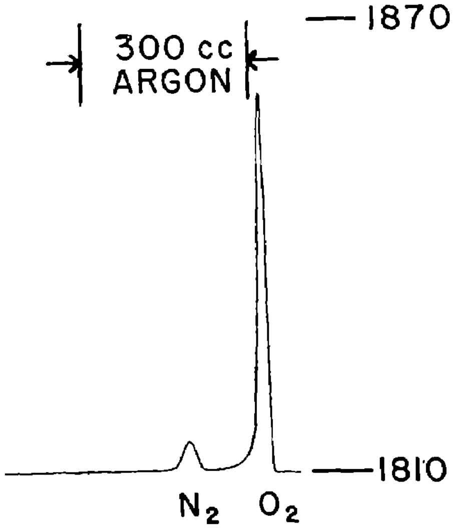

Fig. 2. Analysis of a 2 µl sample of air, analysed by a molecular sieve column, detected by an argon discharge

Since our experiments were performed using commercial helium without further purification, we interpreted our results as having been made possible by the care exercised to introduce as little atmospheric contamination to the gas stream as possible.

With the same equipment and the same care, we have also used argon as the carrier gas for the detection of atmospheric gases. Oxygen, nitrogen, and carbon dioxide each diminish the electrical conductivity of a self-sustained discharge in argon. The sensitivities of the discharge to carbon dioxide and oxygen are higher than that to nitrogen (Table 1) although not so high as the sensitivity to organic vapours. Fig. 2 shows the response of an argon discharge to a 2-µl. sample of air analysed by a molecular sieve column.

Table 1. SENSITIVITY OF THE ARGON DISCHARGE TO ATMOSPHERIC GASES

Gas	Sensitivity Increase in voltage across discharge for an increment in concentration of 1 vol. per million vol. argon
Oxygen	2·0
Nitrogen	0·06
Carbon dioxide	6·5

The high sensitivity of the argon discharge to oxygen in the absence of organic vapours suggests its potential utility for the detection of oxygen in argon–oxygen mixtures.

In both our previous reports on the use of the self-sustained discharge we described a mode of operation in which a constant current is passed through the discharge and the voltage across it measured. We have found this approach to be feasible whether the current is held constant within the limits of electronic regulation and the voltage across the discharge is measured directly or whether a large value resistor is used in series with the source of impressed voltage and the voltage across the discharge is indicated by the relatively limited changes in current. We prefer the first approach for two reasons:

(1) The voltage across a self-sustained discharge remains essentially constant in gas of constant composition despite moderate changes in current. The current, on the other hand, is a function of the series resistance as well as the impressed voltage. The voltage across the discharge thus serves as a better indicator of the gas composition.

(2) A self-sustained discharge, in our experience, is most stable over a limited range of current. It seemed, therefore, most desirable to keep the current at a value near the most stable part of that current range.

Constant current sources suitable for this purpose which provide a separate voltage signal proportional to the voltage across the discharge are now available commercially.

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Wetting Phenomena in Gas Chromatography Capillary Columns

IN some instances poor efficiency with capillary columns has been reported, especially when glass capillaries were used. This may be due to two quite different phenomena : bad liquid-phase distribution on the smooth wall of the capillary caused by non-uniformity of radius, or non-wetting properties of the stationary phase. Giddings[1] has theoretically discussed the first reason; but experiments on it are lacking. This communication is concerned with the second point only. If the liquid phase does not spread on the wall, it coalesces into droplets: the larger these droplets the lower the efficiency. They may even come to plug the column, resulting in a saw-tooth gas flow pattern. The wetting properties of the stationary phase thus appear to be very important.

We have measured the contact angle θ of several usual liquid phases on metal, glass and polymer materials often used to make capillaries[2]. By plotting cos θ for a given substrate against the surface tension γ of the corresponding liquids one can obtain a maximum value γ_c compatible with spreading on this solid[3]. We have thus a means of evaluating both liquids and solids for use in capillary columns. Values of γ_c are given in Table 1.

It must be pointed out that this critical tension may vary with temperature, so that for a given liquid–solid pair a critical temperature may exist above which θ is zero[4]. This may explain some discrepancies in reported observations. Others may result from:

(*a*) The solvent used to deposit the stationary liquid film in the capillary. The solution may fail to spread on the wall, and another solvent should be sought. It is important to know that poor efficiency may result from non-spreading of the solution, even in cases where the liquid phase itself is capable of

Table 1

Solid	γ_c (dyne/cm)	Solid	γ_c (dyne/cm)
Stainless steel	24·0 ± 0·5	Polytetrafluoro-ethylene	19 ± 1
Aluminium	27·4 ± 0·5	Polyethylene	33 ± 2
Copper	27·0 ± 0·5	Polyethyleneglycol-terephtalate	40 ± 2
'Pyrex' glass* acetone washed	28·0 ± 0·5	Polyamide 6–6	41 ± 2
'Pyrex' glass* sulphochromic acid washed	44·0 ± 1·0		

* Values for 'Pyrex' are reproducible for a given cleaning method.

spreading; droplets of solution left behind when the column is emptied do not distribute evenly in a short enough time.

(*b*) Surface-active agents which may reduce surface tension of the liquid phase and thus facilitate the spreading of the liquid. The favourable effect of products like 'Alkaterge *T*' reported by Averill[5] may be due partly to this phenomenon.

(*c*) In general, roughening the wall of the capillary will decrease the contact angle and may be used to advantage.

(*d*) On the contrary other surface-active agents may be adsorbed on the surface of the capillary and in some cases reduce the solid–liquid adhesion. Owing to the viscosity, the slowness of coalescence may, however, give time for desorption of the surface active agent. Compounds of this kind may be expected mainly from the sample under analysis and, in spite of the small quantity present, may reach a concentration large enough to produce a local disturbance of the liquid film, travelling with the solute; a monolayer may be sufficient. This may produce a gradual alteration of the column with consequent loss of efficiency.

Measurements of contact angle and surface tension were made in air by photographing the profile of sessile drops. The nature of the carrier gas is not likely to alter appreciably the value of the critical tension.

We are extending this study to temperatures often met with in gas chromatography (50°–150° C).

We thank Miss M. Raison for some confirmatory measurements made by the dipping-plate method.

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Unpaired Electron in Nitroso-*bis*(dimethyldithiocarbamato)iron(II)

We have examined the electron spin resonance spectrum of $[Fe(NO)\{S_2CN(CH_3)_2\}_2]$ to investigate how the unpaired electron, which is in an anti-bonding orbital in free nitric oxide, distributes itself in a metal complex. The magnetic moment of a powdered sample at room temperature is 2·24 B.M., which shows the complex to have one unpaired electron with an appreciable orbital magnetic moment. The electron spin resonance spectrum of a benzene solution consists of three equally intense lines with a separation of 0·0011 cm^{-1}. This triplet structure arises from an interaction between the unpaired electron and the nitrosyl nitrogen atom and indicates that the unpaired electron is in an orbital which embraces the nitrosyl group. This interaction is greater than that in free nitric oxide[1] in which the splitting is 0·00099 cm^{-1}.

Single crystals of this complex have also been studied by electron spin resonance and the dependence of the spectrum on the orientation of the crystal with respect to the field has been determined.

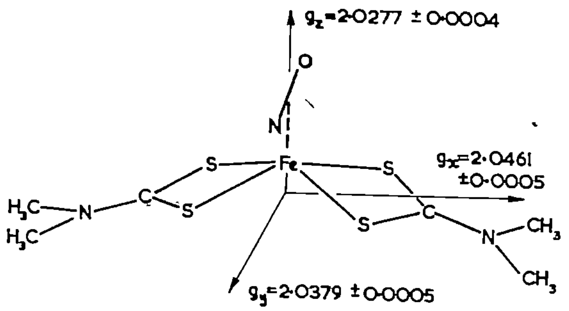

Fig. 1

Since the structure of the isomorphous $[Co(NO)\{S_2CN(CH_3)_2\}_2]$ is known[2] it is possible to determine from these results the directions of the principal *g* values with respect to the molecule. These are shown in Fig. 1. It is seen that the N—O direction is not important in determining the directions of the principal *g* values, as it would be if the unpaired electron were associated only with the nitric oxide ligand[3]. Instead the principal *g* directions are determined by the ligand field at the iron atom produced by the dithiocarbamate groups.

Our results indicate, therefore, that the unpaired electron is not entirely on the nitrosyl group, but is in a molecular orbital which includes both the iron atom and the nitrogen atom of the nitrosyl group, and which has the symmetry of the environment of the iron atom. Further experiments with more sensitive apparatus are planned to investigate whether the unpaired electron is also associated with the sulphur atoms.

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BIOCHEMISTRY

Chemistry of a Case of Juvenile Amaurotic Idiocy

Chemical analysis which would give a hint of the nature of juvenile amaurotic family idiocy is still lacking. The condition is considered to be a metabolic disorder because of the abnormal intracellular deposits in the ganglion cells. Klenk[1] found no increase in the total amount of gangliosides; but Cumings[2] reported an increase in the neuraminic acid level in the cortex in adult cases of amaurotic family idiocy. Tingey[3], in his routine carbohydrate analysis of the brain of the present case (a 15-year-old boy), found no increase in neuraminic acid and no significant abnormality. The present investigation was conducted primarily to examine the nature of the glycolipids and polysaccharides present both in the brain and the liver.

The method used to fractionate the liver was a modification of that previously used[4] in an investigation of glycogen storage disease. In the present case extraction with M sodium chloride and cold N sodium hydroxide failed to extract any detectable glycogen. The very small amount present was presumably protein-bound and required hot 30 per cent potassium hydroxide for its extraction (cf. with normal liver[5]).

It was significant that no storage of uronic acid containing mucopolysaccharides was encountered in the liver. In fact, except where the 30 per cent potassium hydroxide procedure[6] was applied directly to the defatted tissue, no uronic acid was found in any polysaccharide fraction. In that instance the uronic acid was accompanied by glucose, mannose, galactose, and hexosamine in the laevorotatory (– 40°) polysaccharide mixture.

The major carbohydrate-containing macromolecules isolated were: (*a*) a mucoprotein $[\alpha]_D$ – 53° containing 23·5 per cent hexoses (galactose, mannose), hexosamine and 16 amino-acids; (*b*) a neutral mucopolysaccharide containing 44·3 per cent hexoses (glucose, galactose, mannose) and galactosamine; (*c*) an acidic polymeric carbohydrate sulphate $[\alpha]_D$ + 42° containing 26·9 per cent galactose and hexosamine; (*d*) an acidic polymeric carbohydrate sulphate $[\alpha]_D$ – 47° containing 10·2 per cent hexoses (glucose, galactose, mannose) and hexosamine.

The fractionation of both the grey and white matter of the brain was carried out by identical procedures. The acetone-dried tissues[7] were extracted with chloroform-methanol[8] and the crude lipids extracted, partitioned with water to separate out in the water phase a soluble non-dialysable fraction which consisted of those mucolipids of the ganglioside type which contained neuraminic acid. The chloroform phase, constituting the so-called total extracted lipids (Table 1), was successively fractionated on florisil[9] (to absorb phospholipids), and ion-exchange resins[9] (to absorb sulphatides) to leave a mixture of cerebrosides and cholesterol.

Table 1. COMPOSITION OF THE WHITE AND GREY MATTER (g/100 g fresh tissue)

	Cortex	White matter
Water	82·92	73·32
Total solids	17 08	26·68
Total extracted lipids ($CHCl_3$-MeOH soluble)	6·20	12·46
Total phospholipid	2·87	5·10
Cholesterol (free)	1·13	2·65
Cholesterol ester	0·10	0·19
Cerebrosides	0·91	2·17
Sulphatides	0·16	0·41
Water-soluble non-dialysable lipids	0·094	0 019

The carbohydrate content of the grey matter ganglioside (18·15 per cent neuraminic acid, 22·67 per cent hexose and 8·87 per cent hexosamine—mostly galactosamine) approximated more to that of a gargoylism ganglioside isolated by Svennerholm[10] (24·1 per cent neuraminic acid, 24·6 per cent hexose and 11·6 per cent galactosamine) than to that of the mucolipid isolated from a normal brain by Rosenberg and Chargaff[11] (21 per cent neuraminic acid, 33 per cent hexose, and 4·4 per cent *N*-acetylgalactosamine). Klenk and Gielen[1] have reported that ganglioside preparations are mixtures of at least three different components. Recently[12] other workers have reported different neuraminic acid : galactosamine ratios in fractionated gangliosides. It could well be that in juvenile amaurotic idiocy the proportion of the different gangliosides changes. In both the grey and white matter only galactocerebrosides were encountered. Phosphoinositides, phosphatidyl serine, phosphatidyl ethanolamine and phosphatidyl choline were indicated from a chromatographic examination of the grey and white matter total extracted lipids. In comparison with that of a normal 12-year-old child (Cumings *et al.*[13]) the phospholipid, cholesterol, and cerebrosides were all depleted in the present case. The insoluble residues remaining after lipid extraction contained small amounts of neuraminic acid and hexosamine but no evidence was obtained of preferential extraction of mucoprotein or mucopolysaccharide.

We thank Dr. R. M. Norman and colleagues of the Burden Neuropathological Laboratory, Frenchay Hospital, Bristol, for supplying the brain and liver, and the British Council for the award of a scholarship to one of us (S.H.I.B.).

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Fibre Composition of Tough and Tender Muscles of Meat Animals

THE connective tissue content of muscles has been considered to be the principal biochemical entity responsible for toughness in meat. Little attention seems to have been given to the contribution which differences in the composition of muscle fibres themselves might make in rendering a muscle tough. I have examined the distribution of nitrogen within characteristically tough (semitendinosus) and tender (longissimus dorsi) muscles of different species (cattle, lambs, and pigs). The nitrogen components estimated were sarcoplasmic protein nitrogen, myofibril protein nitrogen, stroma nitrogen (by difference) and non-protein soluble nitrogen. The analytical technique used was essentially that of Lawrie's modification[1] of Helander's extraction technique[2].

Table 1 shows the average nitrogen distribution on a fat-free basis in the longissimus dorsi muscles at the 11th and 12th ribs and in the semitendinosi muscles in 8 Hereford Shorthorn bullocks (slaughtered at 32 months of age), 8 lambs of various crosses (age range 8–15 weeks) and 2 bacon weight pigs. Table 2 shows the data of Table 1 calculated on a stroma nitrogen-free basis.

The results show that the stroma nitrogen content is highest in beef muscles and lowest in pig muscles. Also beef muscles have the highest percentage of myofibril nitrogen on a stroma nitrogen-free basis. While the greater toughness of beef semitendinosus compared with beef longissimus dorsi is no doubt principally due to its higher content of stroma nitrogen, it does not seem unreasonable to suggest that, on a stroma nitrogen-free basis, the greater myofibril nitrogen content and smaller sarcoplasmic nitrogen content are to some extent reflected in the greater toughness of the semitendinosus. Extending this argument, it could be suggested that the greater

Table 1. Average Nitrogen Distribution on a Fat-free Basis in Muscles of the Three Species

		Percentage fat-free total nitrogen	Nitrogen components (% of total nitrogen)			
			Sarcoplasmic	Myofibril	Stroma	Non-protein soluble
Longissimus dorsi at 11th and 12th ribs	Cattle	3 89	19·12	54·73	13·62	12 53
	Lambs	3 40	25·56	54·05	8·09	12·31
	Pigs	3·80	23·64	56·71	7·24	12·41
Semitendinosi (distal end)	Cattle	3·79	16·03	55·68	16·94	11·35
	Lambs	3·26	24·44	52 24	11·37	11·95
	Pigs	3·47	21·67	56·87	9·08	12·89

Table 2. Average Nitrogen Distribution on a Fat-free and Stroma Nitrogen-free Basis in Muscles of the Three Species

		Percentage fat-free total nitrogen	Nitrogen components (% of total nitrogen)		
			Sarcoplasmic	Myofibril	Non-protein soluble
Longissimus dorsi at the 11th and 12th ribs	Cattle	4·50	22 13	63·36	14·51
	Lambs	3·70	27·81	58·81	13·89
	Pigs	4·10	25·49	61·14	13·38
Semitendinosi (distal end)	Cattle	4·56	19·30	67·04	13 66
	Lambs	3·68	27·58	58·94	13·48
	Pigs	3·82	23·83	62·55	13·68

sarcoplasmic and smaller myofibril nitrogen contents of lamb and pig muscles, when compared with beef muscles, are reflected to some degree in the greater tenderness of the muscles of these two species.

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Ribonucleic Acid Synthesis in Chloramphenicol-treated *Shigella*

Chloramphenicol markedly inhibits protein synthesis by bacteria. The organisms, however, continue to produce ribonucleic acid (RNA) at an unaltered rate or at one higher than that of cells not under the influence of the drug[1,2]. Several groups of investigators have examined extracts of chloramphenicol-treated *Escherichia coli* to determine the cellular fraction in which the newly formed RNA was located. Pardee *et al.*[3] concluded from their findings that the RNA was either of a low molecular weight or was easily degraded during extraction from the cells. The latter explanation appears likely since the authors did not extract the RNA with a medium containing magnesium ions to minimize the breakdown of ribosomal particles. Using such a medium, Dagley and Sykes[4] and Nomura and Watson[5] detected the appearance of a new ribosomal component in the extracts of treated cells. The latter investigators also noted that soluble or low-molecular-weight RNA continued to be synthesized in the presence of the antibiotic.

In this laboratory, it was earlier observed that chloramphenicol stimulated RNA synthesis in resting cells of *Shigella flexneri* serotype 3[6]. Cell extracts of untreated and chloramphenicol-treated cells have been examined to determine whether the formation of soluble and/or ribosomal RNA was stimulated by the drug.

Strain *B*-1003 of *S. flexneri* serotype 3 was used. The organism was grown in a chemically defined medium[6]. Collected cells were washed in 0·01 M *tris* buffer (*p*H 7·4) containing 0·01 M magnesium acetate. To obtain resting cells, the organisms were resuspended in buffer, aerated to deplete endogenous nutrients, centrifuged, and stored overnight at 5° C. The cells were then washed in the buffer, resuspended in the defined medium, and equal portions added to each of three flasks. One flask of cells serving as a 0 h, or un-incubated, control was immediately centrifuged at 0° C in a Sharples supercentrifuge. Chloramphenicol was added to the second flask to give a final concentration of 2 μg/ml.; the third flask was used as a chloramphenicol-free control. The latter two flasks were incubated at 37° C for 4 h and the cells were then collected on the Sharples supercentrifuge. The packed cells were disrupted by grinding with alumina. Soluble and ribosomal RNA were separated by centrifugation at 100,000*g*. The RNA content of the soluble fraction was determined by the orcinol reaction and that of the ribosomal fraction was calculated as the difference between the total and the soluble fractions.

As shown in Table 1, the synthesis of soluble RNA was appreciably stimulated by chloramphenicol. In contrast, the level of ribosomal RNA synthesis was similar to that of the untreated cells. Thus chloramphenicol-treated cells exhibited a low ribosomal RNA/soluble RNA ratio. It should be pointed out that although the level of RNA synthesis varied among different cell preparations, the ribosomal RNA/soluble RNA ratios were similar in the various experiments. The cell wall and membrane fraction of the disrupted cells were also examined. The RNA content of this fraction from untreated and treated cells was less than 0·5 per cent of the total RNA and did not increase during incubation.

Table 1. Effect of Chloramphenicol on RNA Synthesis by Resting Cells of *Shigella flexneri* Serotype 3

Cell preparation	Cell fraction	Experiment 1			Experiment 2		
		RNA content*	Net increase	Ribosomal : soluble	RNA content	Net increase	Ribosomal: soluble
0 h (before incubation)	Ribosomal	59	—		82	—	
	Soluble	16	—		25	—	
	Total	75	—	3 7 · 1	107	—	3·8 : 1
Incubated 4 h, no chloramphenicol	Ribosomal	94	35		149	67	
	Soluble	22	6		37	12	
	Total	116	41	4·3 1	186	79	4·0 : 1
Incubated 4 h with 2μg chloramphenicol/ml	Ribosomal	99	40		158	68	
	Soluble	48	32		76	43	
	Total	147	72	2·1 : 1	226	119	2·1 . 1

* Expressed as mμM RNA nucleotide/ml. cells.

The significance of these findings from the point of view of the mechanism by which chloramphenicol inhibits protein synthesis is not as yet evident. It appears that the drug stimulates the production of soluble, but not ribosomal, RNA in *S. flexneri* serotype 3. Alternatively the results may indicate that the drug effects a degradation of ribosomal RNA in the cells or prevents the assembly of newly formed ribosomal RNA subunits. Either of these proposed activities could be the result rather than the cause of the blockage of protein synthesis by the antibiotic. A study of the biological activity of the soluble RNA formed by chloramphenicol-treated cells is being carried out.

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Mechanism of the Ether Activation of Phospholipase *A*

IT has recently been shown that with many phospholipases the rate of reaction is appreciably influenced by the electrokinetic state of the enzyme and its substrate. Thus the lecithin-splitting activity of the phospholipase *B* of *Penicillium notatum* requires a negative ζ potential on the substrate's surface before activity commences, while the phospholipase *C* from *Cl. perfringens* needs a negatively charged enzyme and excess positive groups on the substrate[1,2]. On the other hand, the triphosphoinositide phosphomonoesterase of brain[3] requires the negative ζ potential of the substrate to be considerably reduced before activity can begin at an appreciable rate.

The physico-chemical characteristics of a phospholipase *A* reaction in an aqueous system have now been investigated particularly in respect of the ether activation reported by many workers[4]. The enzyme was purified by density gradient electrophoresis of cobra venom (*Naja naja*) using a glycerol gradient The preparation had an isoelectric point of about 5·1 and was electrophoretically homogeneous. The ovolecithin substrate was purified by chromatography on alumina and silicic acid columns. Phosphatidyl ethanolamine was prepared from egg yolk phospholipid by silicic acid chromatography. Analysis[5] showed it to contain about 90 per cent phosphatidyl ethanolamine, the remainder being ethanolamine plasmalogen and the saturated ether analogue.

The enzyme was assayed in an aqueous system[4] in which the substrate was emulsified in buffer and the decrease of acyl-ester groups recorded on incubation. Ca^{++} was found to be obligatory for the attack on both substrates and was used throughout at a concentration of 5 × 10^{-4} N. Addition of 10 per cent by volume of ether enormously activated the hydrolysis of lecithin. However, it was unexpectedly found that the activity of the enzyme towards phosphatidyl ethanolamine was unaffected and often slightly inhibited by the addition of this solvent.

Measurements of the electrophoretic mobility of lecithin particles[1] in *p*H 6 5 collidine buffer containing 5 × 10^{-4} N Ca^{++} showed them to be slightly negatively charged. On incubation with enzyme at 30°, there was a small increase in the mobility consistent with the release of a small quantity of fatty acid and consequent appearance of carboxyl groups in the organized surface of the lecithin micelle. However, when ether was added to saturate the buffer (10 per cent by volume) incubation with the enzyme caused virtually no change in the electrophoretic mobility of the lecithin particles, in spite of the liberation of greatly increased amounts of fatty acid and lysolecithin. It was found, furthermore, that artificially produced mixed micelles containing lecithin, lysolecithin and fatty acid were very negatively charged in collidine buffer and that this charge was reduced almost to zero by the addition of ether. Addition of fatty acid to lecithin micelles produced inhibition of the enzyme system, even in the presence of ether when very few of their carboxyl groups appeared on the surface.

It is suggested, therefore, that the enzyme, which is negatively charged at this *p*H (6·5), attaches itself to negative sites on the lecithin micellar surface by a calcium bridge. Such sites may be those tertiary acidic groups of the phosphate moieties of the lecithin which are not compensated by the quaternary ammonium groups of the choline. The appearance of carboxyl groups on the surface of the micelle competes with these negative sites for the Ca-enzyme$^+$ complex, and the reaction is suppressed Ether causes a reorganization of the micelle so fatty acid carboxyl groups are no longer present on the surface, or, alternatively, it removes the fatty acid from the substrate micelles entirely. In agreement with this theory, the addition of a long-chain anion to the lecithin (dicetylphosphate or dodecyl sulphate) caused inhibition of the ether activated system and at the same time produced a negative ζ potential on the substrate. No reversal of the inhibition was obtained on addition of cetyltrimethyl ammonium bromide to these particles in sufficient quantities to reverse the charge and produce a positive ζ potential.

Phosphatidyl ethanolamine micelles are negatively charged at *p*H 6·5 and this charge increases with increasing *p*H, presumably due to the suppression of the ionization of the amino group. Correlated with this liberation of free tertiary acidic groups of the phosphate moiety on the substrate's surface is an increase in the rate of enzymatic hydrolysis up to *p*H 9·3. In contrast lecithin, the mobility of which does not change appreciably between *p*H 6 and 10, does not show a similar increase in enzymatic hydrolysis with increasing *p*H. On incubation of phosphatidyl ethanolamine with enzyme at *p*H 9·3, it becomes only very slightly more negatively charged even when the reaction has gone to 50 per cent completion. This suggests that the fatty acids liberated are not being orientated in the micelle–water interface, perhaps because of repulsion by the large excess of negative charges on the surface. Addition of ether to the system only reduces the negative charge of phosphatidyl ethanolamine particles very slightly and those of the substrate after incubation by the same amount. It would seem, therefore, that this substrate has many more negative sites available for the attachment of the Ca-enzyme$^+$ complex and can more -

adequately compete with the few carboxyl groups which appear on the surface as the reaction proceeds. Consequently ether has no activating effect. In contrast when mixed micelles of phosphatidyl ethanolamine and fatty acid are prepared artificially the negative charge of such particles is much higher than that of the phospholipid alone. This indicates that, in this case, carboxyl groups are present on the surface and, in agreement with this, it is found that the enzymatic hydrolysis of the substrate is inhibited by the presence of the fatty acid.

To summarize, it is suggested that a phospholipase *A*-calcium complex links with anion sites (phosphate) on the surface of the lecithin micelle. The carboxyl groups of the fatty acids liberated in the reaction appear to a limited extent in the micelle–water interface, and inhibit the reaction by competing with the anion sites for enzyme. Saturation of the system with ether removes carboxyl groups from the surface and the reaction can proceed. With phosphatidyl ethanolamine micelles, there are many more free anion sites on the surface. The few carboxyl groups appearing on the surface as the reaction proceeds only have a very limited competing effect and consequently ether does not activate.

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Positional Specificity of Phosphatide Acyl Hydrolase (Phospholipase *A*)

SNAKE venom phospholipase *A* was shown by de Haas and van Deenen to release the fatty acid constituent present in the β-ester position of mixed-acid phospholipids obtained by a synthesis *de novo*[1–3]. This result confirmed those of Tattrie[4] and Hanahan *et al.*[5], who worked on fatty acid distribution in egg lecithin, using phospholipase *A*, phospholipase *D* and pancreatic lipase. Hence, the positional specificity of phospholipase *A* appeared to be settled. Recently Bennett and Tattrie[6], however, reported, because of reacylation investigations of snake-venom-formed lysolecithin, that this enzyme may not invariably hydrolyse the β-fatty acid ester bonds. This assumption supports the interpretation previously made by Marinetti *et al.*[7], indicating a preference of the enzyme for liberating long-chain poly-unsaturated fatty acids, irrespective of their position in the phospholipid molecule. For this reason further investigations into the mode of action of phospholipase *A* were made.

The synthetic lecithins and cephalins investigated so far by us contained either two dissimilar saturated fatty acid constituents or one saturated and one mono-unsaturated fatty acid of equal chain-length in different positions. In view of the conflicting opinions, a further check on the specific action of phospholipase *A* required examination of phospholipids containing, in different positions, a poly-unsaturated fatty acid with a chain-length dominating that of the saturated fatty acid constituent. The synthesis of mixed-acid lecithins and cephalins, involving the preparation of defined γ,β-diacylglycerol iodohydrins, has already been published[8,9]. In the present investigation (γ-linolenoyl-β-palmitoyl)-L-α-phosphatidyl ethanolamine and (γ-palmitoyl-β-linolenoyl)-L-α-phosphatidyl ethanolamine were prepared by a coupling of the corresponding mixed-acid γ,β-diacylglycerol iodohydrins with silver benzyl-2-ethyl-phthalimido phosphate, as previously described[10]. Experimental results of the synthesis of phospholipids with polyenoic acyl chains will be described elsewhere. Emulsions of 10 mg of both structural isomeric phospholipids, prepared in 1 ml. of borate buffer, pH 7, calcium chloride $2 \cdot 5 \times 10^{-3}$ M, with a few drops of ether, were incubated together with 2 mg of snake venom (*Crotalus adamantheus*) for 6 h at room temperature. The entire reaction mixture was lyophilized and adequate samples dissolved in chloroform were subjected to chromatography on silica-impregnated paper according to Marinetti[11]. The paper chromatogram demonstrated a complete breakdown of both substrates. The spots of the liberated fatty acid and formed lysophospholipid were treated directly with methanol/hydrochloric acid, and the fatty acid methylesters thus obtained were analysed by gas-liquid chromatography.

Table 1 ACTION OF PHOSPHOLIPASE *A* ON TWO SYNTHETIC MIXED ACID L-α-PHOSPHATIDYL ETHANOLAMINES, CONTAINING LINOLENI ACID IN DIFFERENT MOLECULAR POSITIONS

Substrates	Fatty acid composition afte hydrolysis by phospholipase *A*	
	Liberated fatty acid	Lysophospholipid
(γ-Palmitoyl-β-linolenoyl)-L-α-phosphatidyl ethanolamine	98·5 per cent linolenic acid	99 per cent palmitic aci
(γ-Linolenoyl-β-palmitoyl)-L-α-phosphatidyl ethanolamine	97 per cent palmitic acid	97·5 per cen linolenic aci

The results obtained (Table 1) showed that during the enzymatic hydrolysis of both synthetic phospholipids, the β-fatty acid is liberated, thus confirming our previous conclusions. It should be noted that th results presented here have not been corrected for non-enzymatical 'at random' degradation, which wa shown to occur to a very small extent under the condi tions used. The experiments did not reveal a prefer ential enzymatic release of the long-chain poly unsaturated fatty acid constituent, and hence do no support the reports[6,7] attributing a lack of position specificity to snake-venom phospholipase *A*. I addition, it should be pointed out that experiments o the phosphatide-(mono)acyl hydrolase from be venom and pancreas demonstrated that thes enzymes also exhibit a β-specificity for synthetic phos pholipids.

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PHYSIOLOGY

Electrophoretic Mobility of Serum Post-heparin Tributyrinesterase

MUCH work has been devoted to the mechanism of the clearing action of heparin, and it is now generally accepted that its action is exerted by a lipolytic enzyme called clearing factor. On the other hand, it has been found that the injection of heparin is followed by the appearance in the serum of an eserine-resistant tributyrinesterase, which is considered to be one and the same with clearing factor[1]. Various techniques were used to locate the clearing factor in the serum protein fractions, including fractional precipitation[2] and ultracentrifugal fractionation methods[3]. The findings of the different investigators are not in agreement. Thus, it has been claimed that clearing factor remains in the albumin fraction of serum after half saturation with ammonium sulphate[2]. However, Graham *et al.*, using ultracentrifugal and ethyl alcohol fractionation, found the enzyme in the globulin fraction[3]. Because of this discrepancy, investigations were made in order to study the electrophoretic mobility of the clearing factor (post-heparin tributyrinesterase[1]) in comparison with the fractions of the proteinogramme.

Our investigation was carried out under the following conditions: A volume of 0·04 ml. serum was applied in a linear fashion to a Whatman No. 3 paper strip. Electrophoresis was carried out in an Elford apparatus at 4° C temperature, in a veronal buffer ionic strength 0·05 and *p*H 8·6 for 9 h. The distribution of the enzymes on the paper strip was examined after electrophoresis by the following procedure: The strip was divided into two other strips of 1·5 cm and 3 cm width. The first was dried and stained with bromophenol-blue in order to obtain the electrophoretic pattern of serum proteins, and afterwards was used as a marker. The other strip, immediately after electrophoresis, was cut up into 1-cm pieces while still wet. These pieces were transferred to small test-tubes containing 1·0 ml. of distilled water. They were allowed to stand for 12 h at a temperature of 4° C. To each of the eluates the tributyrinesterase activity was measured, by Tuba–Hoare's method[4], with some modifications, as follows. 1 ml. of veronal buffer *p*H 8·6 and 0·03 ml. of glycerol tributyrate was added to each tube and after vigorous shaking the mixtures were incubated for six hours at 37° C. The enzyme action was finally stopped by adding 4 ml. of alcohol. The tubes were then centrifuged and the butyric acid content of supernatant was measured by titration with sodium hydroxide (0·025 N) using phenolphthalein as indicator. The tributyrinesterase activity of the eluates was reported to the serum protein fractions of the bromophenol stained strip (marker).

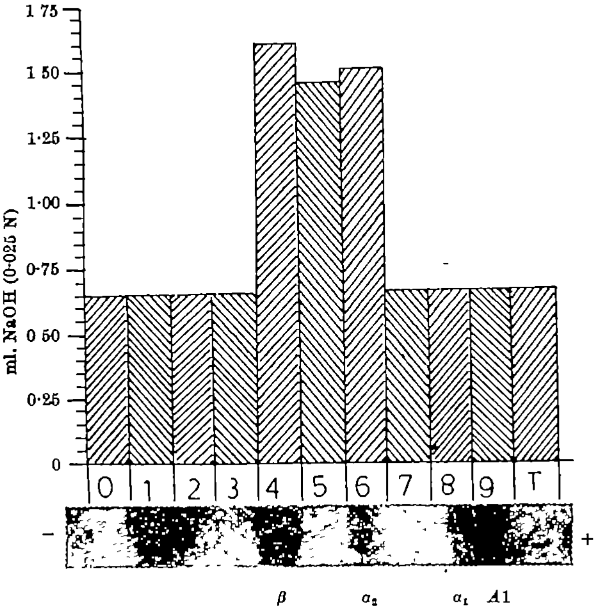

Fig. 1

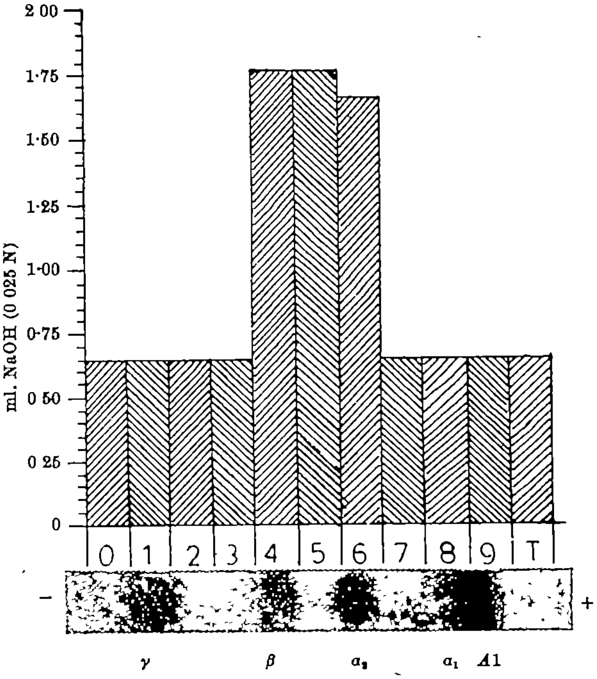

Fig. 2

In a preliminary series of experiments the electrophoretic mobility of normal serum tributyrinesterases was studied in sera received from 20 non-heparin-treated normal individuals. In a second series of experiments the electrophoretic mobility of post-heparin tributyrinesterase was studied in sera received from 10 healthy subjects 5 min after intravenous injection of 2·000 units of heparin.

These sera were centrifuged for 30 min at 3,000*g*/min in order to remove the platelets and leucocytes[5]. They showed a definite increase in tributyrinesterase activity (about 20 per cent) in comparison with the sera of the same subjects received before injection.

The results of our preliminary experiments are presented in Fig. 1. It is evident that tributyrinesterase activity was found on the paper pieces corresponding to the zone defined from the anode side of the α_2-globulin fraction and the cathode side of β-globulin fraction. In Fig. 2 are presented the results from the electrophoretic study of post-heparin sera. It is evident here that tributyrinesterase activity was recovered both in the α_2-globulin and β-globulin fractions and in the zone in between. No enzymatic activity was recovered in the albumin, α_1- or γ-globulin fractions.

It can be concluded, therefore, that serum post-heparin tributyrinesterase is an α_2- or β-globulin and has an electrophoretic mobility identical to this of normal serum tributyrinesterases.

Our conclusion is in agreement with the ultracentrifugal and ethyl alcohol fractionation findings of Graham *et al.*[3].

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Capillary Blood Standard Bicarbonate Values of Conscious and Anæsthetized Individuals

THE clinical significance of blood standard bicarbonate has not yet been firmly established. Relatively little information is available at present concerning the effect of surgical anæsthesia on this new parameter in capillary or arterial blood. Payne and Conway[1] recently concluded that normal values for arterial blood standard bicarbonate were significantly lower than those reported by Astrup *et al.*[2] on capillary specimens. The latter workers contend that comparable standard bicarbonate values are obtained from arterial and capillary blood. Evaluation of acid-base balance during anæsthesia in our hospital revealed that standard bicarbonate concentrations of capillary blood from conscious and anæsthetized subjects were consistent with those of Astrup *et al.*[2].

Capillary blood collected from 15 clinically healthy medical students, analysed in Astrup equipment, yielded an average standard bicarbonate of 23·4 ± 1·6 m. equiv./l. The value is close to the mean (22·9 ± 1·5 m. equiv./l.) quoted by Astrup *et al.*[2] for capillary blood of 65 apparently normal persons. In line with these findings, Robinson[3] observed average standard bicarbonate-levels of 24·4 m. equiv./l. in arterialized venous blood of 20 fit males. Likewise, Papadopoulos and Keats[4] noted a mean arterial standard bicarbonate-level of 24·7 ± 0·7 m. equiv./l. in 20 subjects prior to orthopædic or gynæcological surgery.

A total of 124 standard bicarbonate estimations were made on capillary blood taken from 24 patients before, during and following major surgery. Anæsthesia was maintained by orotracheal administration of *cyclo*propane, nitrous oxide or halothane, following induction with thiopental and succinylcholine. Ventilation was controlled both by manual and mechanical methods. Standard bicarbonate-levels during anæsthesia ranged from 18·5 to 28·5 (mean 22·9 ± 2·3 m. equiv./l.). Although a majority of values fell within normal limits reported by Astrup *et al.*[2], 20 were above 24·8 m. equiv./l. and 8 dropped below 21·3 m. equiv./l. In anæsthetized patients, Robinson[3] as well as Papadopoulos and Keats[4] observed means of 23·3 and 21·8 m. equiv./l., respectively. The latter figure obtained on arterial blood tends to approach the lower standard bicarbonate-level (18·14 ± 1·94 m. equiv./l.) found by Payne and Conway[1] also in arterial blood of 22 patients anæsthetized with halothane. Individuals studied by Papadopoulos and Keats[4], however, were subjected to prolonged hyperventilation during surgical anæsthesia. Available evidence indicates that a modest to moderate compensatory metabolic acidosis frequently accompanies respiratory alkalosis[5].

Capillary blood collected from 16 patients during the first 2 hours following surgery yielded a mean standard bicarbonate of 23·6 ± 2·2 m. equiv./l. (range 18·6–26·5). These data compare favourably with the average obtained on medical students. A similar relationship between control and post-operative standard bicarbonate-levels was noted by Payne and Conway[1]. A reasonable explanation for the low values reported by these workers may stem from the use of arterial rather than capillary specimens. Ideally, arterial blood should be used for acid-base evaluation, but the collection procedure is not without danger to the patient. Woolmer[6] suggests that under general anæsthesia there is sufficient vasodilatation to effectively arterialize capillary blood.

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Effect of Potassium Depletion on Lactic Dehydrogenase Activity in the Rat Nephron

IN potassium depletion, the kidney is unable to concentrate the urine properly[1,2]. Striking morphological changes have been found in the region of the medulla and papilla[3,4,5]. In addition, marked changes in enzymatic activity, located mainly in the medulla, have been demonstrated by means of histochemical staining methods[6]. In the work reported here, lactic dehydrogenase activity was measured in various functional units of the kidney of normal and potassium-depleted rats through the use of the quantitative histochemical techniques originated by Lowry[7].

Male Sprague-Dawley rats, weighing between 100 and 150 g, were depleted of potassium over a 4-week period by feeding them a virtually potassium-free diet which contained a sodium polystyrene sulphonate resin and supplements of vitamins and minerals[8]. They drank a solution of 0·45 per cent sodium chloride and 0·45 per cent sodium bicarbonate. The test and control animals were pair fed. The controls were not given resin, while potassium chloride (50 m.equiv./l.) was added to the drinking water. Within three weeks the rats on the depletion diet became lethargic, lost weight, and developed a shaggy coat[8]. The mean serum potassium-level was decreased significantly from 5·7 m.equiv./l. (*S.E.* 0.41) for the control animals to 2·9 m.equiv./l. (*S.E.* 0·32) for the potassium-depleted group ($P = 0{\cdot}00027$). After urine was collected on sheets of sterile aluminium foil, the animals were anesthetized with ether, the abdomen was opened, and blood was drawn from the inferior vena cava for chemical analysis. A 2-mm. thick cross-section was cut from the right kidney, extending from the renal capsule to the papilla tip. It was mounted on a microtome sample holder and quickly frozen in liquid nitrogen. The tissue was sectioned at 16μ in a cryostat at −20° C, lyophilized and used for microdissection, as described previously[9,10]. The individual structural units of the nephron were identified by means of alternate stained sections and dissected out from the dry sections[10]. Both in the control and the potassium-depleted animals the loops of Henle in the inner medullary zone appeared dark and narrow in the dry sections ; the collecting ducts, light and broad. The nephron fragments were weighed on a quartz fibre 'fishpole' balance[11] and placed in a micro test-tube for the enzyme assay. The lactic dehydrogenase (LDH) assay has been described previously[12]. LDH activity in serum and urine was determined by the same assay method, diluting serum samples 1 : 100 and urine samples 1 : 10 with the substrate reagent.

LDH activities found in various parts of the nephron and in the serum and urine of the potassium-depleted and control rats are summarized in Table 1. Potassium depletion affected only the inner medullary zone (Table 1(*a*)). A significant increase of the LDH activity was noted in the collecting ducts of the inner medullary zone (287 versus 67 M/kg/h, P = 0·0382). A decrease in LDH activity which was not significant was seen in the loop of Henle (157 versus 219 M/kg/h, P = 0·0996). The experiment was repeated with a larger number of rats (Table 1(*b*)). Again a significant increase in LDH activity occurred in the collecting ducts of the inner medullary zone (273 versus 145 M/kg/h, P = 0·00529), and a decrease in the loop of Henle which was not significant (123 versus 156 M/kg/h, P = 0·0725).

Table 1. LACTIC DEHYDROGENASE ACTIVITIES IN THE KIDNEY, SERUM, AND URINE OF NORMAL AND POTASSIUM-DEPLETED RATS*

Material analysed	Control	Potassium-depleted	P†
(*a*) 4 weeks depletion :	[2]	[2]	
Glomerulus	71 ± 5·6 (12)	57 ± 8·2 (10)	
Proximal convoluted tubule	152 ± 8·1 (14)	137 ± 23 (12)	
Distal convoluted tubule	231 ± 18 (17)	232 ± 20 (12)	
Outer medullary zone	88 ± 26 (9)	101 ± 8·7 (12)	
Inner medullary zone, collecting duct	67 ± 4·6 (9)	287 ± 43 (10)	0·0382
Inner medullary zone, loop of Henle	219 ± 15 (10)	157 ± 15 (12)	0·0996
Papilla, collecting duct	196 ± 21 (14)	156 ± 12 (22)	
Small artery	66 ± 5·1 (9)	66 ± 5·0 (12)	
(*b*) 4 weeks depletion :	[4]	[4]	
Inner medullary zone, collecting duct	145 ± 23 (16)	273 ± 19 (16)	0·00529
Inner medullary zone, loop of Henle	156 ± 13 (16)	123 ± 7·8 (16)	0·0725
Papilla, collecting duct	53 ± 3·4 (19)	62 ± 4·1 (29)	
(*c*) 2 weeks depletion :	[5]	[5]	
Serum	35·6 ± 5·1	36·5 ± 4·9	
Urine	0·14 ± 0·038	4·40 ± 2·3	0·1007
4 weeks depletion :			
Serum	51·3 ± 4·0	58·4 ± 10·0	
Urine	0·29 ± 0·095	0·79 ± 0·19	0·0463

* Results are given as mean values with the standard error of the mean, and in parentheses the number of tissue fragments analysed. Activities in the tissue fragments are expressed in moles of DPNH oxidized per kg dry weight per h incubation at 37° C. Activities in serum and urine are expressed in millimoles of DPNH oxidized per litre per h incubation at 37° C. Number of animals in each experiment is given in brackets.

† The bilateral probability P was calculated by means of Student's *t*-test with the number of degrees of freedom equal to the total number of animals less two. Only values of P = 0·10 or below are shown.

The results of these two experiments cannot be combined directly, because they represent experiments at different times with different groups of animals. However, the bilateral probabilities can be combined by the method of Fisher[13], using the formula :

$$\chi^2 = -2 \ln \frac{P_1 . P_2 . . . P_n}{2^n}$$

finding the combined probability from a table of χ^2, assuming $2n$ degrees of freedom and multiplying by 2 to obtain the bilateral probability P. For the collecting duct this gives a combined bilateral probability P = 0·0013 ; and for the loop of Henle, P = 0·026. It is concluded, therefore, that potassium depletion caused an increased LDH activity in the collecting ducts of the inner medullary zone and a decreased LDH activity in the loop of Henle.

The serum LDH-level remained unchanged at 2 and 4 weeks of potassium depletion (Table 1(*c*)). A considerable increase in urinary excretion of LDH was noted at 2 weeks of depletion. This change was still noticeable at 4 weeks of depletion ; the combined bilateral probability was P = 0·018. The increased urinary excretion of LDH may be related to the partial loss of this enzyme from the loop of Henle.

The primary structural lesion in the kidney of the potassium-depleted rat is mainly in the lower reaches of the nephron, in the medulla, especially in the collecting ducts[3,4]. The main functional effect of potassium depletion consists of an inability to concentrate the urine[1,2,4]. This is followed by conservation of potassium[14], which can probably be considered an adaptive response. A number of other enzymatic changes in the kidney have been reported in potassium depletion.

Muntwyler *et al.*[15,16] found increased activities for glutaminase and carbonic anhydrase in the whole kidney homogenates of potassium-depleted rats which were not protein depleted. After repletion for one week, they observed that these activities had almost returned to normal. They found no changes in the activities of *d*-amino-acid oxidase and arginase. By histochemical staining methods, Pearse and MacPherson[6] observed an increased activity of TPN-diaphorase in both limbs of the loop of Henle and in the papillary collecting ducts, as well as increased activities of acid phosphatase and phosphorylase in the papillary collecting ducts and of esterase in the proximal convolution and the pars recta. They also reported a decreased activity of TPN-diaphorase in the papillary collecting ducts. More recently, Davis[17] detected an increase in the pyruvate kinase activity in the medulla of the potassium-depleted rat. His observation of a much smaller increase in LDH activity is understandable in the light of our finding of opposite changes in different parts of the medulla, since he worked with a whole homogenate of the medulla.

It would seem that the histological changes in the medullary collecting ducts and the increased activities of glutaminase, carbonic anhydrase, TPN-diaphorase, acid phosphatase, phosphorylase, and pyruvate kinase in potassium depletion could all be part of an adaptive mechanism aimed at the conservation of potassium. The increased lactic dehydrogenase activity in the medullary collecting ducts described here fits into this pattern. Both LDH and pyruvate kinase play an important part in the cellular energy metabolism as a link between the glycolytic pathway, mainly located in the cytoplasm, and the Krebs tricarboxylic acid cycle, mainly located in the mitochondria. In view of the counter-current exchange between the loop of Henle and the adjacent collecting ducts[18], one could speculate that the simultaneous decrease of LDH activity in the loop of Henle might be part of the same adaptive response, aimed at the conservation of potassium[19].

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Changes in Multilocular Brown Adipose Tissue in the Rat following Hypothermia

BROWN fat is involved in reactions to stress and in hibernation. The observations reported here show that it may also be altered in the rat after hypothermia. The Giaja closed jar technique was used for cooling and the method of Goldzveig and Smith for re-animation[1].

At autopsy 14–16 days after a single cooling to just below 2° C rectal temperature, hooded rats tended to have paler and less red interscapular brown fat than uncooled controls. The lobes lying dorsal to the submaxillary glands showed the same tendency. The experimental animals were still underweight (mean $=$ 165 g, $N = 14$) compared with the controls (mean $=$ 195 g, $N = 15$) at autopsy, but since there is evidence that the brown fat is darker and redder in states of food deprivation[2–4] and stress[5,6] it is unlikely that the colour differences found here were a simple function of the reduced food intake that follows hypothermia. Both groups of animals in this experiment had similar experience on a conditioned avoidance task.

In another experiment, growing male albino rats were repeatedly cooled to 2° C (Table 1). The finding of Andjus[7] that repeatedly cooled rats come to withstand hypothermia better was confirmed. 6–8 days after the last cooling the brown fat lobes dorsal to the submaxillary glands were dissected out. They were paler than those of controls and reduced in extent. Subsequent histology showed that there were marked changes. The homogeneous appearance of the sections was disrupted (Fig. 1). The white fat of cooled rats was also greatly reduced. Scarcely any was visible around the kidneys. Judged from this the animals would be considered to have been in a state of severe inanition. Yet their total body-weights were not far below those of controls (Table 1). Moreover, in another group of animals, the food of which was restricted so that their body-weights stayed below those of cooled animals, much more perirenal white fat was visible, and the lobes of brown fat sampled appeared histologically normal. This shows that the changes in the sections taken from cooled animals are not simply dependent on body-weight losses.

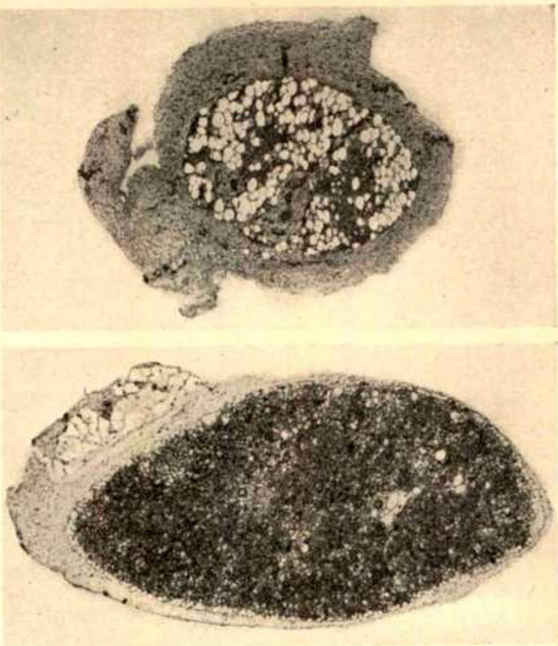

Fig. 1. Multilocular adipose tissue (× *c*. 28). Fixed in Bouin, stained with eosin and hæmatoxylin, cleared in xylol. Top, experimental (1 *P*7); bottom, control (2 *P*7)

The relative difficulty in reducing the brown fat has led to the theory that it is an ultimate reserve[8]. From the present results it seems that hypothermia can result in a selective utilization of adipose tissue including perhaps the brown fat reserve. Preliminary investigations have also shown that in a state o selective deposition of fat such as occurs in hypothalamic hyperphagia the brown fat is increased though histology will be necessary to determine whether this is due to an increase in multilocular cells or ordinary unilocular fat cells.

Table 1

Animal No.	Weight, 30th day	Treatment (30–64th day)	Weight at autopsy 70–71st day
3 *P*1	60	Cooled to 15 ± 1° C.	200
4 *P*1	84	6 ± 1° C., 3° C., 2° C.,	221
1 *P*7	57	2° C, 2° C, 2° C	229
2 *P*1	62		210
2 *P*7	57		237
3 *P*7	43		210
1	50	Controls	222
3	53		208
5	46		219
7	52		233
		31st–71st day	
2	53		180
4	43	Food restriction	181
6	50		185
8	52		171

The difference between the colour changes after hypothermia and food restriction suggests that brown fat may function as a special as well as an ultimate reserve. It has been found that in the hedgehog this tissue is particularly affected when animals emerge from hibernation[9]. It may be that recovery from artificially induced hypothermia provides circumstances for the utilization of brown fat in the rat that are normally lacking.

Experiments are in progress to determine whether these changes can be seen in other areas of brown fat, and with different temperature treatments.

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PHARMACOLOGY

Potentiation of the Effects of Indirect Sympathomimetic Amines by Reserpine and Bretylium

IT has been shown[1,2] that the effects of sympathomimetic amines, called indirect (tyramine, phenylethylamine, phenylethanolamine, ephedrine, amphetamine), are abolished by pretreatment with reserpine. However, immediately after an injection of reserpine, the effects of indirect sympathomimetic amines are potentiated[2,3]. On the other hand, it has been reported that bretylium enhances the effects of tyramine[4].

The object of the work recorded here is to try to differentiate the potentiating effects of reserpine and bretylium and, in addition, to find an eventual difference between some indirect sympathomimetic amines. We used the preparation of pithed rat prepared by Shipley and Tilden's method[5].

The hypertensive effects of phenylethylamine and amphetamine were greatly increased by small doses of reserpine, but those of phenylethanolamine and tyramine were augmented to a smaller extent. The threshold dose of reserpine potentiating phenylethylamine and amphetamine was 0·05–0·1 mg/kg, but the threshold doses for increasing tyramine and phenylethanolamine effects were greater (0·25 mg/kg). The potentiation disappeared rapidly with repeated injections.

This potentiating effect of reserpine was lost in the rat pretreated with large doses of reserpine (10 mg/kg intraperitoneal 24 h before the experiment), but could be obtained if *nor*epinephrine (0·05 μg) was injected at the beginning of the experiment.

Injection of 5 mg/kg of bretylium produced a hypertension and, after the return of blood pressure to the initial level, potentiated the tyramine effect, but did not increase phenylethylamine and amphetamine hypertension.

This potentiating effect of bretylium could not be obtained in the rat pretreated with large doses of reserpine. However, after administration of small doses of reserpine, bretylium still increased the tyramine hypertension, and the same is true of the reverse process.

These results show: (1) That the potentiating effects of bretylium and reserpine to the indirect sympathomimetic amines seem to be correlated to the presence of *nor*epinephrine in the body, but that the mechanisms of the two sensitizations are not exactly the same. (2) That the effects of indirect sympathomimetic amines depend on the presence of *nor*epinephrine in the body, but all the sympathomimetic amines do not act in exactly the same way.

To explain these results it is suggested that *nor*epinephrine is present in the body in several forms or in several storage areas and that these forms or storage areas are sensitive to the drugs in different ways.

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Table 1. INFLUENCE OF RESERPINE AND BRETYLIUM ON THE HYPERTENSIVE EFFECTS OF SOME SYMPATHOMIMETIC AMINES

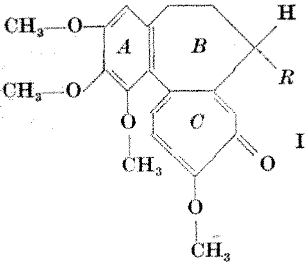
	Treatment with 0·1 mg/kg of reserpine			Treatment with 0·25 mg/kg of reserpine			Treatment with 5 mg/kg of bretylium		
	Before (mm Hg)	After (mm Hg)	Potentiat. per cent	Before (mm Hg)	After (mm Hg)	Potentiat. per cent	Before (mm Hg)	After (mm Hg)	Potentiat. per cent
Epinephrine, 0·05 μg.	5 ± 1	6 ± 2	0	9 ± 3	11 ± 3	0	—	—	—
*Nor*epinephrine 0·05 μg.	17 ± 1·7	17 ± 1·7	0	17 ± 2·5	14·5 ± 2	0	15·5 ± 1·8	20 ± 3·4	27 ± 6
Tyramine, 25 μg.	13 ± 1·5	13 ± 2·5	0	14 ± 1·7	21 ± 3*	53 ± 8*	15 ± 2·4	28 ± 3*	89 ± 16*
Phenylethylamine, 25 μg.	13 ± 1·5	33 ± 4·2*	140 ± 14*	13·5 ± 2·5	53·5 ± 7·5*	315 ± 61*	17 ± 2·3	21·5 ± 2·6	25 ± 4·3
Phenylethanolamine, 25 μg.	8 ± 2	10 ± 1·8	30 ± 11	10 ± 1·1	18 ± 3·8*	86 ± 5*	—	—	—
Amphetamine, 20 μg.	11 ± 1·48	24 ± 4*	115 ± 50*	—	—	—	13 ± 1·2	14 ± 1·5	0

* $P < 0·01$.

Desacetylamino-colchicine: a Derivative of Colchicine with Increased Cytotoxic Activity in Mammalian Cell Cultures

COLCHICINE, the main alkaloid of *Colchicum autumnale*, is well known for its action on the mitotic spindle, resulting in an arrest of mitosis in metaphase. The synthesis of derivatives of this compound is of interest for several reasons. Some were used to elucidate the chemical structure of colchicine. In addition, many of them were tested for their ability to inhibit the growth of tumours. Furthermore, such compounds made possible an analysis of relationships between structure and biological activity of the colchicine molecule. In earlier investigations of the relation between structure and activity it was demonstrated that compounds lacking the acetyl group on the side-chain may be equally as active as colchicine[1,2]. In addition, the methoxy group on the tropolone ring could be replaced by a free or substituted amino group without loss of biological activity[3].

Recent developments in the chemistry of colchicine[4] made available a number of nitrogen-free derivatives of colchicine. Of these, desacetylamino-colchicine (I) was of particular interest since its structure is identical with that of the parent compound, colchicine (III), except for the absence of the acetylated amino group on ring *B*. Therefore, it seemed possible to decide, using this compound, whether the amino group of colchicine was necessary for the molecule to exert its specific action on the mitotic spindle.



I, $R = -H$
II, $R = -NH_2$
III, $R = -NH-CO-CH_3$

In order to compare the inhibitory activities of colchicine and its desacetylamino derivative on cell reproduction, I used *in vitro* cultures of a transplantable murine mast cell tumour (cell line P–815–X_2)[5,6]. The culture techniques used and the medium have been described previously[7]. Additional components of the medium were: L-asparagine (0·1 mM), L-serine (0·1 mM) and *tris*-buffer (3 ml. of 2 per cent *tris*-(hydroxymethyl)-amino-methane in 0·15 N hydrochloric acid per 100 ml. of medium). Furthermore, leukovorin was replaced by folic acid (10 mg/l.). Approximately 200,000 cells were incubated in 2 ml. of this medium, which contained various concentrations of the inhibitor to be tested. After 24 h, cell reproduction was measured by hæmocytometer counts. The results obtained in a typical experiment are illustrated in Fig. 1. It can be seen that the inhibitory activity of desacetylamino-colchicine was approximately 10 times as high as that of the natural alkaloid, colchicine. In similar experiments the activity of desacetyl-colchicine (II) was found to be lower by a factor of 3 than that of colchicine. This is in good agreement with earlier reports[1]. It was concluded, therefore, that it is not the lack of the acetyl-group but rather the lack of the amino-group which is responsible for the increased activity of desacetylamino-colchicine, as compared with colchicine.

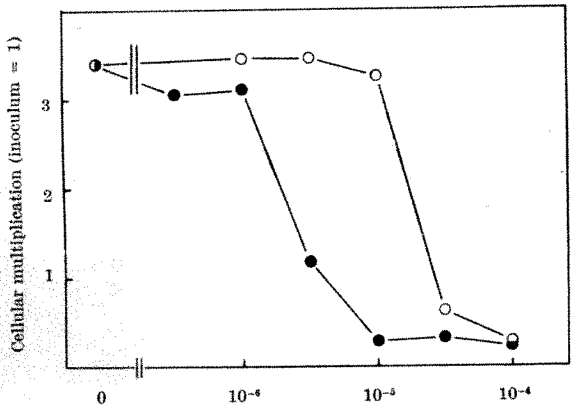


Fig. 1. Inhibition of cell reproduction by colchicine and desacetylamino-colchicine. ○, Colchicine; ●, desacetylamino-colchicine

The specific action of the inhibitors on the mitotic spindle was analysed by measuring the fraction of cells in metaphase, and in anaphase-telophase, respectively, 12 and 24 h after the addition of the inhibitor. The cells were fixed by mixing an aliquot of the cell suspension with an equal volume of ethanol–acetic acid–water 5 : 2 : 3. Afterwards the cells were centrifuged and resuspended in a small volume of 0·025 per cent crystal violet in 1 per cent acetic acid. In this suspension the cells in metaphase and those in anaphase-telophase were determined by hæmocytometer counts. Colchicine caused a pronounced accumulation of cells in metaphase, while at the same time the number of cells in anaphase-telophase fell below the control-level. The results summarized in Table 1 indicate that the metaphase block produced by desacetylamino-colchicine was of the same degree as that observed with colchicine. Similar results were obtained 24 h after the addition of the inhibitors.

Table 1. METAPHASE BLOCK OBSERVED AFTER 12-H EXPOSURE OF CELL CULTURES TO COLCHICINE AND DESACETYLAMINO-COLCHICINE

	Metaphases Percentage of total cells	Anaphases + telophases Percentage of total cells
Control (no inhibitor)	1·36	1·15
Colchicine 3×10^{-5} μmoles/ml.	23·5	0·53
Desacetylamino-colchicine 3×10^{-6} μmoles/ml.	30·6	0·36

This work was supported by the Schweizerische Nationalfonds zur Förderung der wissenschaftlichen Forschung. I thank Prof. A. Eschenmoser, Eidgenössische Technische Hochschule, Zürich, for supplies of desacetylamino-colchicine and desacetyl-colchicine.

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Gustatory Thresholds in Patients with Hypertension

THERE is generally acknowledged to be a relationship of some sort between intake of salt and hypertension[1,2]. Dahl[3] has shown that groups habitually consuming large amounts of dietary salt show a greater incidence of hypertension than groups consuming lesser amounts. He has suggested that 'prehypertensives' eat more salt prior to the onset of their hypertension than do normotensive patients. Because of the possible importance of altered gustatory sensation, we decided to measure taste thresholds in hypertensives.

A method was used similar to one described by Richter and MacLean[4]. The subject was presented with a number of rows of cups with 4 cups in each row. Three of the 4 cups contained distilled water while the fourth contained either a salt or a sugar solution; the location of the fourth cup was at random.

The salt (or sugar) cups in successive rows went from most dilute to most concentrated. Salt solutions used for testing ranged from 0·00125 per cent to 0·64 per cent sodium chloride, doubling in concentration at each dilution so that there were 10 dilutions in all. Dextrose was used for testing threshold for sweetness; concentrations ranged from 0·1 per cent to 6·4 per cent, also increasing each time by a factor of two, giving seven dilutions for tasting. Solutions were mixed as per cent by weight/volume. For example, to prepare a 6·4 per cent solution of dextrose, 64 g of dextrose were dissolved in distilled water and then diluted until the total volume equalled 1,000 c.c. This method of preparation, although it has some theoretical disadvantages[5], seemed perfectly satisfactory for the purposes of our study.

The subject was asked to taste the fluid in each cup, spit it out, rinse his mouth with distilled water and proceed to the next cup. He was allowed to taste each cup more than once if he wished. When he completed all 4 cups in one row, the subject was asked to indicate which one contained the salt or sugar. If the selection was difficult, he was forced to guess. Comments made by the subjects were recorded (for example, 'different', 'quinine water', 'Epsom salts', etc.). After completion of one set of cups, the subject was asked to repeat the entire procedure. Two thresholds were determined: the lowest concentration at which a subject could correctly detect a difference on both tests, and the point at which the subject correctly recognized and could name the substance in solution as salt or sugar on both tests. No errors in selection of a cup at higher concentrations than threshold were apparent when these criteria were utilized. On the basis of previous work by Pfaffmann *et al.* and preliminary work of our own, it did not appear that fatigue or adaptation was likely to occur with repeated tasting[6].

The first group of ten hypertensive patients was drawn from the outpatient hypertension clinics. Three were females, seven males; three were white and seven Negro; their ages ranged from 28 to 57 years. The second group of ten hypertensive patients was selected at the State Penitentiary and all were male. Two were white, eight Negro; their ages ranged from 38 to 63 years. Hypertension was diagnosed when diastolic pressures were regularly over 100 mm Hg; no subject was on restricted salt intake or drug treatment. Each of the total of 20 patients had a control of the same sex and race, and age within two years. These controls were drawn from the hospital environment for the hospital group, and from the penitentiary for the prisoner group. Race was thought to be particularly important in view of published reports of differences in tasting ability between white and Negro groups in the United States[7,8]. Age has also been thought to be important[9].

Each subject was tested 1½ h after the last meal and was asked not to smoke for a like period, although there is evidence to indicate that smoking does not affect taste thresholds[10]. The first group of hypertensives and their controls were told that they were to be tested for ability to taste salt or sugar. The second group of hypertensives and their controls were asked to taste the solutions and to make their own identification, but this group was tested only for salt.

The results were analysed by two-tailed t-tests for matched data, based on the logarithms of the dilutions rather than on the raw data, because of the logarithmic progression of dilutions utilized. Comparisons were made for salt both on thresholds for 'difference' and on thresholds for actual identification of specific taste; for sugar these two thresholds coincided. If the first (or 'difference') threshold for salt were used, no difference could be noted between hypertensives and controls ($t = 1{\cdot}03$ for the first group, 0·90 for the second). Hypertensives did show, however, a highly significant diminution (see Table 1) in their ability to taste 'saltiness' as compared with their controls ($t = 3{\cdot}07$, $P < 0{\cdot}02$ for first group; $t = 3{\cdot}75$, $P < 0{\cdot}01$ for the second). There was no difference in threshold for sugar between hypertensives and their controls ($t = 0{\cdot}03$).

Table 1. 'SALTINESS' THRESHOLDS OF HYPERTENSIVES AND CONTROLS (mg %)

Hospital group		Penitentiary group	
Hypertensives	Controls	Hypertensives	Controls
0·04	0·01	0·16	0·16
0·04	0·16	0·16	0·08
0·08	0·02	0·32	0·08
0·02	0·01	0·04	0·04
0·16	0·04	0·32	0·04
0·16	0·02	0·02	0·02
0·16	0·04	0·08	0·02
0·08	0·08	0·16	0·16
0·04	0·01	0·16	0·04
0·32	0·02	0·32	0·08

Our data suggest that hypertensives may eat more salt because they cannot taste it as well as can normotensive individuals. Obvious next moves are to see if the abnormality described in this paper exists in siblings, offspring and parents of hypertensives, and to follow normotensive individuals whose taste pattern suggests that which we have noted in hypertensives. Such non-hypertensive groups would have advantages over the subjects reported in this communication in that there would be fewer complications by reason of such factors as natriuresis secondary to vascular damage, or dietary alterations imposed by medical advice or by the reading of lay literature on hypertension. Confirmatory data in such subjects would greatly strengthen the hypothesis that salt intake is an ætiological factor in hypertension rather than merely a reflexion of the disease or its treatment.

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HÆMATOLOGY

Double Exponential Nature in Chromium-51 Red Blood Cell Survival Curves in Hæmoglobin E Thalassæmia and Relation with Reticulocytes

SEVERAL cases of hæmoglobin E thalassæmia were investigated for the survival of erythrocytes using auto red blood cells labelled *in vitro* with chromium-51. A standard method such as that described by Mollison[1] was adopted for the present investigation with the necessary precautions. Blood samples were collected at 10 min after the injection and afterwards on 1, 2, 3 and 5 days and thereafter every third day for a few weeks. Five ml. of blood sample was measured out, the plasma removed, hæmolysed with water to the original volume, and counted in a well-type scintillation counter at the completion of the test for each subject. The specific activity was plotted semi-logarithmically against time without any correction for chromium-51 elution. A pronounced bending in the curves was observed between 2–4 days, particularly in cases of patients. Normally, erythrocyte survival curves with chromium-51 could be fitted with an exponential type of decrease at least for the first few weeks with a little depression at the beginning due to a rapid elimination at the earliest stage[2]. Eadie[3] has, however, discussed the precise nature of the survival curves.

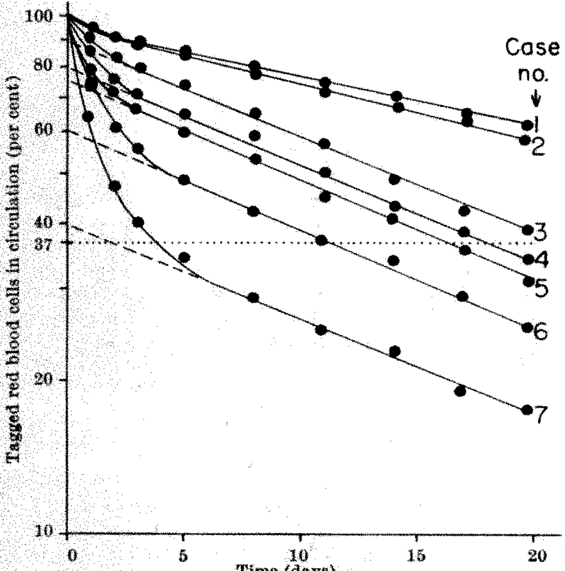

Fig. 1. Level of red blood cells labelled with chromium-51 in blood with time

Fig. 1 shows the survival curves of the red blood cells labelled with chromium-51 of the two normal subjects and five hæmoglobin E thalassæmia patients. The present curves for the patients show clearly the presence of double exponential nature consisting of a fast and a slow component. The gross mean life can be obtained directly from the curves as the time required to fall to 37 per cent survival, which ranges from 4 to 21 days for the five patients. These curves can, however, be analysed using an equation:

$$Rt = A e^{-r_a t} + B e^{-r_b t}$$

where Rt denotes the fraction of the tagged red cells in circulation at any time t, A and B represent the percentages of the so-called fast and the slow fractions, and r_a and r_b stand for their respective rate constants.

Table 1. ANALYSIS OF CHROMIUM-51 RED BLOOD CELL SURVIVAL CURVES AND RELATION WITH RETICULOCYTES OF HÆMOGLOBIN E THALASSÆMIA CASES

Case No.	Rectic. (per cent)	T_g (days)	A (fast) (per cent)	B (slow) (per cent)	T_a (days)	T_b (days)
1 N	0·3	44	5	95	0·6	48
2 N	0·3	38	5	95	0·6	42
3 Th	4	21	10	90	0·7	24
4 Th	6	18	20	80	0·9	22
5 Th	7	16·5	25	75	0·9	24
6 Th	12	11	40	60	1·0	23
7 Th	28	4	60	40	1·0	24

The actual analysis of the curves shows the 'fast component' to be a rapidly eliminating fraction having a mean life of about a day, whereas the 'slow component' gives a mean life of 22–24 days (half-life about 16 days) for the patients. It is striking to note that the mean life of this second component for all the five patients is fairly constant, which is about half of that of normal subjects. It is obvious that the variable factors are the fractional quantities A and B, which are responsible for showing a variation in the gross mean life as obtained by chromium-51. Table 1 summarizes the results.

Fig. 2 shows a linear relationship of the gross mean life with the contribution of the components—an

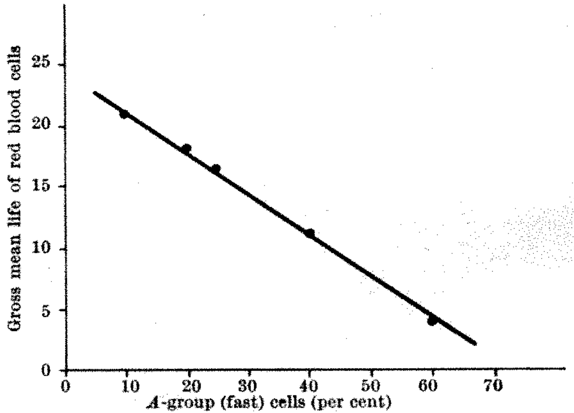

Fig. 2. Relation of the gross mean life of red blood cells of the patients with their 'fast fraction'

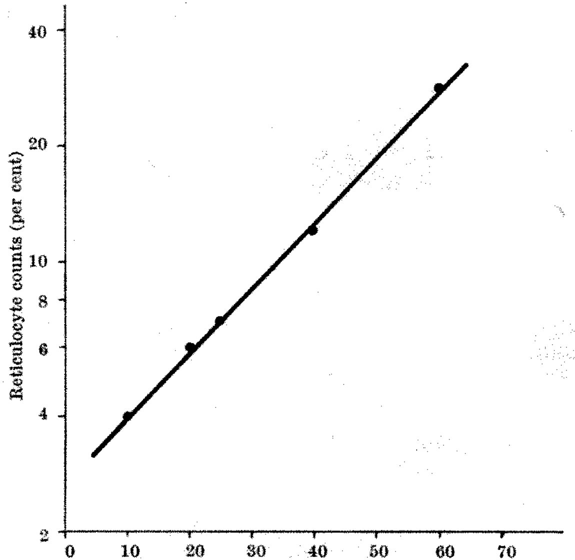

Fig. 3. Relation of the reticulocyte counts of the patients with their 'fast fraction'

inverse relation with the fast group. Attempts were made to correlate any of these fractions with any of the clinical parameters such as index of anæmia, plasma hæmoglobin, fœtal hæmoglobin, plasma bilirubin and reticulocytes. Only the reticulocytes percentage at the beginning of the test could show a relation with the quantities of these fractional components. Fig. 3 shows a semi-logarithmic relation of the reticulocyte percentage with the fast component.

The investigation leads to the conclusion that in all hæmoglobin E thalassæmia patients at least a fraction of the red cell population has fairly constant life span, though short compared with normal, which is independent of any index of anæmia. A rapidly eliminating fraction of the cell population is related to reticulocytes and probably to the very young cells, but there is no indication that they hæmolyse at the very early stage. It can, however, be said that this fraction may be rapidly eluting chromium-51 in the circulation, or alternatively, they may be actually dying out very rapidly due to trauma or other interference caused by the process of labelling which might indicate that they are very fragile in nature.

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HISTOLOGY

Submicroscopic Structure of the Peritrophic Membrane in Arthropods

A PERITROPHIC membrane, in the form of loosely connected concentric layers of substance wrapping the ingesta, has long been described in the mid-gut of different classes of arthropods (insects, myriapods, crustacea and onychophora).

Investigations with the electron microscope on the structure of this membrane have been so far reported only on insects[1-9].

The single layers, obtained by delamination of the concentric layers by mechanical procedures, consist essentially of fibrillar networks. The size and disposition of the fibrils in the networks vary in the different insects. In some species, a thin amorphous film is stretched across the holes of the network[2]. Unorganized fibrils, scattered at random in an amorphous ground substance, are associated with the networks in some insects[5] or are the only components detected in others[2,4,9].

In the work reported here, observations on the peritrophic membrane with the electron microscope have been extended to species belonging to other groups of arthropods : *Glomeris* sp., *Iulus albipes* Koch (Myriapoda), *Eriocheir sinensis* Milne-Edwards (Crustacea Decapoda), *Xiphosura* (= *Limulus*) *polyphemus* L. (Xiphosura).

In this material, the peritrophic membranes were collected by dissection of the midgut, washed and disintegrated in distilled water by shaking or by ultrasonic waves (Siemens, sonic emulsifier).

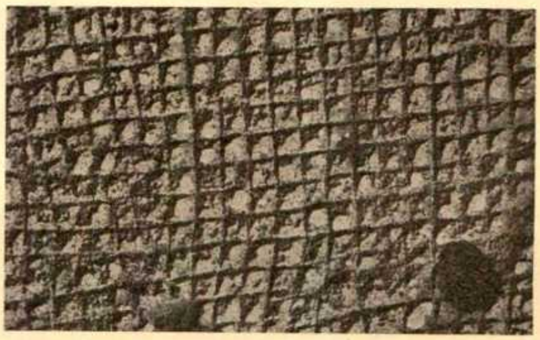
Fig. 1. Peritrophic membrane of *Iulus albipes* (× 23,500). (*Photo: Grégoire; R.C.A.*)

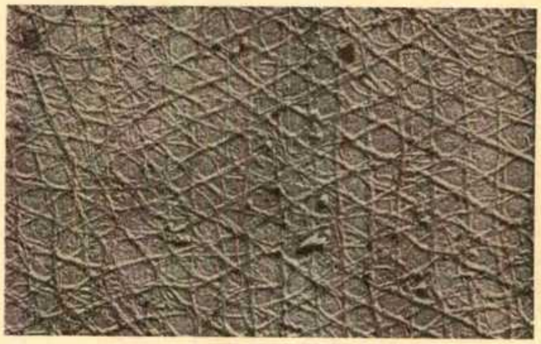
Fig. 2. Peritrophic membrane of *Eriocheir sinensis* (× 32,000)

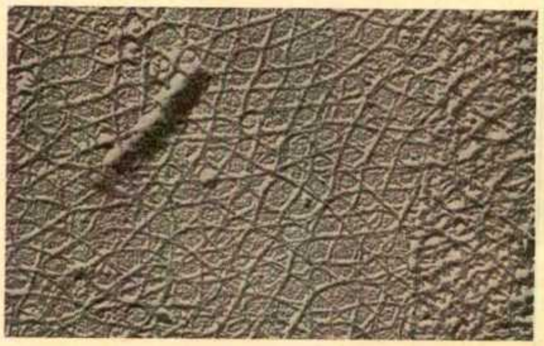
Fig. 3. Peritrophic membrane of *Xiphosura polyphemus* (× 34,400). (*Photo: Grégoire; R.C.A.*)

Drops of aqueous suspensions of these dissociated membranes were deposited on to 'Formvar' coated screens, drained, dried, shadow-cast with palladium, and examined with the electron microscope (R.C.A. Emu-2 or Siemens Elmiskop 1).

As in several insects[5], the peritrophic membranes of these arthropods consist of fibrillar networks. Because of the extreme flexibility of the single layers, easily shrunk, stretched or folded during desiccation on the 'Formvar' films, the patterns of the networks may assume various appearances in the different portions of the preparations. However, in *Iulus albipes*, two systems of parallel fibrillar strands, disposed at right angles or obliquely and delimiting rectangular or lozenge-shaped holes (Fig. 1), were observed in most preparations. In *Eriocheir sinensis* (Fig. 2) three systems of strands enclosed hexagonal holes. A similar structure, though less regularly disposed, was recorded in the peritrophic membrane of *Xiphosura polyphemus* (Fig. 3). Fibrils were especially visible in the strands of the membrane of *Eriocheir sinensis* (Fig. 2). The presence of a typical peritrophic membrane in *Xiphosura* is of particular interest, owing to the fact that this structure has not been described in Chelicerates, with the exception of the old observation of Tulk[10] in *Phalangium opilio*.

These observations suggest that fibrillar networks characterize the submicroscopic structure of the peritrophic membrane not only in insects but also in the other classes of arthropods.

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Intracellular Calcification in a Coccolithophorid

Hymenomonas sp., a euryhaline coccolithophorid chrysomonad, was raised axenically in a chemically defined medium[1] containing lactic acid, modified by raising the concentration of magnesium and calcium to 10^{-2} M, of phosphate to 10^{-3} M and adding carbonate 10^{-6} M and 10 mg per cent ethylenediamine tetraacetic acid. Coccolith formation was discernible macroscopically when these organisms were cultured in constant fluorescent light for 2 weeks at 18°–20° C. Phase-contrast microscopy of the growing cultures showed abundant coccoliths extracellularly and on the surface of the chrysomonad. The appearance of some individual protozoa suggested that coccoliths might be located intracellularly. This observation is supported strongly by the following findings.

Actively growing cultures were centrifuged at 1,200 *g* for 15 min at 4° C. The precipitate, fixed in 10 per cent (v/v) neutral formalin in 95 per cent (v/v) ethanol, was embedded in paraffin and cut serially at 4μ; undecalcified sections were examined with the following histological and histochemical procedures[2]: von Kossa, hæmatoxylin-eosin, periodic acid-Schiff, methylene blue extinction and toluidine blue stains. Bright-field, phase, dark-field and polarization microscopy were utilized on stained and unstained sections.

These investigations revealed intracellular, von Kossa-positive, ovoid bodies which were periodic acid-Schiff-positive, metachromatic and strongly basophilic at *p*H 2·62. The average cell had about 5 of these anisotropic bodies, the variable diameters of which ranged from approximately 1/10 to 1/20 of the cell diameter. Decalcification of the sections with either 0·1 N ethylenediamine tetraacetic acid or 0·1 N hydrochloric acid while under microscopic observation resulted in still discernible intracellular bodies which had become isotropic and had lost the ability to stain with silver nitrate, while all other reactions remained unchanged. The same results were obtained with extracellular coccoliths.

These data are consistent with the hypothesis that in *Hymenomonas*, a representative of the most primitive protozoa, coccolithogenesis is an intracellular, biphasic process, adhering to the general pattern of biological calcifications. It is suggested that the primary formation of the organic matrix of the coccolith is followed by a secondary calcification. The mature coccoliths are then extruded into the surrounding medium. X-ray diffraction powder investigations and chemical analyses of the extracellular coccoliths lend further support to the present histological observations[3].

These observations confirm and extend the reports by Parke and Adams[4], and Paasche[5]. The chemical nature of the matrix, the metabolic processes involved in this primitive calcification and the role of photosynthesis in this process are at present under investigation.

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PATHOLOGY

Effect of Cholesterol Oleate on the Phagocytic Function of the Reticulo-Endothelial System

THE cells of the reticulo-endothelial system in contact with the blood stream are found mainly in the liver and spleen. One of their more important functions is phagocytosis of particulate matter such as bacteria, virus, colloidal dyes or finely divided carbon. It seemed reasonable to assume that the function of these cells may be modified by the nature of ingested material. This hypothesis has been explored by injecting intravenously different suspensions of fatty acid esters of known chemical constitution and afterwards measuring the reticulo-endothelial system activity by the carbon clearance test. Thus it has been shown that glyceryl trioleate stimulates the reticulo-endothelial system function whereas ethyl oleate has a depressant action[1]. This suggested that the alcohol with which the fatty acid was combined might be of importance in determining the biological actions of the ester. These findings have been confirmed and extended[2,3] using various esters derived either from glycerol or ethyl alcohol. Accordingly, it seemed of interest to study the effects of a cholesterol ester and compare its action with the substances previously mentioned.

A 10 per cent suspension of cholesterol oleate (B.D.H.) was made by homogenizing the melted lipid in 5 per cent dextrose water containing 1 per cent 'Tween 20'. The particle size was very variable and many measured between 5 and 15μ. This was injected intravenously into male mice weighing 15–25 g. The granulopectic activity of the reticulo-endothelial system[4] was measured after intravenous injection of colloidal carbon in a dose of 8 mg/100 g. The phagocytic index K measures the rate of clearance of carbon from the blood and the corrected phagocytic index α is derived from the equation $\alpha = \sqrt[3]{K} \times \frac{W}{wls}$ where W = body-weight and wls = weight of liver and spleen. Following doses of 10 and 30 mg of cholesterol oleate the granulopectic activity was measured in groups of eight mice at intervals of 1, 2, 3, 4, 5, and 7 days. The control group is composed of eighteen normal mice. Table 1 records the average of results obtained in this experiment.

Table 1. EFFECT OF 10 AND 30 MG DOSES OF CHOLESTEROL ESTER ON THE PHAGOCYTIC COEFFICIENT K AND a

Dose	Indices	Time in days after injection					
		1	2	3	4	5	7
10 mg	K a	0·018 3·0	0·029 4·1	0·035 4·5	0·044 4·2	0·080 4·5	0·049 4·1
30 mg	K a	0·010 2·1	0·010 1·8	0·016 1·6	0·024 2·5	— —	0·055 3·2
Control	K a	0·044 4·4					

It was noted that the largest dose which could be given without mortality (mainly in the larger mice) was 50 mg. The spleens of all animals in this large dosage group were abnormal on examination by naked eye 24 h later. They were granular, tomato pink and slightly enlarged. Paraffin sections for microscopy were prepared from both livers and spleens. The histology of the spleen is shown in Fig. 1. It appears that cholesterol oleate in a dose of 30 mg causes a severe inhibition of phagocytic function for two or three days. This degree of blockade compares favourably with that obtained by injection of inert colloids such as saccharated iron oxide and unlike the latter is not followed by a phase of hyperactivity. The advantages in the use of this material are, first, the particles are not agglutinated by serum and the risk of embolism is reduced; secondly, the particle size can be controlled by conventional methods of preparation; and thirdly, it is almost certainly metabolized and excreted. Following the larger dose of 50 mg the lymphoid follicles of the spleen remain intact but are surrounded by a fatty red pulp of poor phagocytic capacity. This histological change suggests that cholesterol esters may be useful for investigating the interdependence of phagocytic and lymphoid tissue in immunological reactions. Unfortunately this 50 mg dosage causes focal necrosis in the liver although perhaps this could be circumvented by the use of a more uniform preparation of smaller particle size.

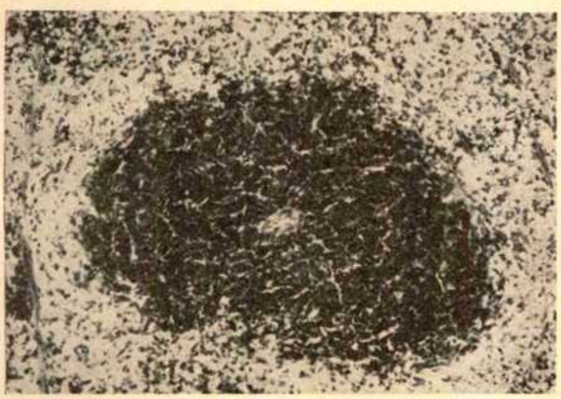

Fig. 1. Spleen after 50 mg cholesterol oleate showing intact Malpighian body surrounded by hypocellular fatty pulp. (Hæmatoxylin and eosin, × 90)

The 10 mg dose produces no striking changes in reticulo-endothelial system function, a finding which contrasts markedly with the stimulating effects of the glycerol esters, triolein and tricaprin. Although the reasons for these differences are not known, the finding lends further support to the view that the reticulo-endothelial system activity can be modified by administration of lipids the chemical nature of which is important in determining the effect produced.

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Anaphylaxis in Mice with Fragments of Rabbit Antibody

AT present there is much interest in the relationship between the sensitizing capacity of antibodies and their affinity for tissues. Ovary and Karush[1] found that univalent fractions I and II (*F* I, *F* II) obtained from purified rabbit anti-haptene antibody by papain digestion were unable to sensitize the guinea pig for direct passive cutaneous anaphylaxis (PCA) or reversed PCA. They postulated that the site necessary for the fixation of antibody to guinea pig tissues was not present on fractions I and II but was carried by fraction III. Reversed PCA could be produced with *F* III and horse anti-rabbit globulin. Ishizaka *et al.*[2] stated that pepsin-digested rabbit globulin, which presumably lacks fraction III, would not induce inflammatory reactions in guinea pig skin and would not fix complement. Taranta and Franklin[3] also found that pepsin-digested antibody did not fix guinea pig complement *in vitro*. It seemed of interest to us to test papain and pepsin digesteo fragments of rabbit γ-globulin in a system in which tissue fixation of antibody does not seem to play a part, namely, anaphylaxis in the mouse. In this species maximal incidence of anaphylaxis with rabbit antibody can be obtained by injecting antibody and antigen intravenously in rapid succession[4].

Swiss-Webster female mice weighing 17–25 g were inoculated intraperitoneally with 5 billion *B. pertussis* organisms to increase their susceptibility to passive anaphylaxis[5]. Five days later the animals were injected intravenously with antibody and antigen in rapid succession. Rabbit γ-globulin was degraded with papain by the method of Porter[6] and with pepsin by the method of Nisonoff *et al.*[7]. Digestion of rabbit γ-globulin with papain results in the production of

Table 1. PASSIVE ANAPHYLAXIS WITH RGG*, *F* I† AND *F* II† AS ANTIBODY

Pertussis-sensitized mice injected intravenously‡ with:	Deaths/Total
2 mg *F* I§ + 1 mg BSA	0/8¶
2 mg *F* II + 1 mg BSA	0/8
2 mg RGG* + 1 mg BSA	4/8
2 mg non-immune RGG + 1 mg BSA	0/8

* Rabbit γ-globulin, 40 per cent antibody; prepared from anti bovine serum albumin rabbit serum by precipitation with sodium sulphate.

† Papain digests of rabbit γ-globulin mentioned above (anti-bovine serum albumin) method of Porter (ref. 6).

‡ Antibody and antigen injected in rapid succession.

§ 5 × this amount of *F* I had no effect.

¶ No symptoms of anaphylaxis.

Table 2. PASSIVE ANAPHYLAXIS WITH PAPAIN OR PEPSIN DIGESTED RABBIT ANTIBODY

Pertussis-sensitized mice injected intravenously with:	Deaths/Total
5 mg *F* I* (rabbit anti-mouse globulin)†	0/5
5 mg *F* II (rabbit anti-mouse globulin)	0/5
0·4 mg rabbit anti-mouse globulin (pepsin digested)	0/5
4 mg rabbit anti-mouse globulin (pepsin digested)	9/13
0·4 mg rabbit anti-mouse globulin (untreated)	6/10
4 mg rabbit anti-mouse globulin (pepsin digested and treated with cysteine)	0/10
10 mg anti-BSA (pepsin) + 1 mg BSA	2/8
1 mg anti-BSA (untreated) + 1 mg BSA	5/9
10 mg anti-BSA (pepsin) + 1 mg bovine gamma globulin (BGG)	0/5

* Antibody fragments from papain digested rabbit γ-globulin.

† 38 per cent antibody.

univalent antibody fragments (*F* I, *F* II). Digestion of rabbit γ-globulin with pepsin results in a decrease in sedimentation coefficient from $7S$ to $5S$ without destruction of its capacity to form specific precipitate, indicating that the $5S$ fragment is bivalent. Treatment of the $5S$ unit with a disulphide-splitting agent such as cysteine results in the formation of fragments with the properties of univalent antibody and $S = 3{\cdot}5$ (ref. 7).

The results in Table 1 demonstrate that 4 of 8 pertussis-inoculated mice succumbed to anaphylactic shock when injected intravenously with 2 mg of rabbit γ-globulin (anti-bovine serum albumin) containing 0·8 mg antibody, and 1 mg of bovine serum albumin. However, mice injected with bovine serum albumin and 2 mg (or even 10 mg) of *F* I or *F* II prepared from the aforementioned rabbit γ-globulin by digestion with papain showed no symptoms of anaphylaxis. The failure of *F* I or *F* II to produce anaphylaxis in mice cannot be explained by the inability of these fractions to combine with bovine serum albumin since *F* I was found to inhibit the hæmagglutination of bovine serum albumin coated red cells by antibovine serum albumin serum.

The results in Table 2 demonstrate that pepsin digested rabbit anti-mouse globulin killed 9 of 13 pertussis-inoculated mice, and pepsin digested rabbit anti-bovine serum albumin killed 2 of 8 animals. Addition of cysteine to pepsin degraded antibody destroyed its lethal effect, as well as its ability to precipitate with homologous antigen. It should be noted that pepsin digested rabbit antibody was only about 1/10 as active on a weight basis as undegraded rabbit antibody. However, it is unlikely that the biological activity of pepsin digested rabbit γ-globulin is due to the presence of small amounts of undegraded γ-globulin in the preparation. If such were the case, cysteine treated pepsinized antibody should kill mice since we have found that the activity of undegraded γ-globulin in mice is unaffected by 0·01 M cysteine. As shown in Table 2, pepsin digested rabbit γ-globulin treated with cysteine had no lethal effects.

The property of antibody which seems to be essential for the induction of anaphylaxis in the mouse is the capacity to form aggregates. Pepsin degraded bivalent antibody produced anaphylaxis in mice, but pepsin digested antibody reduced to univalent fragments by treatment with cysteine was inactive (Table 2), as were univalent papain digested fragments (Table 1). It is possible that anaphylaxis in the mouse is initiated by antigen-antibody aggregates in the circulation which trigger an anaphylotoxin-like mechanism.

It should be noted that the ability of pepsin digested rabbit antibody to kill mice is not likely due to tissue-fixing properties of the antigen with which it combines. Pepsin digested anti-bovine serum albumin plus bovine serum albumin was lethal to mice, but it is unlikely that bovine serum albumin fixes to mouse tissue, since it has recently been demonstrated that bovine serum albumin will not produce reversed passive cutaneous anaphylaxis in mouse skin[8].

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Intracellular Lymphocytes in Leukæmia

IT has recently been reported that lymphocytes may exist inside large cells cultured from leukæmic human bone marrow during the phase of remission[1].

This phenomenon occurred exceptionally also in our material. Bone marrow was grown in Sykes–Moore chambers from a man with chronic lymphatic leukæmia and Coombs's positive hæmolytic anæmia. Large elongated cells developed rapidly. These cells appeared to be surrounded and attacked by lymphoid cells growing in the same bone marrow specimen (Fig. 1). The affair usually ended with the death and disintegration of the large cells.

Bioassays in new-born or young Swiss mice yielded negative results within a relatively short period of observation (5–12 months): 1·6 per cent of 488 mice inoculated with fluids from this and other leukæmic human bone marrow tissue cultures, or with leukæmic human brain extracts, developed generalized lymphatic leukæmia, as compared with 1 per cent incidence of leukæmia in 384 control mice inoculated with non-malignant human bone marrow tissue culture fluids or brain extracts. This is in good correlation with results obtained in other studies[2,3]. (*Note added in proof*. These human leukæmic brain extracts were claimed to have caused a high incidence of ascitic mesenteric lymphosarcomas in adult Swiss mice within 2–5 weeks (Schwartz, S. O., Gordon Research Conferences, Cancer, 1961).)

As to the mechanism of the intracellular existence of lymphocytes in leukæmia, the following considerations are suggested: (1) Histiocytes engulf leukæmic lymphocytes in order to destroy them. (2) The large cells may serve as a feeder layer for the lymphocytes.

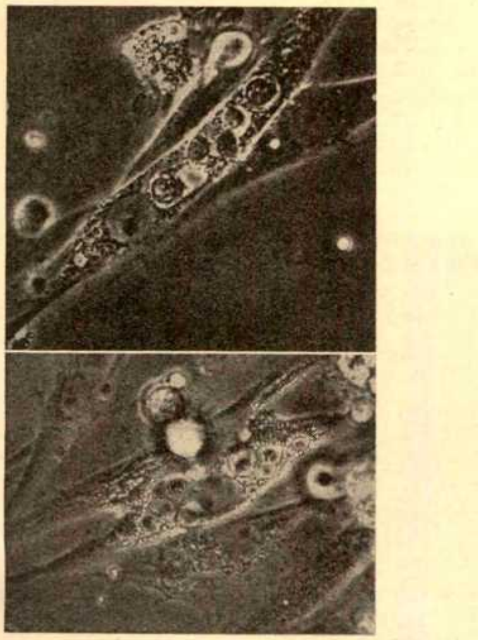

Fig. 1. Two large elongated cells grown from a leukæmic human bone marrow are invaded by lymphoid cells of the same bone marrow tissue culture. (Phase contrast)

(3) Lymphoid cells of auto- or homo-logous immune activity attack host cells. Autoimmune phenomena are frequent in human lymphatic leukæmia[4,5], and occur in mouse leukæmia[6]. Emergence of immunologically hostile lymphoid cell clones may be stimulated by a leukæmogenic virus in mice[7]. Lymphocytes are known to be capable of attacking Rous sarcoma cells[8] and homologous mouse cells[9], leaving disintegrating carcasses of such cells behind.

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ANATOMY

Ion Etching of Dental Tissues in a Scanning Electron Microscope

TOOTH sections have been bombarded with a 5-kV argon ion beam inside the specimen chamber of a scanning electron microscope. This apparatus was designed so that the ion beam could be focused into a 1·5-mm diameter spot on the surface of the specimen, covering the area viewed by the microscope. The ions were extracted from a radio-frequency ion source and were focused by two electrostatic lenses on to the specimen, through two apertures. The pressure in the specimen chamber when the ion source was running was better than 10^{-6} mm mercury. The ion beam density was not uniform over its cross-section, but varied from about 10 m. amp/cm^2 at the centre to zero at the edge; this enabled us to check that, for the current densities we utilized, the nature of the changes in the specimen surface that was produced was not a function of the rate of etching. The bombarded area was kept to a minimum to reduce the amount of material deposited on the electron collection system, and reduce the temperature rise, which we have calculated to be less than 100° C, using the values of thermal conductivity given by Craig and Peyton[1]. The total ion current was kept to about 15μ amp. The micrographs were taken with a 16-kV scanning electron beam and only the high-energy reflected electrons were used to form the picture. This allowed micrographs to be taken with a resolution better than 500 Å., even though the specimen tended to charge under the electron beam. This method of observation also allows pictures to be taken while the surface is under bombardment; but for this work it was found preferable alternately to bombard for, say, 5 min, and then observe the surface at leisure. The ion beam made an angle of about 40° to the specimen normal: the direction is indicated by the arrow on the micrographs. The surface was also viewed at an oblique angle, as is indicated by the magnification symbols on the micrographs (Figs. 1 and 2).

The specimens, which were sections of teeth (human, rodent (*Myocaster coypu*) and marsupial (*Macropus*)), were polished down to a thickness of about 100μ before mounting. They were further polished on metallurgical polishing papers (down to a grade 4/0, John Oakey and Sons, Wellington Mills, London, England), after sticking them with 'Araldite' adhesive to an aluminium backing plate. Before bombardment, the specimens were covered with a 300 Å. layer of aluminium to keep the electrical resistance from the specimen to earth as low as possible; this thickness of aluminium would be removed by the ion beam in less than 10 sec.

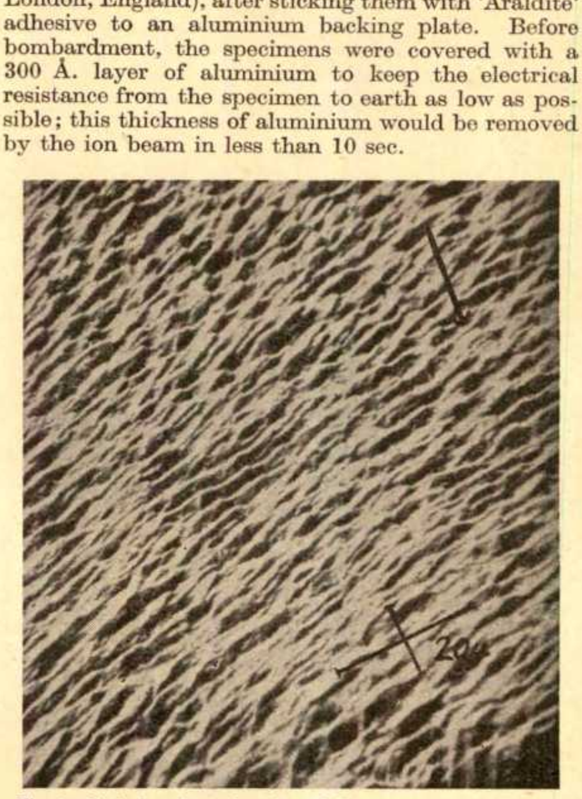

Fig. 1. Etched surface of section of *Macropus* enamel, showing enamel rods running nearly parallel to the surface

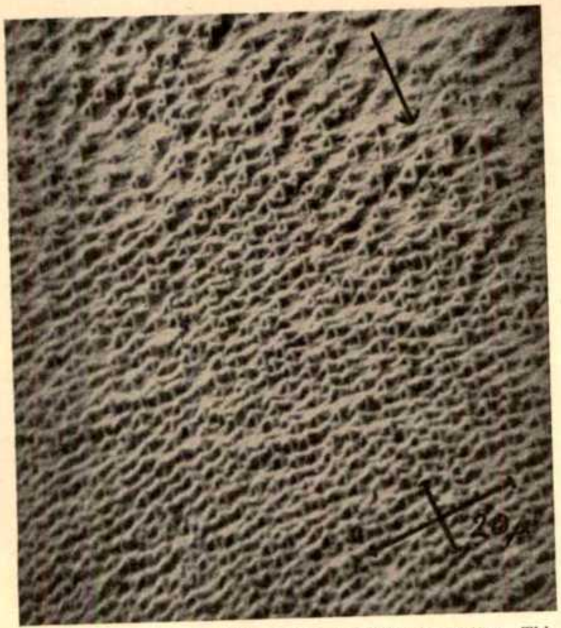

Fig. 2. Etched surface of the section of *Macropus* dentine. This region is close to the pulp chamber, hence the dentinal tubules are close together. The peritubular dentine can be seen 'standing proud' of the intertubular dentine

After observation in the scanning microscope the specimens were also examined by optical methods and by taking replicas, which were studied in a Siemens electron microscope. The surface was found to be hard, and true replicas could be produced without pulling crystallite away from the surface.

For a given ion beam, the rate of etching depends on the chemical composition of the specimen at the surface and on the angles that the ion beam and the crystal lattice make with the specimen surface.

In a region of constant composition, the production of a surface topography due to differential etching-rates can only depend on differences in the orientation of crystalline structural units that have been etched away. Enamel has a fairly uniform composition, but it was etched differentially to produce a surface profile in which the rod structure of the tissue could be visualized (Fig. 1). We suggest, therefore, that the differences in sputtering-rate which we have observed in enamel are related to the differences known to exist in the orientation of the crystallites of hydroxyapatite[2], and that ion beam etching may have a potential as a method for revealing these differences. Dentine was etched away more rapidly than enamel: enamel is known to be the more highly mineralized. Intertubular dentine was eroded more rapidly than peritubular dentine (Fig. 2): the latter is again known to be the more highly mineralized. We suggest that these differences in sputtering-rates are primarily related to these differences in mineralization.

The scanning electron microscope proved of great value in enabling the etching to be followed, until a convenient amount of material had been removed. As the specimen was bombarded in its correct position for viewing by the scanning electron microscope, previously observed areas could be rapidly re-located after each period of bombardment.

Although Figs. 1 and 2 are scanning electron micrographs, replicas were taken of the specimen surface after it had been removed from the scanning microscope, and these were examined in a transmission electron microscope (Siemens Elmiskop I).

Good replicas were obtained, and there was no sign of any material having been pulled away from the surface with the replica, which is a common fault when replicas are taken of surfaces that have been etched with chemical solutions.

We thank Prof. C. W. Oatley for his encouragement and the facilities provided, and the Atomic Energy Research Establishment, Harwell, for financial support. Some of the material was purchased out of a grant from the Advisory Medical Research Committee Fund of the London Hospital and Medical College. We would also like to thank Major J. J. Buxton for his continued help.

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RADIOBIOLOGY

Treatment for Removal of Inhaled Radioactive Ceria ($^{144}CeO_2$) from the Lungs of Rats

INHALATION is one of the most probable routes for entry of radioactive elements into the body. Relatively insoluble, radioactive particles deposited in the lung are normally retained for long periods of time resulting in high, local radiation dosages which may lead to pulmonary damage, for example, fibrosis and carcinoma[1]. Therefore, it is important that methods are available for increasing the rate of clearance of radioactive particles from the lung and from the body. This communication reports preliminary results of tests to remove inhaled ceria ($^{144}CeO_2$) (in equilibrium with its daughter product, praseodymium-144). Cerium-144 is a fission product occurring mainly in insoluble form and accounts for much of the radioactivity remaining 1–4 years after nuclear fission[2]. Cerium-144, with a 290-day half-life, decays by β-γ emission to praseodymium-144, which has a 17-min. half-life.

The techniques used for exposing ten-week-old Sprague–Dawley strain rats to radioactive aerosol were essentially those described by Willard[3]. The rats were exposed for about 15 min. to an aerosol generated from a 0·3 per cent suspension of $^{144}CeO_2$. At the end of the exposure to radioactive aerosols the rats were divided into various groups and sub-groups for subsequent treatment. The number of animals used and the treatment to which they were exposed are given in Table 1. The test materials were administered as aerosols for a period of 30 min., in the same exposure chamber used for administering the radioactive material. A group of rats was treated immediately after exposure, then twice a day for the following three days, and once a day for the next three days, and thereafter every second day until the fifteenth day. Another group of rats was given the same treatment regimen, except for omission of the treatment immediately following exposure to $^{144}CeO_2$. Rats were killed in the first experiment by intraperitoneal injection of sodium pentobarbital one week after the last treatment (21 days post-exposure), and in the second experiment, on the day following the last treatment (15 days post-exposure). The lungs and liver were removed and prepared for counting by wet ashing[4]. Samples were counted with an end window proportional counter.

Table 1. EFFECT OF DIFFERENT AEROSOL TREATMENTS ON REMOVAL OF INHALED ^{144}Ce–^{144}Pr FROM RATS

Rats were treated by 30-min. exposures to aerosols produced from 1 per cent phenylephrine hydrochloride, 25 per cent DTPA, and 3 per cent 'Pluronics *F*68' solution. The animals were killed in the first experiment one week after the last treatment (21 days after exposure), and in the second experiment the day following the last treatment (15 days after exposure)

Experiment	Treatment regimen		^{144}Ce–^{144}Pr content of tissues*			
			Retained in lungs		Retained in liver	
	Aerosol	Time started†	nc‡	Per cent of controls	nc‡	Per cent of controls
1 (3 animals per group)	Controls (untreated)	1 hr.	240 ± 26	100	64 ± 12	100
	Phenylephrine hydrochloride		62 ± 8·0	26	12 ± 2·2	19
	Phenylephrine hydrochloride + DTPA		22 ± 5·1	9	2·1 ± 0·3	3
	DTPA		16 ± 3·4	7	2·7 ± 0·5	4
	Phenylephrine hydrochloride	24 hr.	88 ± 27	37	13 ± 1·6	20
	Phenylephrine hydrochloride + DTPA		35 ± 11	15	7·3 ± 4·4	11
	DTPA		55 ± 9·0	23	7·3 ± 2·7	11
2 (5 animals per group)	Controls (untreated)	1 hr.	940 ± 120	100	65 ± 8·6	100
	Phenylephrine hydrochloride		920 ± 100	98	60 ± 7·8	92
	Phenylephrine hydrochloride + DTPA		77 ± 11	8	5·6 ± 0·5	9
	DTPA		95 ± 26	11	6·4 ± 1·2	10
	'Pluronics *F*68'		220 ± 59	23	13 ± 5·4	20
	Phenylephrine hydrochloride	24 hr.	860 ± 98	91	56 ± 8·4	86
	Phenylephrine hydrochloride + DTPA		290 ± 19	31	14 ± 2·3	22
	DTPA		290 ± 58	31	14 ± 1·9	22

*The ^{144}Ce–^{144}Pr content of the carcasses, as well as that of the lungs and livers, was decreased by the therapy.

†Post-exposure.

‡Mean and 95 per cent confidence intervals around the mean.

DTPA (diethylenetriamine pentacetate) was the most effective agent for removing radioactive particles from the lungs. The lungs of the DTPA-treated animals contained 90 per cent less ^{144}Ce–^{144}Pr than control animals. ^{144}Ce–^{144}Pr was excreted without significant deposition in liver, in which it generally accumulates. DTPA exerted its effect not only when administered immediately after inhalation of radioactive particles, but also when treatments were started on the next day, although to a lesser degree. Experiments now in progress using whole-body counting techniques indicate that DTPA is also effective in removing ^{144}Ce–^{144}Pr from the lungs of dogs.

'Pluronics *F*68' (Wyandotte Chemical Co.), a non-ionic dispersing agent, was less effective than DTPA. Possibly the action of this dispersing agent was limited to the upper part of the respiratory tract; thus accounting for its lesser effectiveness. In a separate experiment the difference in concentration of DTPA and 'Pluronics *F*68' aerosols did not account for the greater effectiveness of DTPA in removing ^{144}Ce–^{144}Pr.

The results of the phenylephrine hydrochloride treatments were not conclusive.

These experiments show that the chelating agent, DTPA, very effectively removes relatively insoluble ^{144}CeO$_2$ particles from the lung. These encouraging results suggest that other insoluble radioactive particles may be similarly removed from the lung if appropriate chelating agents are found. In selecting the chelating agent consideration must be given to the possibility of translocating radioisotopes from the lung to more radiosensitive organs. In the present case, ^{144}Ce–^{144}Pr cleared from the lung was also removed from the body.

We thank the Geigy Pharmaceutical Co. for supplying the DTPA, and Mr. M. D. Snyder for making the aerosols and for his valuable help during the experiments. We also thank Mr. W. Skinner, Mrs. E. T. Edmerson and Mr. J. P. Herring for preparing and counting samples.

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Balance of Potassium, Rubidium and Cæsium between Japanese People and Diet and Assessments of their Biological Half-Times

THE biological half-times of radionuclides of the alkali metals have been determined by several investigators by using laboratory animals and even man; excretion and retention analyses were used following a single dosage of radionuclide for the calculation. These methods, however, are subject to criticism because of the extrapolations of animal data to man and also the use of a single dose for a long-term administration; this is especially the case with cæsium-137, which has been incorporated in man through foodstuffs as a result of world-wide contamination from nuclear weapon testings.

The biological half-time of cæsium-137 is short and an equilibrium is attained between the human body and diet within a year. When equilibrium has been attained, the ratio of the levels of cæsium-137 in the human body and diet should be the same as that of non-irradiated cæsium. Thus it would be possible to presume the levels of cæsium-137 in man on the basis of diet-levels. This consideration inspired an examination of the distribution of common alkali metals in man and his diet.

Measurements were made of potassium, rubidium and cæsium in the human soft tissues, muscles and bones from autopsies collected in Tokyo during August 1959–August 1960. The complete data and discussions were published elsewhere[1]. The total-body amount of potassium and cæsium was estimated from the concentrations in muscle, bone and soft tissues, and the weight percentage composition of standard man, assuming the total-body consists of 43 per cent of muscle, 14 per cent of bone with bone marrow and the remainder of soft tissues. In the case of rubidium, the average ratio of rubidium/potassium in the analysed tissue samples ($2·7 \times 10^{-3}$) was multiplied by the total-body potassium for the estimation.

The concentrations of potassium and cæsium were respectively as follows: 2·39 mg and 29·5 mμg in muscle, 1·89 mg and 15·7 mμg in bone and 1·51 mg and 12·7 mμg in soft tissues (per gram of fresh tissues). The estimate for the standard man of 70 kg body-weight showed the total-body amount of potassium as being 136 g or 0·19 per cent by weight; these values are in good agreement with those based on body radioactivity measurements reported by other authors[2]. The concentration of rubidium and cæsium in the total-body was $5·1 \times 10^{-4}$ and $2·0 \times 10^{-6}$ per cent by weight respectively and the total amount in the standard man was 0·36 g and $1·4 \times 10^{-3}$ g, respectively. The estimate of the total-body cæsium recommended by the International Commission on Radiological Protection, namely, less than 10^{-5} g, seems astonishingly low in comparison with the present estimate. On the other hand, rubidium was found approximately one-third of the recommended value of 1·2 g.

Total diet samples were supplied by Dr. H. Hayami of the National Institute of Nutrition, Japan. The complete diet consumed by a member in a family was collected from ten families chosen at random from a number of sites in Japan. The category of family was divided into three: urban adult, rural adult and rural children. The composite sample of ten families was analysed for potassium, rubidium and cæsium. The results are shown in Table 1. The daily intake of the alkali elements by Japanese people would lie somewhere between the values for urban and rural, and an approximate estimation by a simple averaging resulted in 1·4 g of potassium, 1·5 mg of rubidium and 0·01 mg of cæsium as the daily intakes by an adult Japanese.

Reliable statistical data for the average body-weight of adult Japanese are not available, but 56 kg is believed as a justifiable value which is equivalent to 80 per cent in standard man. On this basis, the total-body amount for Japanese was computed as shown in Table 2.

Assuming a single exponential function in excretion and the complete absorption from the gastro-intestinal tract, the biological half-times (days) of potassium, rubidium and cæsium were calculated as 54, 140 and 76, respectively, as presented in Table 2.

Table 1. DAILY INTAKE OF POTASSIUM, RUBIDIUM AND CÆSIUM BY JAPANESE PEOPLE

Category	K (mg)	Rb (mg)	Cs (μg)
Urban adult	1,231 ± 244 (9)	1·26 ± 0·22 (7)	8·9 ± 2·6 (9)
Rural adult	1,638 ± 426 (9)	1·65 ± 0·33 (7)	11.0 ± 2·5 (9)
Rural children	1,014 ± 329 (6)	1·44 ± 0·87 (5)	8·6 ± 4·2 (6)

No. in parentheses represent number of composite (10 families each) samples of complete diet. The means and errors are based on assumed normal distribution.

Table 2. ESTIMATES OF BIOLOGICAL HALF-TIMES OF POTASSIU RUBIDIUM AND CÆSIUM ON THE BASIS OF DAILY INTAKE AND TOTA BODY AMOUNT (56 KG BODY-WEIGHT)

	K (g)	Rb (mg)	Cs (mg)
Total-body amount (Q)	110	300	1·1
Daily intake (q)	1·4	1·5	0·01
Q/q	79	200	110
Biological half-time (day) ($Q/q \times 0·693$)	54	140	76

These values are in agreement with the half-time (days) in total-body recommended by the International Commission on Radiological Protection namely, 58, 45 and 70, respectively, except fo rubidium. The reason for the higher value o rubidium is not clear.

C. R. Richmond presented the value of 35 days fo potassium on the basis of the daily intake and bod content: 2·6 g and 0·19 per cent of the body-weigh respectively[3]. The value of 2·6 g, which was derive from the observed urinary excretion by E. C. Anderso *et al.*[4], seems surprisingly high as compared with th dietary intakes in Japan.

The value of Q/q in Table 2, namely, the total-bod amount divided by the daily intake, serves for th presumption of total-body burden of a radionuclide an equilibrium is assumed between the human bod and diet. The daily intake of cæsium-137 multiplie by 110 will represent the total-body burden in a long term administration.

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BIOLOGY

Depletion of Carbon-13 in Young Kauri Tre

MEASUREMENTS have been carried out of th carbon-13/carbon-12 ratio in a New Zealand kau (*Agathis australis*) a thousand years old. This tre grew in the Omahutu Forest, in the north of Ne Zealand. In 1912 it was felled. The stump, fro which the specimen was taken, suffered in a fore fire in 1931. It is estimated that the fire remove the outer 10–30 growth rings.

Nine samples were analysed for carbon-1 The result of this analysis is shown in Fig. 1*b*, wher $\delta^{13}C$ has been plotted against the age of the sampl $\delta^{13}C$ is defined as the per mille enrichment of th carbon-13/carbon-12 ratio of the sample with respec to that of the P.D.B.-Chicago Belemnite Standard.

In 1954 Craig[1] determined the variation of δ^{13} in a *Sequoia* tree over a different time-interval an noted that the low value found for $\delta^{13}C$ in the youn tree had to be ascribed either to a change in $^{13}CO_2$/$^{12}CO_2$ in air or to differences arising within the tre itself (Fig. 1*a*).

Craig's result for *Sequoia* and the present result fo kauri are similar in shape, but 2,000 years apart i time. They would seem strongly to support a explanation which involves the difference betwee an older and a younger tree, rather than universa changes in the carbon-13/carbon-12 level of the ai

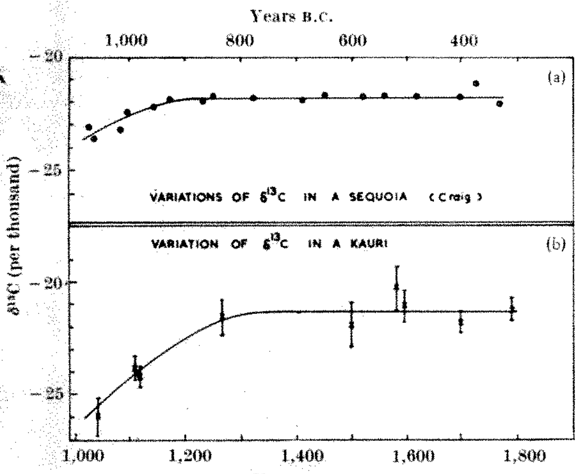

Fig. 1. δ^{13}C-values versus time for kauri (New Zealand) (*b*); and *Sequoia* (*a*).

The depletion in Craig's *Sequoia* is less than that in the kauri specimen described here.

A young tree obtains its carbon dioxide at a lower height above the ground than an older one. On this basis Keeling[2] expects Craig's *Sequoia* to be depleted in carbon-13 in the centre if a local cycling of carbon-dioxide occurs in the area where the tree grows. Whether such an explanation would fit the present results depends on the conditions which prevailed in the area during the period of time when the tree was growing.

However, a *Pinus radiata* from a well-ventilated location in Wainuiomata (near Wellington) was found to have the following δ^{13}C values:

Wood from centre (1880–83): –29·2 ± 0·5
Wood from outside (1955–59): –23·7 and –25·1 ± 0·5

It seems that the only alternative to differences in the carbon-13/carbon-12 ratio of the carbon dioxide absorbed by young and old (or small and tall) forest trees is the existence of differences in fractionation which arises in the trees themselves. For trees growing in exposed areas this interpretation seems to be the only possibility.

I thank Mr. J. R. Hulston of the Institute of Nuclear Sciences for analysing the samples.

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Resistance to Aluminium and Manganese Toxicities in Plants related to Variety and Cation-Exchange Capacity

ALUMINIUM and manganese toxicities are now recognized as being major factors in the soil acidity complex[1]. The adaptability of crop plants to acid conditions is of practical interest, and the question arises as to whether selection for persistence on acid soils can be made indirectly via selection for resistance to aluminium and manganese toxicities. Dessureaux[2] has achieved some success in selection for resistance to manganese toxicity in *Medicago sativa*.

Twenty varieties of *Lolium perenne* and *L. multiflorum* were screened for resistance to aluminium toxicity. Twenty seeds of each variety were sown in 800 gm. of silica sand contained in wax cartons (Mono Containers, Ltd.) of 500 ml. capacity, with three replications. The cartons had provision for drainage and were irrigated three times a week with 150 ml. of culture solution : 0·0015 M $(CaNO_3)_2.4H_2O$, 0·00075 M $MgSO_4.7H_2O$, 0·00075 M KH_2PO_4, 0·0001 M $FeC_6H_5O_7$ plus micronutrients and 50 p.p.m. aluminium as $Al_2(SO_4)_3$. Plants were gathered at the end of six weeks, and during the growing period they received aluminium in the culture solution during the first two and last two weeks.

Table 1. RESISTANCE OF RYEGRASS VARIETIES TO ALUMINIUM TOXICITY

Variety	Resistance to aluminium toxicity as per cent decrease in total yield from the control (zero = complete resistance)	
Ba. 6280	23	
Melle, pasture	27	
Westerwolth, Sceempter	36	
New Zealand, Short rotation	40	
Westerwolth, Mommersteeg	41	
S. 321	46	*L.S.D.* (P = 0·05) for figures within the bracket = 5·0.
S. 24	52	
S. 22	53	
Irish	53	
Danish leafy Italian ryegrass	55	
Hunsballe, late flowering	60	
Ayrshire	63	
New Zealand, Certified Mother seed	63	
Northern Ireland Devon Eaver, certified	64	
S. 23	69	
Heraf, pasture	69	
S. 101	73	
Danish, early flowering	73	
Sceempter	74	
Kent, indigenous	74	

Table 1 presents the results as percentage decrease in total yield from the control grown without aluminium. It is apparent that wide variation in resistance to aluminium toxicity exists between commonly grown ryegrass varieties. Within most varieties individual plants were found with unusual resistance to aluminium. This suggested the practicability of selection for increased resistance, and from an initial selection of the most resistant plants the 4th selection generation has now been reached. It was found that for later selection generations the concentration of the aluminium in the solution had to be increased.

A less-extensive comparison of ryegrass varieties for resistance to manganese toxicity, using a similar technique, with 25, 50 and 150 p.p.m. manganese as $MnSO_4.4H_2O$, has shown the varieties Heraf pasture and *S*. 321 to be highly resistant, and Melle pasture and Hunsballe late flowering to be susceptible. Selection for resistance to manganese toxicity has also been carried out.

The results in Table 1 suggest that, in general, resistance to aluminium toxicity is independent of geographical origin and hence the soil type on which the varieties were originally developed. This is clearly indicated by the Aberystwyth varieties (with prefix *S*. or *Ba*), which cover a wide range of relative resistance. This conclusion is not surprising, as the soils of plant breeding stations and seed growing farms are unlikely to be allowed to become so acid that high available aluminium becomes a factor in selection. Comparison of the relative resistance of varieties suggests that the annual and biennial varieties tend to show high resistance. This may in part be a reflexion of the normally greater seed size of these varieties, giving more rapid emergence and establishment, as we have indications that seed size and depth of sowing are factors influencing response to screening techniques.

A possible factor in resistance was cation-exchange capacity, and this has been examined. Selections resistant and susceptible to aluminium and manganese toxicities were grown in solution culture with and without aluminium for a period of 8 weeks. Determinations were made on the washed roots using the modified procedure of Crooke[3], and are presented in Table 2.

Table 2. CATION-EXCHANGE CAPACITIES OF ROOTS OF VARIETIES RESISTANT AND SUSCEPTIBLE TO ALUMINIUM AND MANGANESE TOXICITIES

Variety	Root cation-exchange capacity (mean of at least four determinations) m.equiv./100 g dry wt.	
	Control solution	Solution with 15 p.p.m. Al
Danish (susceptible to Al)	23·0	18·7
Ba 7486 (intermediate)	18·5	16·0
Ba 7485 (resistant to Al)	14·9	12·0
	Control solution	Solution with 15 p.p.m. Al
Danish (susceptible to Mn)	23·0	21·7
Ba 8200 (resistant to Mn)	16·2	19·7

It is clearly seen that, of the varieties tested, resistance to both aluminium and manganese toxicities is associated with low cation-exchange capacity. The effect in solution culture of a toxic level of aluminium is to lower the cation-exchange capacity of both resistant and susceptible varieties. Crooke[3] found a variable effect of heavy metal toxicity on cation-exchange capacity, an increase being most common, although manganese caused a reduction.

Data on the cation-exchange capacity of cultivated plants indicate that Leguminosae have approximately twice the cation-exchange capacity of Gramineae[4-6]. Further, that within either major group, species making most demands on soil fertility tend to have a higher cation-exchange capacity than do species less exacting in their requirements. It is possible that the association of aluminium and manganese toxicity resistance with low cation-exchange capacity is an extension of this tendency for plants of low fertility requirement to have a lower cation-exchange capacity. In accordance with the Donnan theory, confirmed by experiment[7-9], low cation-exchange capacity favours monovalent to divalent cation uptake. Thus the lower cation-exchange capacity of toxicity-resistant selections may possibly be effective in increasing the uptake of monovalent cations at the expense of the polyvalent cations aluminium and manganese.

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Role of *Dendrophoma* and *Phoma* Species in the Rotting of Jute

IN the course of an investigation designed to investigate the fungal flora growing on jute materials under different conditions of decomposition, species of *Dendrophoma* and *Phoma* were isolated from weather-exposed jute fabric for the first time, using special media. The exposure period was June–August in the monsoon season, and was attended with intermittent heavy rain and sunshine. Isolations were made on 'Cellodextrin' agar and on 'Czapek–Dox'-jute extract agar containing 1 per cent glucose. Similar experiments with conventional media had previously failed to reveal these genera. They were not encountered on jute fabric exposed to 100 per cent relative humidity within the laboratory, nor on fabric rotting in contact with soil or within the bale.

There were altogether four isolates, two of each genus. They are probably different strains of the same species, according to the Commonwealth Mycological Institute, Kew, who very kindly identified the genera. The cultures are being maintained there as *Dendrophoma* 90887 and 90888, and *Phoma* 90889 and 90890.

Phoma species have previously been implicated with the breakdown of cotton fabric on aerial exposure[1-3], and individual species have been shown to cause considerable loss in fabric strength[2,3]. Siu[4] classifies them as moderate decomposers of cellulose. To our knowledge, there is no reference to *Dendrophoma* as a decomposer of vegetable fibres or cellulose, though obviously the two genera are closely related.

Table 1. LOSS OF WEIGHT OF FILTER PAPER

Fungus	Loss (%)
Dendrophoma sp. 192	25·8
Dendrophoma sp. 193	24·5
Phoma sp. 194	21·5
Phoma sp. 195	23·6

Table 1 shows the cellulose-decomposing ability of the four isolates, as determined by loss in weight, after 14 days, of filter paper suspended in 'Czapek–Dox' salt solution. Fig. 1 shows *Dendrophoma* sp. 193 attacking jute fibre.

The organisms form dark-coloured colonies on malt agar, with dark, usually sub-globose pycnidia containing hyaline, elongate spores. *Dendrophoma* sp. 193 tended to form sterile pycnidia after a few transfers on this medium. The cultures are being maintained here on an agar medium containing only jute extract and potato extract, with a strip of filter paper placed

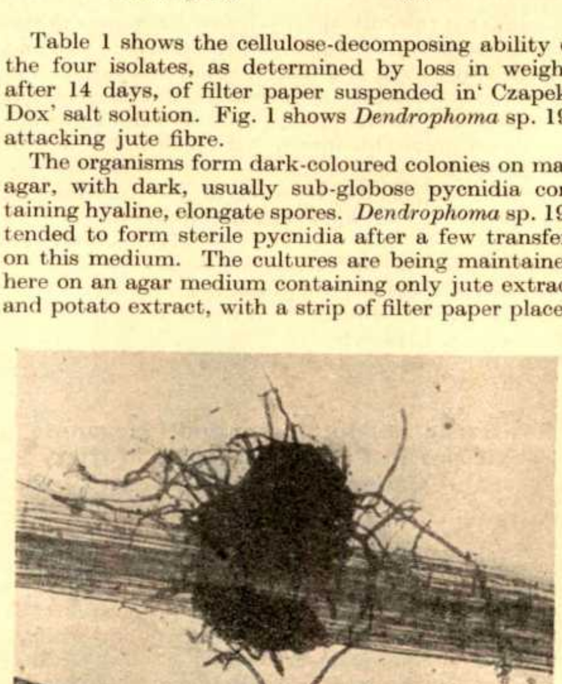

Fig. 1. *Dendrophoma* sp. 193 attacking jute. (× 110)

on the slant. The *Phoma* cultures form heavy mycelial and pycnidial growth on this medium, whereas only pycnidia, concentrated on the paper strip, are seen with the *Dendrophoma* isolates.

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'Nests' of the Marine False-Scorpion

During the period April 9–15, 1962, large numbers of *Neobisium maritimum* (Leach) were collected at two places in the Isle of Man, at Chapel Bay, Port St. Mary, and at Port Erin between Spaldrick and Ghaw ny Pharick. All stages, proto-, deuto-, trito-nymphs and adults, were taken from their silken chambers and also collected as free-moving individuals. The silken chambers were preserved irrespective of whether they were occupied or not.

This species has been taken in small numbers in large-scale surveys of the rock crevice fauna at Rhoscolyn, Anglesey[1], and Wembury, Plymouth[2]. The silken chambers ('nests') have been observed by other workers[3-5]. In the Isle of Man both silken chambers and free-moving individuals were most abundant in the outer regions of deep-seated crevices from about high-water springs to the bottom of the *Fucus–Ascophyllum* zone. They have been removed from more shallow and thinner crevices and also from pockets in the mud and sand deposits of the inner regions of the larger crevices.

Table 1

Numbers	♂	♀	Trito-nymph	Deuto-nymph	Proto-nymph	Total
Of free-moving individuals	109	104	25	20	18	276
From silken chambers	6	21	92	80	151	350
Total	115	125	117	100	169	626

It is evident from Table 1 that most of the free-moving individuals were adult while the majority of those removed from silken chambers were nymphs. The adults are distinguished by their genitalia, and the three nymphal stages by the number of trichobothria on the chelæ[6].

The chambers, constructed by individuals of the same stage, vary both in size and shape from almost flat, from narrow crevices, to dome-shaped almost spherical constructions in the wider crevices. It was rare to find them singly (Fig. 1); usually a number were present close together (Fig. 1) or abutting on each other. In one crevice 124 chambers were present on an area 50 cm × 35 cm. Rarely were they applied to both walls of the crevice and normally they were present on the rock wall surface. The examination of one chamber, partially constructed by a tritonymph, suggests that the base is constructed first and the dome built later. The base, however, is not firmly applied and thus the chambers are easily scraped off the rock with a knife. The silken chamber remains complete, and even after long preservation in 70 per cent alcohol with 5 per cent glycerine added, it still retains a small bubble of air. Empty chambers all showed a lateral slit which presumably marks the place at which the pseudoscorpion leaves the chamber.

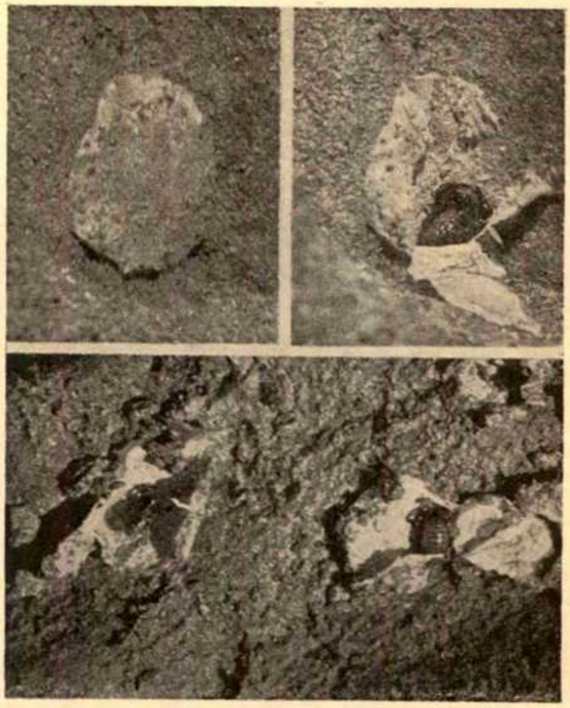

Fig. 1. Top left, silken chamber; top right, same chamber opened to reveal hibernating adult; bottom, two chambers opened to show a tritonymph (left) and an adult (right). (× c. 3)

The new chambers are built of white silk and do not incorporate extraneous matter. They vary from the diaphanous constructions of protonymphs to the dense, stout and tougher chambers of the older stages. Older chambers, mostly vacated, have sand and mud particles adhering to them.

Pseudoscorpions are known to build silken chambers for hibernation, moulting and brooding[5,7]. Of the 794 chambers collected 350 were occupied and each contained a pseudoscorpion. The numbers of each stage are shown in Table 1, and it is evident that these chambers contain a high proportion of nymphs. No brooding chambers (that is, chambers containing females with egg masses attached at the genitalia) were found, and no chamber contained a pseudoscorpion plus its own exuvia. Thus, in the absence of any evidence of moulting, it is concluded that the occupied chambers have been used for hibernation.

All the evidence suggests[5] that each pseudoscorpion builds its own chamber, but in 62 cases these had been constructed within old chambers. The older part often contained the exuvia of the previous inhabitant. Godfrey's[4] observations on the 'nests' of *Chthonius tetrachelatus* (Preyssler) containing apparently a double lining to the wall of the chamber may prove, therefore, to be a misinterpretation.

The unoccupied chambers (444) were divided into those which contained exuviæ (169) and those which did not (275). The trichobothria on the moulted chelæ are distinct, and thus the exuviæ can be allotted to their respective nymphal stages. The difference between exuviæ and dead individuals is difficult to judge, but there is no doubt that most of the nymphal remains are of the former type. The remains of adults proved more difficult. No post-adult moults have been described for this order (although this possibility cannot be completely ignored) and thus these adult remains are more likely to represent individuals which have died during hibernation. In some cases, the shrivelled and withered nature of these adult remains suggests that this is so.

An examination of the unoccupied chambers (169) containing exuviæ and dead individuals revealed that the remains belonged to 30 proto-, 45 deuto-, 66 trito-nymphs and 43 adults (184), the discrepancy in the totals being due to the presence of the remains of more than one stage in a 'double' chamber

The collection of free-living individuals suggests that either the majority of the adults do not build hibernating chambers or that, if they do, they have recently vacated them. Though it is impossible to separate the generations, the high proportion of tritonymph exuviæ suggests that at least some of these adults may have recently moulted. The majority of the nymphs, on the other hand, do build hibernating chambers and the high numbers of protonymphs indicates that some of last year's generation pass the winter in this stage.

I thank Mr. R. F. Newton, technician in this Department, for the photographs.

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Serological Aspects of Immunity in Invertebrates

WHILE most of the scattered attempts to produce antibodies in invertebrates have failed[1], some of the reactions which have been produced in host animals are interesting in themselves. For example, it has been clearly demonstrated that caterpillars develop a temporary, but significant, resistance to bacteria following injections with several kinds of substances[2-4], and that in some cases they even produce heat-stable bactericidal substances[5]. Of great potential significance has been the work of Cantacuzène[6-8], who induced slow antibody-like responses in several marine crabs, in a marine worm, and possibly in an ascidian. His work was done during 1919–28, and therefore lacks some of the quantitative and control data which might fully establish the findings. The findings are of such significance, however, that there is great need to repeat his work and to re-investigate the problem in the same hosts.

During the summer of 1961 at the Station Biologique de Roscoff, where Cantacuzène did most of his work, I examined the responses of the marine worm, *Sipunculas nudus*, and of the spider crab, *Maia squinado*, to a marine protozoan parasite, *Anophrys sarcophaga*. This ciliate is a natural parasite of the shore crab, *Carcinus*, reaches high density in the host blood, and eats the amœbocytes[9]. Ciliates were introduced into the cœlomic cavity of the worm, and the subsequent reaction of the blood to the ciliate studied *in vitro*. Little or no reaction of uninoculated animals was observed after repeated bleedings over a period of five weeks. When, however, the ciliates (in doses of 300 to + 3,000 in 0·2 c.c. of host crab blood) were injected into worms, eight worms produced within 20–48 h a heat-labile substance in the blood plasma which immobilized parasites within 1–2 min. The parasites then lost their cilia, rounded up, and within 10–15 min were lysed so completely that only their outlines remained.

The capacity to destroy ciliates was also provoked in the worm by injections of normal crab blood and of bacterial suspensions. Injection of unsterilized fresh sea-water evoked only a weak and irregular response. Though I tested the sera against *Anophrys* only, the reaction was so readily produced by the several kinds of stimuli that it is probably active against other ciliates as well.

During the process of immunization, or reaction, no change in the *p*H of the blood was observed, and the site of the origin of the substance is not known. It may well be present in one of the numerous cell-types of the cœlomic fluid, and may then be released from such cells by specific stimuli. The lysin is evidently labile, since blood which was positive at the time of withdrawal often lost its activity after standing at room temperature. A positive effect was demonstrated in serum at dilutions of 1 : 20 in sea-water, but under more stable conditions higher titres might be found. 'Immunized' worms maintained a high activity in their plasma for 5–8 days, and then apparently lost it. It reappeared again rapidly following reinjection of the animal. Repeated injection of four worms during a period of 4–8 weeks failed to raise the titre or apparent strength of the activity. No disease was provoked by the injections or bleedings.

The sipunculids, which were obtained from two separate coastal areas, had been kept in the laboratory for periods varying from a few days to several months. Of a total of 82 tested before injection, 10 had slight to moderate 'natural' lytic activity which disappeared within a few days. These positive results in controls may be related to trauma during collecting or to infection which has been recently overcome.

The spider crab, *Maia*, has been described as resistant to infection with *Anophrys*[9]. For this reason the effect of *Maia* sera on the *Anophrys* was studied. Most, but not all, of the spider crab sera immobilized or agglutinated the ciliate within 5–10 min, primarily by causing them to stick to each other by the long tail cilium. Except for further agglutination of the other cilia no other effect was demonstrated and the protozoa remained alive during the several hours of observation. However, 14 per cent (5 of 36) of the crabs completely lacked this protective substance, and when two of these were injected with the ciliates, they rapidly developed a fatal infection, with parasites swarming in the blood. These 'fatal' parasites also were agglutinated by the serum of the resistant animals, but not by the serum of the susceptible crabs. The presence of an agglutinin which immobilized the parasite in most, but not all, cases and which was characteristic for specific animals during the five weeks of investigation, then suggests either that the serum factor is a genetic factor for resistance, or that it is acquired during the course of exposure to the parasite.

These two sets of results suggest that completely different types of protective substances may be found in different invertebrates, and that the presence of the lysin or destructive substance may be evoked in measurable quantities within 1–2 days after the injection of the specific substance or other organic material. The nature of the lysin, the types of substrate on which it is active, and the varieties of stimuli needed to evoke it are unknown.

I thank Dr. George Teissier, director of Station Biologique de Roscoff, for making the facilities available for this research, and for his encouragement during the course of the work.

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Functional Activity of the Anterior Pituitary after Prolonged Cultivation *in vitro* and Transplantation into Pituitary-deprived Female Rats

RELIABLE data on the specific functional activities of the anterior pituitaries which take place during their cultivation *in vitro* are scarce[1–3]. In our previous work we have shown that the infantile rat pituitary kept *in vitro* for prolonged periods and transplanted into hypophysectomized rats will stimulate the growth of the host animal[4]. It is, however, known that for the synthesis of the somatotrophic hormone the anterior pituitary shows a considerable degree of autonomy.

For the examination of the gonadotrophic hormone content, on a biological level in anterior pituitaries after longer periods of cultivation, *in vitro* glands of 3-week-old rats were used. After 21 and 28 days of life *in vitro* the cultivated glands were transplanted into adult (100 g) hypophysectomized female rats. The 3-week-old anterior pituitaries were selected for cultivation after it was established that one whole normal anterior pituitary of this age, grafted in the anterior eye chamber or under the kidney capsule, will cause the appearance of a large number of pure epithelial cells in the vaginal smear of about 50 per cent of adult (2 months old) hypophysectomized female rats.

The cultivation was carried by the watch-glass technique of Dr. H. B. Fell, after the glands had been mounted on a grid of glass rods[5]. The medium used contained rat plasma, chicken plasma and chicken embryo extract in the proportion of 8 : 2 : 6, and the incubating temperature was 33°–34° C.

Following the cultivation, 5 whole anterior pituitaries were grafted in each eye of the host animal, and 3 days later 5 more minced pituitaries were injected into the thigh muscle. (In the course of cultivation the mass of the explanted pituitary undergoes a constant decrease. After 3 weeks of life *in vitro* it becomes reduced to probably no more than a quarter of its original size.) Five to six days following grafting the vaginal wall of 5 of 10 animals (into which 3-weeks-cultivated anterior pituitaries were transplanted) reacted by a sloughing off of a large number of pure epithelial cells. Five host animals did not react. Hypophysectomized rats into which 4-weeks-cultivated anterior pituitaries were transplanted gave no reaction.

The conclusion which can be drawn from this is clear. Fifteen whole pituitaries after three weeks of cultivation contain a sufficient amount of the hormones to stimulate the shedding-off reaction of the epithelial cells of the vaginal wall in the hypophysectomized rat, whereas after 4 weeks of cultivation the amount present in the same number of glands is not sufficient to do it.

The question may be asked whether the hormones eliciting the vaginal reaction have been synthesized *in vitro* or in the host animal following transplantation or whether they had been manufactured in the gland before explantation, stored in the tissue in the course of cultivation, and reintroduced in the hypophysectomized host. The possibility that the hormones are synthesized in the host following transplantation in any appreciable amounts, we believe, can be ruled out, as the vaginal reaction occurs once, and only once. The same is the case when the normal gland is transplanted. It will be more difficult to decide whether the hormones were synthesized *in vitro* or before explantation. The fact that the vaginal reaction following transplantation occurs only once and the glands kept *in vitro* for 28 days gave no reaction speaks in favour of the latter hypothesis. The appearance of the cornified epithelium in the vaginal smear following the grafting of the cultivated anterior pituitaries, at the same time, serves as an index for the presence in the grafted explants of both the follicle-stimulating and the luteinizing hormones[6]. The repeatedly observed failure of the fully developed follicles to give rise to corpora lutea, on the other hand, shows that the quantity of the luteinizing hormone in both the normal infantile and the cultivated pituitaries must have been very small.

In a few cases we injected the medium directly surrounding the explanted pituitary tissue into hypophysectomized rats but without success.

It is worthy of being noted that in the cultures of anterior pituitaries *in vitro* presence of thickly granulated acidophile cells in the explanted gland can be followed for many weeks by means of the Severinghaus technique. With the use of the same technique highly modified basophile cells have been observed after 40–50 days of cultivation, and cells with reduced number of granules up to 30 days of cultivation when the Bodian impregnation technique was applied[7].

Finally, we wish to state that our results obtained on the cultivated *in vitro* rat anterior pituitaries transplanted into hypophysectomized female rats are in complete agreement with those of Cutting and

Lewis[2] on the cultivated rat pituitary transplanted into immature female guinea pigs.

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MICROBIOLOGY

Branched and Multiple Forms of *Euglena gracilis*

I HAVE observed in a number of cultures of *Euglena gracilis* (Pringsheim, *Z* strain) organisms of double or branched form having two, four or even eight flagellate anterior ends, these being joined to a central globular structure as shown in Figs. 1 and 2.

Close examination has failed to reveal any dividing wall within the organisms, and the photographs show a dark mass in the central globe which is evidently the nucleus. The multiple forms appear to have arisen through successive cytoplasmic divisions, unaccompanied by nuclear division.

The locomotion of the double forms is erratic, the direction of movement depending on which anterior end may be for the moment in control. The quadrupl and octuple forms are only capable of rolling motio and do not move from place to place through th medium. The causes of the occurrence of these form are not wholly clear, though the presence of a hig concentration of calcium ions has been seen t encourage their development.

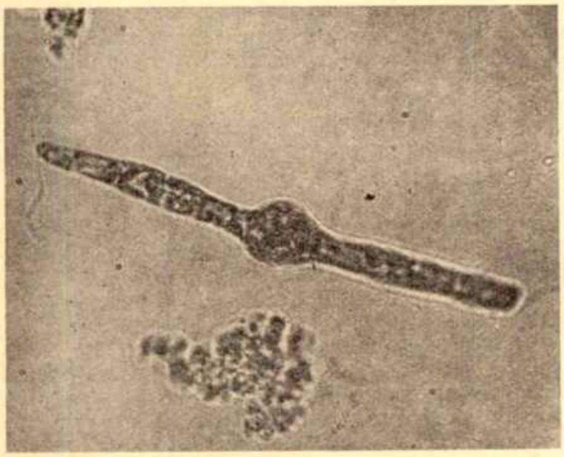

Fig. 1

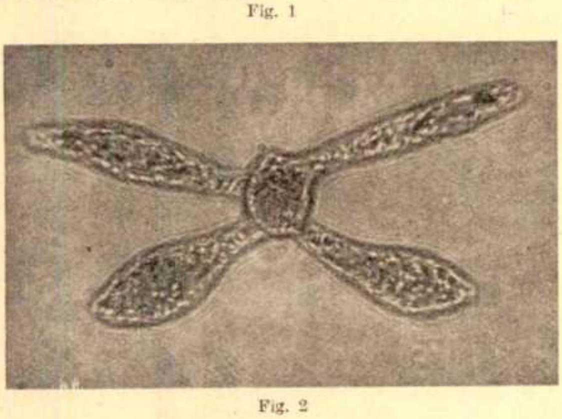

Fig. 2

The double forms of *Trachelomonas* seen b Pringsheim[1] may be a related phenomenon, but h describes these double organisms as having tw (not one) nuclei and nowhere reports quadruple o octuple forms corresponding to those I have seen i *Euglena*.

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Lysine Decarboxylase Activity as a Simple Test in Differentiation of Enterobacteriacea

MANY efforts have been made to find simp methods for rapid identification of Enterobacteriacea Most of the tests are based on the ability of tl bacteria to grow on selective media, or to fermer specific sugars, alcohol derivatives, etc. Such tests, i which bacteria are able to grow on selective media, aı usually time-consuming, and positive results aı recorded, at the earliest, only after 24 h.

There are only a few known tests which are base on pure biochemical action between a mass of bacter and a specific substrate. One of these tests is tl ability of *Proteus* species to decompose urea to an monia and carbon dioxide. Such tests give goc results within a few hours. The activity of variou bacteria capable of decarboxylating amino-acids to tl respective amines and carbon dioxide at *p*H ran 4–6 was comprehensively investigated by Gale[1].

Lysine decarboxylase, which is found in variou bacteria and in *Salmonella*, decarboxylates lysine cadaverine and carbon dioxide, usually in acid ran of *p*H. The cadaverine produced shifts the *p*H of tl media towards the basic range of *p*H.

Møller[2–4] tried to adapt ability of *Salmonella* decarboxylate amino-acids for establishing a simp method for identification of the bacteria. By incuba ing *Salmonella* under a paraffin oil layer in a mediu containing lysine as an amino-acid for decarbox ation, he achieved good results within 1–3 days.

Altmann[5] simplified this test by incubating a ma of bacteria in a broth containing casitone, glucose, aı lysine for 2 h, and extracted the amine which w formed by chloroform. By addition of ninhydrin the chloroform containing cadaverine a deep viol colour appeared, which indicated that the bacter strain under test contained a lysine decarboxyla enzyme. By this method all *Salmonella* strains test by him gave positive results with the exception of *S* *paratyphi A.* and *Sal. emek.* On the other hand, *E. coli* strains gave positive results.

Though in the method proposed by Altmann t incubation time is short, the test is rather laboriou and worth while only for differentiation betwe *Salmonella* and *Shigella*; it does not differentia between *Salmonella* and *E. coli* strains.

In order to avoid many preparatory steps, wh maintaining a relatively short incubation time, simplified test is proposed here, based on the fact th in a solution containing 1 per cent lysine in we citrate buffer cadaverine is produced by the splitting of the lysine molecule by lysine decarboxylase, which causes a shift of *p*H towards the basic range. The method used was as follows:

0·5 ml. of 1 per cent L-lysine dissolved in citrate buffer M/2,000 adjusted to *p*H 5·0 and 4 ml. of 0·01 per cent bromo-thymol blue added to 100 ml. of the solution, in serological test-tubes, served as substrate. Heavy inoculum of the bacteria under test taken from a slant by a bacteriological loop served as an enzyme. The test-tubes were kept in a water bath at 37° C for 4 h. By that time, cadaverine that was produced by enzyme action on lysine shifted the *p*H from 5·0 to 6–7, which was seen by the change of the indicator colour from yellow to blue and was recorded as positive.

Table 1. COMPARISON BETWEEN LYSINE DECARBOXYLASE ACTIVITY IN *Salmonella* AND *E. coli*

Strain	μl. Carbon dioxide evolved during 2-h incubation	μl. Carbon dioxide evolved during 4-h incubation
Salmonella paratyphi A	61	111·7
Salmonella reading	156·7	341·7
Salmonella newport	70·6	157·6
E. coli 125	6·0	11·5

The enzymatic system contained equal numbers of bacteria in 0·5–0·1 M citrate buffer in side-arm, 1 per cent lysine in 2·0 ml. 0·1 M citrate buffer in central well; total volume 2·5 ml. Incubation was at 37° C for 4 h. Figures given are after subtracting blanks.

Eighty-eight strains of *Salmonella* were tested, including strains such as *Sal. paratyphi A*, *Sal. paratyphi B* and *Sal. typhi*, with the exception of one *Sal. emek* strain. All the *Salmonella* strains gave positive results; all 13 *Shigella* strains tested were negative; and out of 130 *E. coli* strains tested, all but one gave negative results under these conditions.

All five *Sal. paratyphi A* which were tested gave positive results, indicating that this test is more sensitive than the tests proposed until now.

Though it is well known that many *E. coli* strains contain lysine decarboxylase enzyme, it may be suggested that, under test conditions, the lysine decarboxylase precursor in *Salmonella* is more sensitive than in *E. coli* strains, and causes rapid stimulation of enzyme activity in these bacteria.

In order to check results obtained by this method, three positive *Salmonella* strains—*Sal. reading*, *Sal. paratyphi A*, *Sal. newport*—and one *E. coli* strain were placed in a Warburg apparatus, and manometric readings were taken for 4 h. Output of carbon dioxide was measured, and compared with change in colour occurring in the test-tube.

From the results, which are recorded in Table 1, it can be seen that all *Salmonella* strains were positive and evolved more than 100 µl. carbon dioxide in 4 h, the highest reading being 341·7 µl., while the output of carbon dioxide from *E. coli* was negligible. These values correlate well with test-tube findings.

In conclusion, it is suggested that the method proposed is simple, gives good results in a short incubation time, and enables rapid differentiation between *Salmonella* and other Enterobacteriaceæ.

I thank Miss L. Horenstein of Kupat Holim Laboratories, Haifa, for supplying various bacteria strains.

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GENETICS

Temperature Response of the Genetically Stable *R* Locus in Comparison with the Mutable *V* Locus in a Clone of *Nicotiana*

VARIEGATION for flower colour in certain *Nicotiana* cultures results from changes in the functional state of a genetic locus, *V*. The dramatic frequency with which these spontaneous changes may occur in somatic tissue is illustrated in Fig. 1*A*. The germinal changes in state, while potentially reversible, have sufficient persistence to permit their genetic detection by means of altered progeny segregations. Therefore, by genetic criteria, the *V* locus alterations have all of the characteristics of reversible mutations. Each of the functional states appears to possess genetic integrity. The question arises of whether to view these different states as nuclear differentiations or as alterations of genetic code. If the former view were taken, the phenomenon should likely be considered 'paramutation' in the generalized sense utilized by Brink[1].

Although the detailed mechanism is at present obscure, it is of interest to know to what extent the characteristics of unstable genetic systems may be shown also by stable genetic systems. There is evidence from earlier work with the *Nicotiana* material[2-4] for similarities, as well as differences, in response to environmental stimuli applied in common. These and other experiments may elucidate

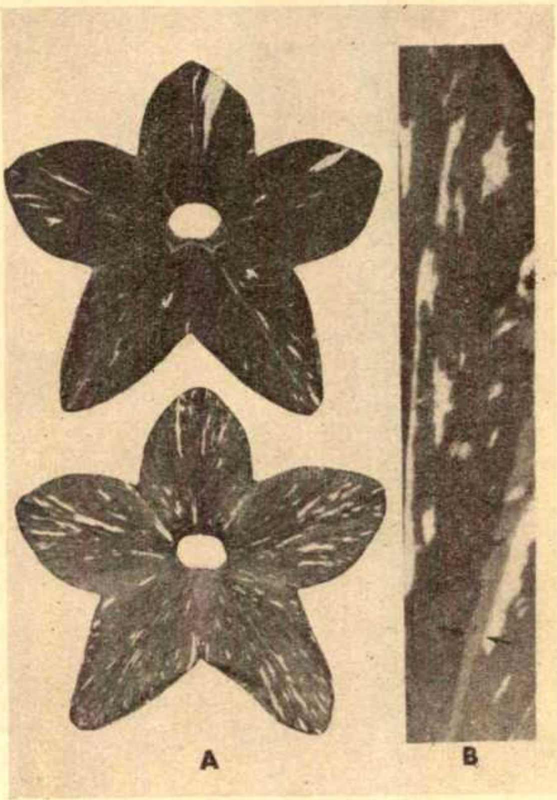

Fig. 1. Flowers of *Nicotiana* clone *S*5230-5: *A*, upper, cultured at 60° F.; *A*, lower, cultured at 80° F. (*c.* × 0·90). The frequency of speckled sectors (associated with the mutable gene *V*) is greater at the higher temperature. *B*, section of petal lobe (*c.* × 4·05) showing (between arrows) a lighter sector of purple tissue (associated with the stable gene *R*) on the background of normally red tissue, which contains more numerous speckled sectors

Table 1. SUMMARY OF SOMATIC AND GERMINAL RESPONSES TO TEMPERATURE FOR BOTH A MUTABLE (SPECKLED) AND A STABLE (PURPLE) GENE IN *Nicotiana* CLONE *S*5230–5. (SEE TEXT).

Line	Plant variable measured	Temperature treatment (°F.) 50	60	70	80	Mean	Regression* coefficient
	Somatic scoring, complete						
1	126 Petals, speckled sectors	1,096	1,397	1,386	1,564	—	—
2	126 Petals, purple sectors	2	5	4	9	—	—
3	Epidermal cells per 10^{-4} cm.^{2}	7·52	8·33	9·45	9·90	8·80	1·34†
4	Speckled sectors per cm.^{2}	8·0	10·2	10·1	11·4	9·91	1·02†
5	Purple sectors per cm.^{2}	0·015	0·036	0·029	0·066	0·0364	4·01
	Somatic scoring, final period						
6	Epidermal cells per 10^{-4} cm.^{2}	7·86	7·56	9·40	10·20	8·76	1·01‡
7	Speckled sectors per cm.^{2}	6·8	9·4	9·5	16·2	10·49	2·83†
8	Purple sectors per cm.^{2}, tran.§	0·0269	0·0345	0·0408	0·0489	0·0378	1·94†
9	Purple sectors per cm.^{2}	0·0218	0·0437	0·0655	0·1092	0·0601	—
	Progeny segregation						
10	Total progeny observed	89	89	89	75	—	—
11	Percentage recessive, speckled	14·6	22·5	31·5	37·3	26·0	2·96†
12	Percentage recessive, purple	31·5	27·0	33·7	34·7	31·6	—
13	Percentage speckled, tran., angles¶	23·1	28·7	34·4	37·4	30·9	1·51‖
14	Percentage purple, tran., angles¶	35·1	31·0	35·9	35·7	34·4	0·20‡

* Regression expressed as percentage of mean per °F.
† Significant at 0·01 level.
‡ Not significant at 0·05 level.
§ Transformation used is $\sqrt{y+0\cdot5}$.
‖ Significant at 0·05 level
¶ Transformation used : angle = arc sin $\sqrt{\text{percentage}}$.

similarities between mutable and stable genes, and may bear on the broader genetic and epigenetic problem of heritable control over development[5].

The plant material used here is superior to other material for approaching several of these problems experimentally. The origin and genetic behaviour of the variegated cultures have been reported previously[6]. The specific clone used (*S*5230–5) is heterozygous for the mutable *V* gene (v_{s_3}/v_s) and for the stable *R* gene (*R*/*r*), which affect flower colour. Replicated cuttings of the clone were grown at four constant temperature greenhouse conditions: 50°, 60°, 70°, and 80° F. The somatic effect of temperature on the mutable gene system was compared with that on the stable gene system in the same tissue. This was done by counting speckled sectors (changes from v_{s_3} to v_s phenotype, Fig. 1*A*) and purple sectors (changes from *R* to *r* phenotype, Fig. 1*B*) on 126 flower petals from each treatment. Similarly, germinal effects on the same gene systems were studied by counting speckled and purple individuals in progeny obtained after self-pollinating the treated plants. Detailed procedures appear in earlier papers[2,3].

The analysis for somatic scoring and progeny segregation is summarized in Table 1, which presents for the first time the stable gene response compared with the previously published[2] response for the mutable gene. Lines 1–5 of the table show the somatic results for the complete experiment in which sectors were counted within one square centimetre on each petal. Data were taken also to estimate the number of epidermal cells per unit petal area, and permit calculation of 'mutation' rates per somatic cell division for each treatment[2,3]. The average for the stable gene in this experiment is 10^{-6}. However, temperature affects cell size, increasing at higher temperatures the population of cells sampled per cm.^{2}. This leads to the expectation of more sectors per unit area at higher temperatures, even without a direct effect of temperature on the sectoring mechanism. This technical correlation has been dealt with by appropriate analyses of variance and covariance. The results are concisely summarized in the last column of Table 1, showing linear regressions on temperature for each variable.

Results for the final third of the scoring period, after the plants have been longest exposed to the different temperature treatments, are unequivocal, and are given in lines 6–9. Somatic sectoring for both the stable and mutable genes show highly significant, correlated regressions on temperature.

The progeny segregations, observed simultaneously under the same field conditions for all treatments, are given in lines 10–14 of Table 1. No significant temperature effect on germinal response for the stable gene is detected, but final decision is reserved because this consequence could be due to excessive sampling error in the 50 degree point alone. Germinal response for the mutable gene *is* significant and correlated with its somatic response.

The similar somatic responses to temperature for both the *R* and the *V* loci, and their previously reported[4] parallel variation with flower-node position, suggest that stable loci, as well as unstable loci, may undergo heritable changes differentially in varying cellular environments.

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Protein Synthesis in Species Hybrids of Birds

THE introduction of the method of starch-gel electrophoresis by Smithies[1,2] has opened up a new field in genetical and phylogenetical studies. Smithies *et al.* showed the presence of a genetical polymorphism in the haptoglobins[3] and the transferrins[4] in man. These findings have already proved to be of genetical and anthropological interest.

Several investigations on apes and monkeys have also been carried out. Thus it is known that lower primates usually have fast-moving transferrin components compared with human beings and chimpanzees[5,6]. In different primate species so far studied, there are generally small differences concerning the main serum protein components. Thus the albumin, cærulo-plasmin, slow α_2-globulin and main haptoglobin (Hp 1–1 band) components show about the same electrophoretic mobility in human beings, chimpanzees and monkeys[5]. The difference between different

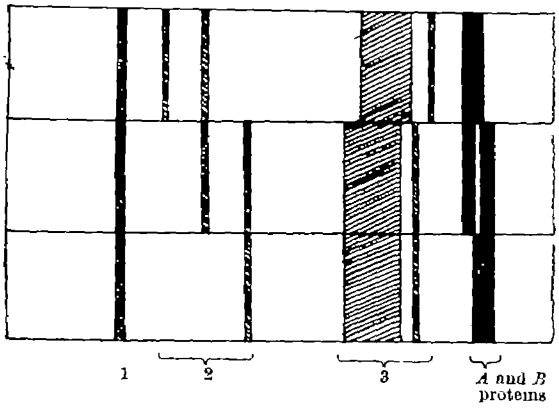

Fig. 1. Schematic diagram showing the main serum protein components in canaries, goldfinches and the hybrids between these two species. Component 1 occurs in all individuals of both species, components 2 and 3 vary within both species and the *A* and *B* proteins are apparently species-specific. Top, *B* goldfinch, middle, *AB* goldfinch × canary, bottom, *A* canary

Macacus species[5,7] is restricted to the presence of different polymorphic transferrin patterns.

From a systematic point of view, it would be of special interest to find qualitative serum protein differences between closely related species. Such differences normally exist between phylogenetically fairly distant groups, for example, between serum proteins of mammals and birds. Qualitative differences between closely related groups have rarely been described. Thus Zweig and Crenshaw[8] have reported differences between turtle species of the genus *Pseudemys*.

We have examined by means of starch-gel electrophoresis the sera of 15 goldfinches (*Carduelis carduelis*), 13 canaries (*Serinus canaria*) and 6 hybrids between these two species. A discontinuous buffer system was used[9]. The serum protein patterns in the two species showed marked similarities, but a difference was observed between the main fast-moving components (proteins *A* and *B* in Fig. 1). All 13 canaries had the fast-moving protein *A* and all 15 goldfinches had the slow component *B*. In the six hybrids both proteins were present, but each was present in apparently half the concentration found in the parental species (Fig. 1). Thus there exists a dosage effect similar to that found in human transferrin heterozygotes.

We have also examined another pair of species, the domestic pigeon (*Columba livia*) and the ring-dove (*Streptopelia risoria*). Fertile male hybrids between these two species are known to exist. The protein patterns of pigeons and ring-doves are rather similar to those of goldfinches and canaries. Between pigeons and ring-doves there was also an electrophoretic difference in the main fast-moving components. We have not yet, however, obtained any serum samples from hybrids between these two species. In view of the interesting results concerning species-specific cellular antigens in doves and pigeons[10], studies of the serum proteins of these species and of their hybrids would certainly yield some significant complementary information.

Sibley and Johnsgard[11], examining avian sera by paper electrophoresis, concluded that serum proteins are of very little value in taxonomic investigations of birds. We think, however, that by the method of starch-gel electrophoresis, investigations of serum proteins of birds may be very rewarding, for example, the use of the *A* and *B* proteins for taxonomic studies in the *Carduelis-Serinus* group.

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PSYCHOLOGY

Alternatives in Testing Recognition Memory

RECOGNITION is often possible where recall is not. The inference that memory is superior when tested by the former procedure has been challenged by Davis *et al.*[1], who showed that the number of alternatives from which the items seen previously must be selected was a crucial factor governing performance. When the number in the recognition test is as great as that which could reasonably be considered in recall, recognition performance is no better than recall.

They then analyse their results in terms of the informational measure suggested by Judd and Sutherland[2], on the basis of which they conclude first that the information transmitted in the recognition conditions increases with the number of alternatives, and second that in informational terms recall is at least as efficient as recognition, and in the case of two-digit numbers is significantly better.

We wish to report a demonstration that performance in recognition tests depends not only on the number but also on the nature of the alternatives utilized. It is shown that subjects' incorrect responses are non-random, thus contradicting assumption 7 of the Judd and Sutherland measure, which requires that wrong responses be equally distributed over all symbols.

Fifteen two-digit numbers were presented to a total of 100 subjects at the rate of 1 per 6 sec with an exposure of 5 sec. Two orders of presentation were used, 50 subjects being run with each. Immediately after the presentation was completed the subjects wrote down all the numbers they could remember. Instructions printed on their recording sheets told them that 15 numbers between 10 and 100 exclusive had been exposed.

Errors of intrusion were noted. Two sets of 15 numbers were then drawn from the 74 possible alternatives; one consisting of the numbers which occurred most frequently as intrusions; the other, of those which had occurred least frequently. These two sets were then used as alternatives in two 30-item recognition tests.

Two fresh groups, each of 20 subjects, were given the recognition tests. The 15 items were exposed as before and in the same orders. One half of each group was given recognition tests with frequent intrusions as alternatives; the other halves had a test with rare intrusions as alternatives. The mean number of correct items identified per subject was 10·5 with the former halves, 13·1 with the latter, a difference which is statistically significant ($P < 0 \cdot 01$). For comparison, the mean score per subject in the original recall test was 9·6 items.

Our results indicate that when memory for an item is imperfect the subject is able to identify certain of its characteristics. If a number of items in the set of alternatives from which he has to choose have similar characteristics, then he will be unable to tell which item is new and which has been seen before. If only one has them, then he can correctly identify it. Furthermore, since our results were obtained from a group of subjects rather than intensive study of individuals, there would seem to be some communality in the characteristics used by different persons for encoding numbers in memory.

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The *T* Concept

THE concept of tendermindedness has been at the centre of a controversy ever since Eysenck[1] attempted to relate it to political attitudes. First Christie[2] then Hanley and Rokeach[3] sought to discredit the ideas put forward by Eysenck. To some extent the issue has been difficult to settle because there has been no external criterion against which T could be validated. Until now it has been open to whatever interpretation the various parties have cared to place on it. Nor has the scale at present used by Eysenck to measure T ever been factor analysed.

In the present work the T scale was administered to a quota sample of 122 adults obtained from two London boroughs by the random route method. Age, sex, occupation, and political and religious affiliations were also obtained.

Factor analysis revealed the primary component to be a measure of what might best be described as 'moralistic religiosity'. The top-eight items, with loadings from 0·496 up to 0·748, all make direct references to religion, the Church, God, Christ, and life after death. No other religious items appear in the scale. The next ten items all exude a strong moralistic aroma; four are directly connected with sexual behaviour, and the rest are all redolent of moral turpitude in one form or another. These items carry loadings from 0·298 to 0·466 on the primary component.

The last three items, with loadings over 0·200, are of interest in that they have a humanitarian quality and score in precisely the opposite direction to that required by the T concept and the key used by Eysenck. The remaining humanitarian items follow this trend with lower loadings.

To confirm this general finding, religious affiliations were used as an external criterion. Those with religious affiliations scored higher on T than those without, the difference being significant beyond the 0·001 level on a two-tailed test.

Political affiliations follow the pattern built int the scale by Melvin[4], in that there is little to choos between Socialists and Conservatives, while Libera appear higher on T. Previously reported correlatio between Communism and Fascism and toughminde ness are easy to understand in view of the explici opposition of these creeds to the established churc Nor, in the circumstance, is it difficult to believe tha T is correlated with neuroticism as recently reporte by Eysenck[5].

The essential weakness of the T scale as it stands i made clear by comparing Torquemada and Eichman both of whom were noted for their ruthless efficienc and hideous inhumanity. Given his religious affili tions and moralistic fervour, it would have bee virtually impossible for Torquemada to have appeare as other than a distinctly tenderminded soul, despit his authoritarian temperament. Eichmann, on th other hand, although of a very similar dispositio would emerge as extremely toughminded simpl because his particular creed was diametrically oppose to religious dogma other than its own.

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Green and Stacey strongly support my ow interpretation of 'tendermindedness', as well as givin it statistical backing. In my description in *Psycholog of Politics* (p.132), I wrote: "the tenderminded set opinions appears to be dominated by ethical, mora istic, super-ego, altruistic values". The relationshi with liberalism reported by Green and Stacey also as predicted and found by us. It is difficul therefore, to follow their reasoning when they spea of "the essential weakness" of the T scale. They see to demonstrate this by referring to hypothetic scores on the scale which might be made by To quemada and Eichmann. It need scarcely be sai in a scientific journal that hypothetical data n obtained in an actual experiment but merely postu lated to suit the convenience of the author have litt empirical value, quite apart from the fact that I hav always been at pains to stress that the scale wa relevant only to the country where it was constructe and validated, and only at the time when this proce was carried out; extension of its validity could clearl only be made on the basis of empirical demonstratio I would never have claimed that the scale as it stoo had much relevance to conditions so utterly differe as those obtaining in Spain 300 years ago, and cannot, therefore, regard the hypothetical demonstr tion of Torquemada's tendermindedness as being any way relevant to the argument. Eichmann equally hypothetical toughmindedness I would n wish to doubt, however, although here, too, empiric evidence would be desirable.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, October 8

Plastics Institute, Reinforced Plastics Group (at the Wellcome Building, Euston Road, London, N.W.1), at 4 p.m.—Symposium in "Design Considerations in Three Different Fields".

Institute of Biology (at the French Institute, Queensberry Place London, S.W.7), at 5.15 p.m.—Prof F. Morel (French Centre for Nuclear Studies, Saclay). "Some Aspects of Kidney Function as Revealed by Tracer Studies" (Third Louis Rapkine Memorial Lecture)

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London W.C.1), at 5.30 p.m.—Mr. J. R. Lucas: "The One Concept of Probability".

Institution of Mechanical Engineers, Applied Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Testing Machines".

Royal Institute of Chemistry, London Section (joint meeting with the Ewell County Technical College Chemical Society, at the Ewell County Technical College, Reigate Road, Ewell), at 7 p.m.—Dr. H. J. Cluley "Complexometric Analysis".

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7.30 p.m.—Miss A. I. Ross: "Aids for the Disabled".

Tuesday, October 9

University College, London (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. S. E. Toulmin: "Heresy in Science".*

London School of Economics and Political Science (at Houghton Street, Aldwych, London, W.C.2), at 5 p.m.—Mr. P. O. Lapie "Europe in the Making".* (First of ten lectures on "The Common Market". Further lectures on October 23, November 6, 20, December 4, January 15, 29, February 12, 26 and March 12.)

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers

Society of Chemical Industry (joint meeting of the London Section and the Chemical Engineering Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. C. E. Faulkner and Mr L. E. Parsons: "The Use of Computers for the Control of Continuous Processes".

British Computer Society (at Northampton College, St. John Street, London, E C.1), at 6 30 p.m.—Mr. Saul I. Gass (Washington) "The Role of Digital Computers in Project Mercury".

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, S.W 1), at 6 p.m.—Annual Meeting. 6.30 p m—Mr. H A. Dean "From Headline News to Accepted Practice" (Chairman's Address).

Plastics Institute, London Section (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. E. C. Bernhardt: "Thermoplastics Processing the Science—the Industry. Recent Advances and Urgent Tasks Ahead".

Royal Institute of Chemistry, London Section (joint meeting with the Brighton Technical College Chemical Society, at Brighton Technical College), at 7 p.m.—Mr R C. Chirnside: "Gemstones and Jewels—Natural and Synthetic".

Royal Society of Medicine, Psychiatry Section (at 1 Wimpole Street, London, W 1), at 8 p m.—Dr. Henry Wilson "Psychiatry and the Medical Registrar" (Presidential Address).

Wednesday, October 10

School of Pharmacy, University of London (in the Assembly Hall, 29–39 Brunswick Square, London, W.C.1), at 8 p.m—Sir Zachary Cope: "The Influence of Pharmacy upon Surgery".

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m—Dr. K. A. Kermack "Vertebrate Fossils from Basutoland" (demonstration), Dr. R. Dearnley "The Lewisian Complex of South Harris, with some Observations on the Metamorphosed Basic Intrusions of the Outer Hebrides" (paper).

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, S.W 1). at 5 p.m.—Dr. G. B. Wetherill. "Sequential Estimation of Quantal Response Curves".

British Coal Utilization Research Association (in the Lecture Theatre, Institution of Civil Engineers, Great George Street, London, S W 1), at 5.30 p.m.—Mr. Herbert Seidl: "The Horizontal Cyclone as a Coal Burner" (Eleventh Coal Science Lecture).

Institution of Mechanical Engineers (at 1 Birdcage Walk. Westminster, London, S.W.1), at 6 p.m.—Prof. Kiyoshi Muto, Dr. R W. Bailey and Mr. K. J. Mitchell: "Special Requirements for the Design of Nuclear Power Stations to Withstand Earthquakes".

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S W.7), at 6 p.m.—Mr. D. M. Corke. "Metal Fatigue".

Institute of Metal Finishing, Organic Finishing Group (at the British Institute of Management, 80 Fetter Lane, London, E.C.4), at 6.30 p.m.—Mr. C. W. Andrews. "Behaviour of Paint Solvents During Evaporation".

Society for Analytical Chemistry (at the Chemical Society. Burlington House, Piccadilly, London, W 1), at 7 p.m—Meeting on "Recent Developments in Polarography". Speakers. Mr. H. M. Davis, Mr. H. I. Shalgosky and Dr. H. W. Nurnberg.

Society of Instrument Technology, Measurement Technology Section (at Manson House, 26 Portland Place, London, W 1), at 7 p.m.—Mr. T. Land and Mr. R. Barber: "Radiation Pyrometry".

Royal Society of Medicine (at 1 Wimpole Street, London, W.1), at 8 p.m.—Sir Stanford Cade, K.B.E., C.B, F.R.C.S.: "Cancer—The Patient's Viewpoint and the Clinician's Problem" (Third Nuffield Lecture).

Thursday, October 11

University College, London (in the Anatomy Theatre, Gower Street, London, W C.1), at 1 15 p.m.—Mr. B. C. Brookes: "The Limits of Human Communication".*

Chemical Society (at the Royal Institution, Albemarle Street, London. W.1), at 6 p.m.—Dr. F. Sanger, F.R.S.: "Amino-acid Sequences in Certain Enzymes" (Pedler Lecture).

Institution of Mechanical Engineers, Thermodynamics and Fluid Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p m.—Discussion on "Design of Turbine Stages with Very Long Blades".

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7 p m—Dr. K. Milne "Survey of Aerials for Radar and Allied Applications"

Friday, October 12

Association of Applied Biologists (in the Lecture Hall, British Museum (Natural History), Cromwell Road, South Kensington. London, S.W.7), at 10.40 a.m.—General Meeting. Symposium on "Harmful Mammals".

Institute of Physics and the Physical Society, Materials and Testing Group (at 47 Belgrave Square, London, S.W.1), at 11 a.m.—Meeting on "Scattering of Elastic Waves".

Institute of Physics and the Physical Society (at 47 Belgrave Square, London, S W.1), at 2 p m—Meeting on "Space Spectroscopy" Speakers: Dr R. Tousey, Dr J Lindsay, Dr. K. A. Pounds and Dr D. W. O. Heddle.

Royal Astronomical Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof Bengt Stromgren. "Problems of Internal Constitution and Kinematics of Main-sequence Stars" (George Darwin Lecture).

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk Westminster, London, S.W.1), at 6 p.m.—Discussion on "Should Engineering Studies be included in Sixth Form Curricula of Grammar and Public Schools?"

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. Konrad Lorenz. "The Function of Colour in Coral Reef Fishes".

Saturday, October 13

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S E.23), at 3.30 p m—Dr Mary E. Gillham: "Some Sea-Bird Islands of the Southern Hemisphere".*

Monday, October 15

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5 30 p m.—Mr. J. Muir, Mr J. Norris and Mr. K C. Overton. "Determination of MCPA and Related Acids by Gas Liquid Chromatography".

University of London (at Queen Elizabeth College, 61–67 Campden Hill Road, London, W 8), at 5 30 p.m.—Sir Harrie Massey, F.R.S.: "Space Research" (Inaugural Lecture)

Institution of Mechanical Engineers, Railway Engineering Group (at 1 Birdcage Walk, Westminster, London, S.W 1), at 6 p m.—Discussion on "Noise and Railways"

Institute of Metal Finishing (at Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr A. Nicholson "Maintenance of Electroplating Plant".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Senior Physicist in the Department of Physics, to be responsible for clinical radiation physics and serve as deputy to the Director of the Department—The Secretary, Board of Governors, Hammersmith Hospital, Du Cane Road, London, W.12 (October 12).

Chief Technician or Senior Technician, to take charge of the glassblowing workshop—The Chief Technician, Division of Chemistry, University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (October 15)

Lecturer in Biochemistry at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 15)

Research Assistant (with an honours degree in botany or agricultural botany), to investigate the effects of storage conditions on the life-span of crop seeds—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (October 15).

Scientific Officer/Senior Scientific Officer (with a first- or second-class honours degree or equivalent, with at least three years postgraduate experience for Senior Scientific Officer), to work as an Applied Mathematician or Applied Physicist in acoustics—The Director, National Physical Laboratory, Teddington, Middlesex, quoting N P L. 76 XY (October 15).

Temporary Research Assistant (with an honours degree in chemistry or degree qualifications in chemistry and geology combined, and experienced in silicate mineral and rock analysis) in Geochemistry in the Department of Geology—The Secretary, University College, Gower Street, London, W C.1 (October 15).

Principal Lecturer (specializing in physical metallurgy), a Senior Lecturer/Lecturer in Industrial Metallurgy, and a Senior Lecturer in Mathematics—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4 (October 16).

Principal Lecturer in Mathematics, and a Lecturer in Mathematics (with qualifications and experience to teach mathematics for diploma in technology courses)—The Registrar (Department 25/E), Bradford Institute of Technology, Bradford 7 (October 17).

Demonstrator (prepared to engage in research within the fields of palæontology, stratigraphy or sedimentary petrology) in the Department of Geology—The Registrar, The University, Liverpool quoting Ref. No. CV/181/N (October 20)

Laboratory Technician at Galway Regional Hospital—The Secretary, Galway County Council, County Building, Galway, Republic of Ireland (October 20)

Senior Lecturer or Lecturer in Experimental Physics at the University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C 1 (October 25).

Senior Lecturers or Lecturers in Experimental Physics at the University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 25).

Lecturer or Assistant Lecturer in the Department of Geography at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C.1 (October 26).

Lecturer (with special reference to the teaching of science) in the Department of Education—The Registrar (Room 22, O R.B.) The University, Reading (October 27).

Reader (with a Ph.D. in physics or a research record of equivalent level, and preferably teaching experience, or experience in leading research workers) in Physics at the University of Queensland, Brisbane, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Brisbane and London, October 27).

Chair of Pure Mathematics in Monash University, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (Australia and London, October 31)

Lecturer (with an honours degree or a higher degree in chemical engineering, or equivalent qualifications, such as an honours degree in science and Associate Membership of the Institution of Chemical Engineers (by examination), and preferably an interest in thermodynamics or experience in the design and construction of process plant) in Chemical Engineering at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

Senior Lecturer/Lecturer (with at least a good honours degree in agriculture or science, suitable postgraduate experience, and with specialized qualifications in one of the following fields (*a*) agronomy or (*b*) plant breeding and genetics) in Plant Science in the Department of Agriculture, University of Malaya—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (Malaya and London, October 31).

Senior Lecturer or Lecturer (veterinary surgeon) in Veterinary Medicine—The Secretary, Royal Veterinary College, Royal College Street, London, N.W.1 (October 31).

Lecturer in Education (Psychology) at the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 1).

Lecturer or Senior Lecturer (preferably with teaching experience) in Zoology at the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 1).

Senior Lecturer (preferably with experience in cardio-vascular physiology or a related field) in Physiology at the University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 9).

Plant Physiologist at the Regional Research Centre, Trinidad (University of the West Indies), to participate in a programme of research on bananas with particular reference to nutrition, timing of crop production and pruning—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 10).

Lecturer or Assistant Lecturer (medically or non-medically or veterinary qualified candidate) in the Department of Histology—The Registrar, The University, Liverpool (November 17)

Lecturer in Pure Mathematics at Monash University, Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 19).

Lecturer (with an honours degree, qualifications and interests in any of the following: plant taxonomy, cytology, genetics, field ecology, and able to teach courses, involving these subjects, to general degree students) in Botany—Head of the Department of Botany University College of Pius XII, Roma, Basutoland (December 1).

Assistant Editor (with good experience of editing professional papers, make-up and progressing, and with some knowledge of engineering or physics) for the *Journal of the British Nuclear Energy Society*—The Secretary, B.N.E.S., c/o Institution of Civil Engineers, Great George Street, London, S.W.1, marking envelope "Staff–Confidential".

Assistant Professor (preferably with a Ph.D. in physiology), of Physiology—Prof. L. B. Lacques, Department of Physiology and Pharmacology, University of Saskatchewan, Saskatoon, Canada.

Grade B Assistant Teacher in the Science Department, to teach physics in O.N C. Applied Physics and Chemistry Courses, and to G.C.E. "O" and "A" levels—The Principal, Reading Technical College, King's Road, Reading.

Junior Laboratory Technician in the Botany Department—The Secretary, Bedford College (University of London), Regent's Park, London, N.W 1.

Master to teach Chemistry with substantial amount of Sixth Form work—The Headmaster, King's College School, Wimbledon, London, S.W.19.

Physics Master, to teach mainly advanced and scholarship level work—The Headmaster, Bishop's Stortford College, Bishop's Stortford, Herts.

Research Student (with an honours degree or equivalent in chemistry, biochemistry or related subject) in the Department of Biochemistry, to work for a Ph.D. degree in the field of the biochemistry of drugs and toxic substances in hens—The Secretary, St. Mary's Hospital Medical School (University of London), Paddington, London, W.2.

Senior Lecturer (graduate) in Animal and Plant Nutrition—The Clerk to the Governors, Essex Institute of Agriculture, Writtle, Chelmsford, Essex.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. Series A. Mathematical and Physical Sciences. No. 1049, Vol. 255 (August 9, 1962): A Spin-Wave Theory of Anisotropic Antiferromagnetics. By D. ter Haar and M. E. Lines. Pp. 1–30. (London Royal Society, 1962.) 9*s*. 6*d*. [178

Ciba (A.R L.), Ltd Technical Notes No. 235, (July, 1962) Laminated Timber by Kingston (Architectural Craftsmen), Ltd. Pp. 10 (Duxford, Ciba (A.R.L), Ltd., 1962.) [178

Political and Economic Planning. Annual Report 1961–62 Pp. 18. (London Political and Economic Planning, 1962) [178

Bulletin of the British Museum (Natural History) Historical Series. Vol. 3, No. 1. Harry Govier Seeley and the Karroo Reptiles. By William Elgin Swinton Pp. 1–39. 13*s*. Zoology Vol. 8, No. 9: The Distribution of the Coral, *Caryophyllia smithii* and the Barnacle *Pyrgoma anglicum* in British Waters By William J. Rees. Pp. 401–418 + plate 12. 8*s*. (London. British Museum (Natural History), 1962.) [178

Other Countries

Organization for Economic Co-operation and Development. European Nuclear Energy Agency. O.E.C.D. 'Dragon' High Temperature Reactor Project—Third Annual Report, 1961–1962. Pp. 196. (Paris Organization for Economic Co-operation and Development—European Nuclear Energy Agency, 1962.) [178

Food and Agriculture Organization of the United Nations. Freedom from Hunger Campaign—Basic Study No. 3: Animal Disease and Human Health. By James H. Steele. Pp. vii + 50. (Rome: Food and Agriculture Organization of the United Nations, London H M Stationery Office, 1962.) 2*s*. 6*d*., 50 cents. [178

Annals of the Natal Museum. Vol 15, Part 19 New Species and Records of South African Marine Mollusca from Natal, Zululand, and Mozambique. By K. H. Barnard. Pp. 247–254. 5*s*. net. Vol. 15 Part 20. Notes and Descriptions of Pipunculidae from the Natal Museum, South Africa. By D. Elmo Hardy. Pp. 255–266. 6*s* net. (Pietermaritzburg: Natal Museum, 1962.) [178

Die Waldungen der Albis- und Zimmerbergkette bei Zürich. mit einer Farbigen Bestandeskarte. Von Ernst Krebs. (Beitrage zur Geobotanischen Landesaufnahme der Schweiz, Heft 40). Pp. 24 + 1 karte. (Bern Verlag Hans Huber, 1962) 7.80 francs; 7 80 D.M. [178

Suomen Geodeettisen Laitoksen Julkaisuka Veroffentlichungen des Finnischen Geodätischen Institutes. No. 54 Astronomical Azimuth Determinations on Trigonometrical Stations in the years 1955–1959. By V. R. Ölander. Pp. 15. No 55 Gravity Survey of Finland in the years 1945–1960 By Tauno Honkasalo Pp. 35 + 3 maps. (Helsinki Geodeettinen Laitos, 1961 and 1962.) [178

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THE ROLE OF THE INDUSTRIAL RESEARCH ASSOCIATIONS IN BRITAIN

THE major interest of *Research for Industry 1961** lies in the report of the Industrial Grants Committee which, from a policy statement issued with the report, appears to be the full-scale review of the research association scheme which the Minister for Science requested from the chairman of the Council for Scientific and Industrial Research, Sir Harold Roxbee-Cox, last November. Moreover, the policy which it outlines has been fully endorsed both by the Council itself and by Lord Hailsham, who has expressed his satisfaction with the analysis and agrees with the Committee's present policy as set forth in this report. Generally, it endorses the view of the Research Council in its 1958 report that research associations are a valuable investment both for industry and Government, and it emphasizes the importance of keeping the research associations flexible so that they can easily and readily adapt themselves to suit, or shape, the future needs of their industries, and thus ensure that this valuable asset keeps abreast of modern developments and is geared to meet the future challenge.

It is to this end the Industrial Grants Committee reaffirms its opinion that, where they have the facilities, research associations should undertake confidential sponsored work for industry without prejudice to their primary co-operative activities. Direct contact in this way with the problems of firms who know what they want and are prepared to pay the full economic cost to have the work done to their own time-scale can well be, as the Committee suggests, invigorating and rewarding to the staff of the association and possibly strengthen the natural confidence and understanding between the research workers and their industrial clients. Against this, it could involve some difficulties in co-operation in the industry generally, where the competitive spirit is strong, and it is to be noted that when this question of sponsored research was discussed by a group at the Conference on the Pattern of Research in British Industry, arranged by the Federation of British Industries at Eastbourne last April, the view was taken that if such confidential work was undertaken by the research association it should be work in the national interest†. No substantial objection to confidential work as such was advanced because it was held that it was usually only a short time before such work became common knowledge through use. On the other hand, the idea of earmarked sponsored research of a civil nature for the Government was unanimously welcomed.

The Eastbourne Conference was largely based on the survey of industrial research in the British manufacturing industry, which the Federation had previously carried out in collaboration with the National Institute of Economic and Social Research, the results of which were published shortly after Lord Hailsham made his request last November. That report, however, scarcely comments on sponsored research beyond noting the absence of any marked demand for facilities, and although the volume of sponsored research carried out by the associations is growing it still accounts for less than 2 per cent of their income. This compares with about 5 per cent of manufacturing industry's total expenditure on research and development in 1958 in payments to outside bodies such as research associations, independent research institutes and industrial consultants for research services and to other firms for technical knowledge. Since rather less than a third of this went to research associations and most of the remainder is believed to have been spent on such technical knowledge, it is probable that for industry as a whole less than 2 per cent also of the total expenditure was for sponsored work. The Industrial Grants Committee holds that there remains an unsatisfied demand in British industry for such services; by meeting this the research associations could provide a valuable contribution. Incidentally, at the Eastbourne Conference the Parliamentary Secretary for Science seemed to be disturbed that any appreciable expansion in sponsored research by the research associations might adversely affect that in the universities and private research institutes.

The next point made in the report is the importance of a strong and stable research corps supported by adequate funds. Over the ten years covered by the study the cost of research is estimated as increasing by 63 per cent (varying from 41 per cent in engineering to 81 per cent in textiles) and whereas expenditure of the research associations rose by 143 per cent, the growth of research in real terms is put at 52 per cent, varying from 85 per cent in engineering to 36 per cent in textiles and 28 per cent in food. Over the period the total income of the associations increased by 115 per cent, industrial contributions increasing by 133 per cent and the grants from the Department of Scientific and Industrial Research by 53 per cent. In effect, industry has borne the cost of the research growth and part of the increased cost of research, but until 1956 the increase in research barely exceeded that of industrial production and as a whole the research associations have consistently failed to earn the maximum grant offered, the percentage of incentive grant taken up varying between 64 and 76.

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* Department of Scientific and Industrial Research *Research for Industry 1961: A Report on Work done by Industrial Research Associations in the Government Scheme.* Pp. iv + 140 + 12 plates (London: H.M.S.O., 1962.) 10s. net. See also p. 115 of this issue of *Nature*.

† Federation of British Industries. *Pattern of Research in British Industry*. Report of a Conference at Eastbourne, 5–7 April, 1962. Pp. v + 84. (London. Federation of British Industries, 1962.) 5s.

An important factor governing the ease with which increased industrial income and the grant it attracts may be collected is the method adopted by the research associations to assess the appropriate industrial contributions. The Industrial Grants Committee identified 21 different methods of raising subscriptions which may be grouped in six broad categories, of which individual negotiation yields the largest total contribution. Nevertheless, in the general criticisms of research associations made at the Eastbourne Conference, the time spent in raising money figured prominently. It was suggested that there were too many associations and that they might be aligned by disciplines rather than by industries. There was strong support for a fixed Government grant rather than one dependent on industry's contribution.

The Industrial Grants Committee has failed to establish any correlation in the period 1949–58 between the ratio of non-grant to grant income of the associations, age, number of members, expenditure, output of industry and private research by industry. Although there is no statistical evidence that the Government grant determines the pattern of growth of research associations, the grants have manifestly contributed to the initial formation of new associations and fostered reasonable subsequent growth. In recent years, instead of making upward adjustments of the block grant at the beginning of the five-year grant period, the Committee has more often made the adjustment in the middle years, so that associations have had adequate forewarning of the intention to stiffen the grant terms and have been able to plan their budgets to avoid discontinuities of income. Discussions at the Eastbourne Conference can, however, have given the Research Council little fresh confidence as to the effectiveness or the popularity of a policy of stiffening the grant, although the Industrial Grants Committee reports that it has seldom been asked to review grant terms for financial reasons during a normal grant period.

There is a wide variation in the distribution of the effort of the associations between basic and applied research, technical liaison and information. The present average distribution is 23 per cent on basic and 47 per cent on applied research, 18 per cent on technical liaison, and 12 per cent on library and information; in more than half the associations the range in each category is 17–29, 39–55, 13–23, and 9–15, respectively. Most of the associations with low expenditure on basic research serve small industries; those with high expenditure on applied research are large and well established. In an important address at the Eastbourne Conference Sir Harold Roxbee-Cox discussed the role of Government in civil research and development, but his only reference to the support of basic research by the Council for Scientific and Industrial Research related to its research grants to universities and its postgraduate awards. He recognized the problem of financing research associations, but gave no hint of any fresh approach apart from the statement that the Council was looking into the possibility of a system of 'earmarked' grants which will encourage the research associations to undertake projects of direct benefit to the community or industry at large, such as highly speculative projects involving the application of advanced technology, new approaches to productivity, and management studies capable of general application. It is hoped that this type of activity will also strengthen the outstanding terms which are already working in some of the associations' laboratories.

This system of earmarked grants was briefly mentioned in the Council's report for 1961, but it received little notice at the Conference where Sir Owen Wansborough-Jones, reporting on a Discussion Group on the effects of Government contracts on research in industry, referred to what appears to be an alternative method in which the initiative came from industry or the universities. Two research associations collaborated with industry and the universities to examine a problem of interest to all of them, but not of primary interest to any one. A similar example was reported from industry. One of these was concerned with basic problems of oxidation of hydrocarbons and the other with the high-temperature properties of steel. The Group considered that this form of initiative was extremely desirable and would be encouraged if it were known that such initiative was welcomed and had a reasonable chance of Government support.

This appears to be the type of initiative that the Minister for Science is seeking, for in a speech at the Conference dinner, he reiterated Sir Harold Roxbee-Cox's plea for greater support from industry itself and added the warning that industry must also assume greater responsibility for spreading information. Without industrial support it would be necessary to close the information centres started recently in large industrial centres. No indication of this is given in the present report of the Council for Scientific and Industrial Research, and, in view of the meagre information forthcoming on that aspect of the Council's activities, it would seem that the policy is self-defeating, however fundamentally sound is the principle that Government support should be given in response to industrial effort or initiative.

The Industrial Grants Committee's present policy in support of co-operative research is to encourage the establishment and healthy growth of co-operative research programmes for industries or groups of firms who believe that such programmes are necessary The first essentials are the determination of the industrial group to set up a programme, a clear statement of how the industrial finance is planned to increase, and a vigorous director of research. If the scale and quality of the effort proposed are regarded as adequate to maintain a healthy research programme and likely to be further developed, gran support is normally given on a scale designed to produce adequate contributions from industry, and while the proportion of total income derived from Government grant will usually diminish in any individual association, the total grant offered to all research associations has increased each year.

The Committee emphasizes that each individual application differs from the others in some respects and that there is no general formula for assessing the appropriate offer of grant. It is satisfied that the policy of progressively stiffening grant terms to established associations has been reasonable and that industry has rightly accepted responsibility for the major part of the associations' growth. It is also satisfied that the present policy, with the additional flexibility now introduced in such matters as foreign membership and sponsored research, is sufficiently flexible to meet the normal conditions of growth. The initiative rests firmly with the members to ensure that the services are such as will attract the income which will earn maximum grant, make special contributions to the general funds to accelerate parts of programmes of special interest and extend membership, or sponsor confidential work. Beyond this, however, the Committee refers to its recommendation that a new and additional form of 'earmarked' grant should be offered for select projects such as research into aspects of industrial health or safety, research which is to the benefit of the community or industry as a whole, or in further support of outstanding teams the abilities of which can be more widely used.

In all this, however, there is nothing new and no indication of how the problem of finance, for example, is to be more efficaciously or economically handled, or of grappling with these particular problems of improving the research associations which were once again raised at the Eastbourne Conference. More fundamental, in fact, appear to be the Committee's observations on the relations between research associations, universities and technical colleges. There is a reasonably close liaison between research workers in research associations and university departments who are interested in the same or related fields of work, and this has been much stimulated by the appointment of academic visitors to the associations. The associations place work in universities, either by contract or by financing bursaries or research fellowships. There are some joint projects, but although the associations sometimes play a part in postgraduate instruction, planning courses jointly with universities, allowing part of the students' work to be done in their laboratories and supplying external examiners, universities are reluctant to approve external laboratories such as research associations for postgraduate training. There are signs that university regulations are being relaxed, but, although the Industrial Grants Committee makes no explicit statement, it would appear that the major responsibility rests on the universities if full use is to be made in future of the facilities of the larger associations.

If the comments of the Zuckerman Committee on the location of research institutions are fully implemented a decisive change in the attitude of some universities may be required, and the Research Grants Committee of the Department of Scientific and Industrial Research as well as the University Grants Committee should be able to assist to that end. The research associations already give all the help they can to the colleges of technology as well as to the technical colleges, and the inclusion of research associations among the approved industrial organizations in which part of the approved course of study for the new award of Membership of the College of Technologists may be carried out affords a means for even closer collaboration in future. The possibilities of such co-operation will obviously be increased if such a policy of location as the Zuckerman Committee recommends is followed, and it is one which the University Grants Committee as well as the Minister for Science should keep clearly in mind.

It could also be assisted by the greater mobility for which the Eastbourne Conference, as well as the Zuckerman report, called. The desire for greater mobility of staff between industry and the research associations as well as the research stations of the Department of Scientific and Industrial Research and the colleges of technology and the universities was a marked feature of the discussions, particularly the support for permanent part-time appointments to the staffs of universities and colleges of technology of outstanding men from industry. Such a step in itself should help to facilitate co-operation between the universities or colleges and the research associations in different ways; it is obvious that the difficulties are often best overcome when they are considered, as they should be, in the light of Britain's needs for manpower and research as a whole.

The Industrial Grants Committee's report adds nothing to the observations of the Research Council in its report for 1961 on the participation of research associations in operational research and the work of the Industrial Operations Unit, or on the projects submitted by the research associations under the scheme for special assistance to industry. Generally, indeed, it seems to throw little fresh light on the research associations or to offer proposals for making them more effective. Their value in relation to other means of co-operation was more searchingly discussed at the Eastbourne Conference of the Federation of British Industries, but neither the Industrial Grants Committee's report nor that of the Eastbourne Conference on the pattern of research in British industry points to any concrete achievement as a result of the inquiry which the Minister for Science instituted last November.

The great value of the Eastbourne Conference lies in the attention it focused on some vital questions which were left unanswered but demand continuous study. Mr. Aubrey Jones, at the outset, like the Minister for Science, but from a different angle, made the point that the stimulus which Government finance had in the past given to research and development in general in industry was likely to decline. After suggesting that Britain needed to consider impartially the possible limitations of the competitive system from the point of view of research and development, he challenged the amount of effort Britain was putting into basic research. He thought that what was needed was a consideration of whether the broad economic interests of the country required the research and development effort in industry to be more evenly spread, and whether or not the gaps could be defined in Britain's civil effort which it should be the purpose of Government effort to try to fill.

What stands out above all is the need for more fundamental and constructive thinking. This was very evident in Sir Harold Roxbee-Cox's address, particularly in his reference to Government assistance to civil development and the use of civil development contracts. It was made quite clear that the Department of Scientific and Industrial Research is not only reviewing the pattern of the research associations but also that of its research stations. It may well be that policy will not be finally determined nor action taken in advance of the report of the Trend Committee now reviewing the Government organization for science under civil agencies. Meanwhile, the Eastbourne Conference has brought to the front again many of the questions such as the size and organization of firms and research units, the location of research units, the training of managements and the nature of and means for effecting co-operation between industry, Government research stations or departments, the universities and the colleges of technology. The answers to these both determine and are influenced by the character of Government organization—of which, from one point of view, the research association is a part.

JOHN VON NEUMANN

Collected Works of John von Neumann

Edited by Prof. A. H. Taub. Vol. 1: Logic, Theory of Sets and Quantum Mechanics. Pp. x + 654. Vol. 2: Operators, Ergodic Theory and Almost Periodic Functions in a Group. Pp. x + 568. (London and New York: Pergamon Press, 1961.) 100*s*.; 14 dollars each volume.

JOHN (Johann Ludwig) von Neumann was born at Budapest in 1903. His mathematical ability was recognized early, and his father, Max, a banker, made unusual arrangements for his education. Von Neumann's first paper was published before he was eighteen, in collaboration with his tutor, Fekete. While registered for the next four years at the University of Budapest, and taking examinations there, von Neumann was studying at Zurich and Berlin. In 1927 he became *Privat-Dozent* in the University of Berlin and transferred to Hamburg in 1929. He was visiting lecturer in the University of Princeton in 1930, and became a professor there in 1931. Two years later he moved to the Institute for Advanced Study as a professor and held this position for the rest of his life. He became an American citizen in 1937. He died in 1957.

In appearance John von Neumann was on the short side, comfortably tubby in his later life, with a round expressive face. Everybody knew him as 'Johnny'. He was universally liked, and a very large circle of friends remember him with the deepest affection. His thought processes were very quick, and he could speak rapidly in several languages. It was entirely consistent with his personality and his interests that he should be fascinated by the mathematical logic of automata and should foresee the enormous possibilities of such machines.

A tribute to von Neumann appeared in a special memorial number of the *Bulletin of the American Mathematical Society*, (64, 1958) containing eight articles by well-known mathematicians. Groups of his papers are discussed in detail and their place in modern mathematics is assessed. Another authoritative article is the obituary notice by Dr. F. Smithies in the *Journal of the London Mathematical Society*, (34, 1959).

Von Neumann was the author or co-author of several books, on quantum mechanics, on the theory of games and economic behaviour, and on functional operators. His papers are now being re-published as *Collected Works* in their original languages, and where necessary, with errata. Volume 1 covers period 1922–29; and Volume 2 the period 1929–34. The first article in Volume 1 is different in type from all the others. It is called 'The Mathematician' and was first published in 1947. This article is an apposite introductory paper, because it gives von Neumann's views about the nature of the intellectual effort in mathematics.

Volume 1 shows the power and range of the youthful von Neumann. Perhaps the common element is the logical development of theory from axioms with an admirable economy in style and algebraic technique but the creative facility of his mind is being continually revealed.

In 1925 was published the first usable version of the axiomatic theory adumbrated by Zermelo in 1908. The purpose was the same as that of Russell's theory of types, namely to avoid the contradiction of informal or 'naive' set theory. Since the Zermelo–von Neumann theory did not allow the famous Frege–Russell definition of the ordinal and cardinal numbers to be formulated, von Neumann constructed an entirely new theory of the ordinals (1928), a theory now in general use.

The elements of von Neumann's theory of games appeared in 1928. This paper, and the extensions and applications contained in the book with Morgenstern give the mathematical foundations of games of strategy and have influenced all workers in this rapidly growing field. The 'minimax value of a game' and the 'pay off matrix' are deep practical concepts which have been applied in computational programmes illustrating the interplay of economic forces. Although still in an early stage, these investigations are of great interest to those working in the social sciences.

Von Neumann was attracted by the thought of placing the quantum theory on a rigorous axiomatic basis. He saw that the proper framework was Hilbert space; but that it was first necessary to extend and clarify the classification of the linear properties of unbounded operators in unitary spaces. Von Neumann's papers on Hilbert spaces, operator and spectral theory, and rings of operators were outstanding and form a substantial part of his life's work. Volumes 1 and 2 contain his earlier papers in this vast field some of them written in terms of quantum mechanics and some written as formal algebraic studies. Both volumes contain papers on more technical problems in quantum mechanics and statistical mechanics some of the former being written in collaboration with E. Wigner.

Volume 2 contains several papers on measure theory and ergodic theory. Ergodic theory is concerned with the asymptotic temporal behaviour of a conservative mechanical system interpreted by unitary operators in Hilbert space. Although these papers represent only a small part of his total contribution to mathematics, their quality is such that even if he had done nothing else, his place in mathematics would be assured.

A celebrated paper in 1933 solved Hilbert's fifth problem for the compact case and the solution was completed almost twenty years later by others to include the open n-dimensional groups. The question was (in modern terminology) whether in every locally Euclidean topological group there exist systems of co-ordinates such that the co-ordinates of the group-product xy are analytic functions of the co-ordinates of x and y. By an averaging process invented by him, von Neumann showed that the answer is 'yes' for a compact group.

Von Neumann's work on continuous groups include two papers in Volume 2 on almost periodic functions in a group.

Prof. Taub and those who have helped him are to be warmly thanked for editing these magnificent volumes. The publishers are to be complimented on the good printing and the modest price. Although none of von Neumann's papers is easy reading, the importance of his work is so great that these *Collected Works* must be given a place in all libraries concerned with modern mathematics.

Several mathematical colleagues have helped me with advice in the preparation of this review.

W. G. PENNEY

BIOCHEMISTRY OF THE CARBOHYDRATES

Carbohydrates of Living Tissues

By Prof. M. Stacey and Dr. S. A. Barker. Pp. xvii+215. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1962.) 45*s*.

As our knowledge of the chemistry of living matter is extended, a study of the polysaccharide and oligosaccharide complexes present in the animal body is of paramount importance. The present book gives a comprehensive survey of these very complicated materials from the point of view of their known distribution, biological activity, constitution, and chemical properties, together with an account of any abnormalities which are known to occur as the result of a specific disease. The authors are to be congratulated for condensing into some two hundred pages most of the background and modern work on these substances. The bibliographies which conclude each of the chapters are such that the reader is at once presented with the major publications, up to and including 1960.

The first four chapters deal with glycogen, hyaluronic acid, chondroitin and chondroitin sulphate *A*, *B* and *C*, and heparin and heparatin sulphate. Methods for the isolation, fractionation, detection and determination of each of these polysaccharides are detailed, and the chemical techniques utilized in the elucidation of their structures are outlined. The enzymatic pathways, in so far as they have been established, in the biosynthesis and degradation of these polymers are described, and the biological activity of each particular substance is stressed. An account is also given of changes in constitution and amount that occur as the result of a particular disease, and in the case of glycogen this is related to enzymatic deficiencies. The close structural relationship between hyaluronic acid and the chondroitin sulphates is excellently illustrated by the formula on p. 82.

Keratosulphate, mucoitin sulphate, the neutral galactans, and a brief account of the lesser-known sulphated and phosphated polysaccharides, and the constitution, biological activity, and inhibition reactions of the human and cows' milk oligosaccharides form the subject-matter of Chapters 5 and 6.

The last three chapters are devoted to the blood-group substances, to the mucoproteins of blood, urine, saliva, cerebrospinal and other fluids, and to the lipocarbohydrates respectively; in the latter are included the cerebrosides and gangliosides. The authors state that the book is presented only as an interim report, and this is particularly true of a number of substances described in the final chapters of which little is known other than the method of extraction, the constituent sugars and perhaps the biological activity.

The occasional footnote explaining a few of the medical terms could, with considerable advantage to the ordinary chemist, have been increased to include all such terminology, but this is a minor criticism. What is more important is that the book is well written, accurate, and attractively produced with clearly printed structural formulæ.

Inevitably much of the data is presented without comment, but the chief merit of the book is that it brings together a wealth of information which previously has not been collected and which can otherwise only be obtained by an extensive search through the literature. At the published price this book is excellent value, and should immediately appeal to every scientist interested in the operation of the animal body, to biochemists, to research chemists, and to all engaged in the medical profession, particularly those studying chemical changes occurring in living tissues during illness.

ELIZABETH PERCIVAL

ORGANIC AND ENZYME-CATALYSED REACTIONS

Mechanisms of Organic and Enzymic Reactions

By S. G. Waley. Pp. xiii+365. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 70s. net.

DR. S. G. WALEY has written a book in which the mechanisms of organic reactions and of enzyme-catalysed reactions are discussed together. He starts with molecular structure, and the general principles of mechanistic organic chemistry. He then considers in some detail the more or less usual list of reaction types which have yielded to mechanistic investigation, that is to say, nucleophilic substitutions, olefine-forming eliminations and their retrogressions, carbonyl reactions, saturated rearrangements and electrophilic aromatic substitutions. What distinguishes this from more conventional accounts of mechanism in organic chemistry is the inclusion of reactions, such as phosphate ester hydrolysis, which are of direct interest to biochemists, and of enzymatic reactions themselves. The author has therefore set himself the unenviable tasks of bringing enzymatic reaction mechanisms into the respectable fold of organic reaction mechanisms and, at the same time, of explaining the mystique of the latter to the uninitiated.

Undoubtedly, this is a worth-while and interesting attempt. Students of biochemistry, whose instruction may include little physical organic chemistry, will find a clear although rather elementary account of organic reaction mechanisms, essentially in the spirit of what the Americans call the 'English School'. Chemistry students, on the other hand, will profit from the view that enzymatic reactions are not essentially different, except in complexity, from any other kind and that a study of their mechanisms is both legitimate and potentially profitable.

However, Dr. Waley's approach has its limitations. The hydrolysis of glycosides, for example, is correctly formalized as a nucleophilic substitution on carbon (p. 166). In the context of this book this formulation carries the implications that the criteria which have been so fruitful in distinguishing in simple systems the so-called S_N1 and S_N2 categories, could, in principle, be used for the enzymatic hydrolysis of glycosides and, further, that such a distinction is valid and useful. Both implications may be wrong. In fact, their acceptance leads Dr. Waley to favour Koshland's view that the glycosidases act by producing two configurational inversions, each the result of a simple S_N2 displacement. This may be so, but there is no evidence supporting it. Enzymatic mechanisms cannot be deduced by analogy with simpler systems ; they can only be understood in relation to the structures of the catalytic proteins, a subject which occupies little space in this book.

Nevertheless, Dr. Waley's account could be read profitably by both students of biochemistry and of chemistry. The style is good and clear and the book is well arranged. The price (70s.) however, is to be regretted. Not many students will be able to afford it.

C. A. VERNON

'PARKER AND HASWELL ZOOLOGY' REVISED

A Text Book of Zoology

Vol. 2. By the late Prof. T. Jeffery Parker and the late Prof. William A. Haswell. Seventh edition, revised and largely rewritten by Prof. A. J. Marshall. Pp. xxiii+952. (London: Macmillan and Co., Ltd.; New York: St. Martin's Press, Inc., 1962.) 70*s.* net.

It is appropriate that this old favourite, originally written more than sixty years ago by the professors of biology in the Universities of Otago and of Sydney, should be revised and much expanded by an alumnus of Sydney, and should appear as he returns to his own country as professor of zoology and comparative physiology in Monash University, a step in a distinguished career of research and teaching on which all his friends congratulate him.

Prof. Marshall has preserved the general plan of this text-book that has well served so many generations of students, but in his revision he has "attempted to retain basic morphology and yet at the same time emphasize functional aspects and, where possible, present animals as living creatures rather than as laboratory specimens". The strong dose of natural history that he has injected into the book brings it very much to life, and he has followed the lack of solemnity that he admires in Simpson's *The Principles of Classification and a Classification of Mammals* by giving many pieces of out-of-the-way information with characteristic humour—students will surely be glad to find a smile here and there and an occasional broad guffaw in a book so crammed with facts.

The book gains greatly from the criticism and collaboration of a number of zoologists whose names make an impressive list; some of them have contributed authoritative sections on the subjects of their special study. The book is further improved by the expansion of the sections on palæontology in spite of the opinion of some zoologists that the previous edition already carried too much information about fossil forms—"it is impossible to gain a good understanding of living animals, either in form or function without an adequate appreciation of what is known of their ancestry". An innovation is the inclusion of the elements of a classification under many of the illustrations in order to "help the student more or less painlessly to familiarise himself with the animal's systematic position". The student will also be grateful for the lavish cross-reference system so that comparative information can be found quickly and "without tediously repetitive consultation of the Index or Contents".

The book is even more fully illustrated than before; not only are there the familiar figures from Wiedersheim, Owen, Flower and many others but also new figures have been introduced from the works of numerous authors. In addition, the work is embellished with many attractive pen drawings and diagrams by Jane Marshall. These, whether showing living animals or reconstructions of extinct creatures, illuminate the text in more senses than one, but their difference in style from the others gives the volume a certain lack of uniformity that although no detriment is slightly disconcerting to the reader on first perusing it. Perfection is impossible: some misprints have slipped through the proof-reading, and an error of fact appears on page 780—a blue whale yields about 135 barrels of oil, not 1,380, equivalent to about 22½ tons, not six.

The book concludes with an extensive list of references and a copious index. With Prof. Marshall's re-writing *Parker and Haswell* starts a new lease of life and will assuredly well serve further generations of students.

L. HARRISON MATTHEWS

EXOTIC FOREST TREES IN THE BRITISH COMMONWEALTH

Exotic Forest Trees in the British Commonwealth

By R. J. Streets. Edited by Prof. Sir Harry Champion. Pp. xii+765+23 plates. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 63*s.* net.

Exotic trees play an important part in organized forestry and this is very true of many countries of the British Commonwealth. Plant collectors and travellers were responsible for introducing small numbers of exotic trees which were treated, in most cases, as individuals or small groups, but a landmark in the large-scale use of exotics is the middle of the eighteenth century, when extensive plantings of European larch were made in Perthshire. Since the middle of the past century, the use of exotic forest trees has spread greatly, both for afforestation and re-forestation purposes. The reasons for this are many, but among the main ones are to increase the national forest estate for economic or other purposes, the need to find species suited to the afforestation of difficult sites such as swamps, peat and spoil dumps, and the replacement by suitable, preferably fast-grown, species of degraded forest and woodland lacking produce of merchantable value. Examples are numerous. Of the many coniferous species being used in Great Britain only the Scots pine is indigenous. Exotic conifers, particularly the Monterey pine, play a very important part in New Zealand's forest economy. Almost the entire yield from the forests of the Republic of South Africa is made up from exotic species. Eucalypts have an important role in many parts of the world as exotics, and teak is the most important exotic forest tree used in Trinidad.

A detailed account of the part being played by exotic forest trees in the British Commonwealth is contained in Streets's book. It is based on Troup's classic *Exotic Forest Trees in the British Empire* (1932, Clarendon Press, Oxford) and it has been brought up to date by the reports submitted to the Seventh Forestry Conference of the British Commonwealth in 1957, but a few territories did not respond to the request for information. Mr. Streets has carried out the work of revision and compilation in a most commendable manner and the result is worthy of this important subject.

After a suitable introduction, the information is presented in two parts. Part 1 occupies some 100 pages and it summarizes, according to individual countries grouped in six geographical regions, experience in the use of exotics. This is given under policy, climate, soils, establishment and species. Part 2 takes up the bulk of the volume and in it is described the experience obtained with individual species, listed in alphabetical sequence of generic names. Where appropriate, failure of a species in a particular territory is noted.

Compiler, editor and publisher are to be congratulated in providing a standard work which is very readable, well set out, makes for easy reference and is suitably illustrated. It will readily find its proper place among essential forestry literature.

C. J. Taylor

A NEW BULB BOOK

Collins Guide to Bulbs

By Patrick M. Synge. Pp. 320+56 plates. (London: William Collins, Sons and Co., Ltd., 1961.) 30*s*. net.

Bulbs are Nature's contribution to the pre-packed age: "from no other group of plants", as Mr. Synge observes, "can one obtain a quicker return, since the embryo flower is already present in the bulb when we buy it". Colchicums will flower on the dealer's counter; *Sauromatum* will expand its stinking spathes on the barren expanse of the parlour mantle-shelf, while fragrant paperwhites and hyacinths will survive and give a fair account of themselves in circumstances which would spell instant death to any less tolerant race of plants. Even the choicest and most difficult of bulbs will frequently save the reputations of the vendor and unskilled purchaser by producing a brave show of flowers in their first (and often last) year of trial. Small wonder that bulbs are so generally popular, and small wonder they suffer neglect and maltreatment that would call for instant action by a society for the prevention of cruelty to plants were such a worthy organization to exist.

Mr. Synge's lucid and comprehensive treatise is likely to increase the popularity of bulbous plants, and his sensible advice on bulb-culture may well save many a purchase from an untimely fate. Although the rabid specialist may discover omissions in this book, most readers will find everything they are likely to grow, or wish to grow, in their gardens and greenhouses, as well as descriptions of many rarer subjects, some of them strangers to the pages of our bulb catalogues, and seldom seen outside botanic gardens. The genera and species are arranged alphabetically, which makes for easy reference, even if it offends systematists and sometimes results in the separation of such close allies as *Acidanthera*, *Gladiolus* and *Watsonia*. The text, though of necessity terse, is here and there peppered with personal observations and short digressions that serve to evict boredom, and reassure the reader that the author not only knows, but also grows, many of the plants he lists. The numerous colour illustrations are for the most part excellently clear and informative, though occasionally a little too harsh in tone, so that the lavender-blue *Crocus* threatens to rival *Tecophilaea cyanocrocus* in an excess of brilliance. The glossary and useful bibliography round off a treat, for which, most of us must agree, thirty shillings is a modest reckoning.

R. D. Meikle

NUCLEAR PROPULSION

Nuclear Propulsion

By M. W. Thring. Pp. v+300. (London: Butterworth and Co. (Publishers), Ltd., 1960.) 50*s*.

This book consists of a number of chapters by various authors on themes having some relevance to the problems of nuclear propulsion. There is no introduction by the editor to indicate the plan underlying this collection of articles or the type of reader to whom it is addressed, but it would appear to be a write-up of a set of lectures given to a general technical audience with only a background knowledge of nuclear reactors.

Separate chapters cover the physics and design principles of nuclear reactors and the thermodynamics of jet and rocket propulsion. This is then followed by chapters on nuclear marine propulsion, nuclear aircraft and the nuclear rocket. There is some discussion of materials problems and the book concludes with a chapter on medical and biological aspects of life in sealed cabins.

The range of topics is wide and detailed treatment cannot be expected. More important, the discussion is based on the technology of 1959 and much has changed since then. Although work on the United States nuclear aircraft propulsion project has been discontinued, the nuclear rocket has received increased impetus while the problems of marine nuclear propulsion have continued to receive serious study.

The individual chapters are well written and cover their subjects with admirable compactness. It is a book which is worth reading once, for background knowledge, but it would have been greatly improved if the editor had restricted its scope. There is not a lot in common between the problems of marine and rocket nuclear propulsion.

J. Fell

FURTHER COMMENTS ON ATTEMPTS BY H. MOISSAN, J. B. HANNAY AND SIR CHARLES PARSONS TO MAKE DIAMONDS IN THE LABORATORY

By PROF. KATHLEEN LONSDALE, D.B.E., F.R.S.
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THE recent successful laboratory crystallization of diamond, in many parts of the world, from carbon dissolved in suitable media under high-pressure, high-temperature conditions has aroused new interest in the attempts made in the nineteenth and early twentieth centuries to make synthetic diamonds. Is it possible that Moissan, Hannay or Parsons really did succeed, in spite of earlier doubts ?

In the case of Moissan and Parsons, none of the specimens they made could be traced after their deaths, although one might have supposed that if genuine, these specimens would have been deposited in the safe keeping of a university or national museum. On the other hand, neither Moissan nor Parsons seem personally to have published any denial of their original claims that they had synthesized diamond. There is, however, plenty of evidence that Sir Charles Parsons was finally convinced that he had never made diamonds; that he realized that his Bakerian Lecture to the Royal Society (1919) was based on an error; and that he took steps to see that a correction *was* published. This evidence I give later on.

The case of Hannay is different. There are, in the British Museum (Natural History), specimens which purport to be the remains of a sample submitted by Hannay to Prof. Story-Maskelyne for examination. These 12 specimens were examined by F. A. Bannister and myself, using X-ray diffraction techniques, in 1942, and the results were first published in *Nature*[1], where various references are given, and afterwards in the *Mineralogical Magazine*[2]. These publications led to considerable correspondence, both published and private, and it seems worth while now to give some extracts from this private correspondence, as well as further X-ray data, obtained more recently, on these 'Hannay diamonds'.

One question that arose was whether in fact the specimens in the British Museum are indeed the remainder of those tested by Story-Maskelyne at Hannay's request. In 1944 I wrote as follows to Dr. E. H. Kraus, who had raised this point: "They correspond exactly to Professor Story-Maskelyne's description of them in *The Times* letter" (February 20, 1880) "and they were labelled in the handwriting of a museum employee who was known to have been most careful and reliable". (This statement was made on the authority of Dr. L. J. Spencer, himself meticulously careful in such matters.) "The fact that there is no other record in the British Museum of a study of the specimens is not very surprising, for at this time the British Museum was in the throes of moving from Bloomsbury to its present quarters in South Kensington, and many records were lost or destroyed. But the existence of the specimens themselves is, of course, listed in the records, and from that point of view they are as authentic as any of the other specimens of that time."

The X-ray diffraction studies made by Dr. F. A. Bannister at the British Museum in 1942 established beyond doubt that 11 of the 12 tiny British Museum crystals were diamond, and my more detailed X-ray study of one of them, using the powerful 5-kW equipment at the Royal Institution, proved that it was a type II diamond (that is, that it did not show 'extra spots' regarded as typical of type I). The British Museum authorities were naturally not willing to allow all these unique specimens to be removed from the Museum for individual and prolonged testing during the uncertain conditions of war-time; and our investigations therefore ceased at this point. At the time it was believed that type II diamonds were very rare and that even if only one out of the eleven 'Hannay diamonds' were of type II that would be strong presumptive evidence that they were synthetic and not natural. On April 5, 1943, I wrote to Lord Rayleigh: "If type I diamonds are 'perfect' (in the X-ray sense) and type II diamonds are 'mosaic' in structure, as all our experiments seem to indicate, then it is fairly certain that if and when a reliable laboratory method of making diamonds is discovered, it is type II diamonds that will be obtained, since no laboratory experiment can ever hope to reproduce the ideal 'annealing' conditions found in Nature's workshop in the depths of the earth". By this time, however, I was not so sure of the *rarity* of *natural* type II diamonds, for in the same letter I wrote: "I am now coming to the conclusion that diamonds giving type II effects are perhaps not so rare as was formerly supposed. It is true that in one parcel of 500 diamonds that I have recently examined with a direct vision spectroscope, only one was transparent beyond λ3000, but on the other hand two much smaller assortments of industrial diamonds contained some 50 per cent type II specimens". Later on, Dr. H. Judith Grenville-Wells[3] found a high proportion of type II (by a study based on ultra-violet transparency) in a collection of 175 *small*, good natural diamonds. Recently, Dr. Milledge (formerly Grenville-Wells) has examined two more of the British Museum 'Hannay diamonds' and has found that these do show the 'extra spots' typical of type I. It is clear, therefore, that this part of the evidence is no longer a point in Hannay's favour.

Two questions then arose: whether other evidence of an 'internal' nature could be found that would suggest whether these diamonds were natural or synthetic; and whether Hannay himself was trustworthy ? It is easier now to deal with the first of these two questions. All the diamonds recently synthesized, and studied by us, have been rather imperfect. They have all given diffraction patterns on which some spots are poorly shaped and on which there are definite signs of distortion. So far we have found none of type I, and attempts are at present being made to determine the source of the type I diffraction phenomena by the 'doctoring' of synthetic diamonds under controlled conditions—as yet without success[4]. We understand that there are now synthetic diamonds made which do have good morphological habits, but we have not yet had the opportunity of studying their X-ray diffraction (Laue and diffuse) patterns. Unlike the synthetic diamonds which we *have* studied, the 'Hannay diamonds', although laminated and splintery in appearance, give very good, clean diffraction patterns without much 'spot distortion'. They do, in fact, give just the kind of diffraction pattern which would normally be expected from splinters of natural diamond. If my opinion were now asked, I would be bound to say that I think that the 'Hannay diamonds' *are* splinters of natural diamond, or at least that there is no 'internal evidence' to the contrary.

The question of Hannay's trustworthiness is far more difficult to answer. As Lord Rayleigh wrote to me (November 26, 1943): "Some people at close quarters with Hannay seem to have been convinced of his good faith. On the other hand, you will probably agree that there was a formidable array of instructed opinion that disbelieved". This current disbelief seems to have been based on the inherent unlikelihood that a young man of twenty-four years of age (as he was in 1879), a chemical engineer without a formal training in physics, could have succeeded in what was, after all, a very empirical 'long shot' experiment, however persistently repeated; and also on some fully justified criticism of some of his other published work, criticism which he never attempted to refute. Lord Rayleigh, in a letter to *Nature*[5], mentions also a paper by Hannay that was rejected by the Royal Society, but there is no mention in the Royal Society records of such a paper. I am informed that if submitted at all, it would have been recorded, even if rejected. Later disbelief has been based on the failure of all attempts to repeat Hannay's experiments under the conditions that he described. In a sense, however, this particular criticism is discounted by the fact that he claimed success in only a very small percentage of his own experiments, added to the difficulty of repeating experiments with materials precisely similar to those available more than eighty years ago. It seems, therefore, that the diamonds in the British Museum are almost certainly natural diamonds; but whether Hannay himself knew this is uncertain, and will probably never be proved.

The case of Moissan is somewhat parallel except that no 'internal evidence' is available since no specimens are available. His methods have been repeated many hundreds of times without the formation of any diamonds. I recently inquired of Prof. J. Wyart, head of the Department of Mineralogy and Crystallography at the Sorbonne, whether he could supply me with any further information, for possible publication, about Moissan's experiments. He replied as follows:

"Dans le Laboratoire de Moissan, il ne reste aucune trace de l'appareil et des cristaux de diamant. Henri Le Chatelier, qui a été son successeur au Laboratoire de Chimie générale, ne croyait pas au diamant de Moissan et dans son livre 'Science et industrie' (Flammarion, 1925) il dit à la page 195: 'Personne ne croit plus au diamant de Moissan; même de son vivant, en dehors de ses élèves, peu de savants ont pris au sérieux cette prétendue découverte.' Cette opinion de Le Chatelier était aussi celle de F. Wallerant, qui occupait la chaire de Minéralogie de la Sorbonne et qui a été mon premier maître. Il avait vu les diamants de Moissan, et Wyrouboff, très connu pour ses travaux de chimie et de cristallographie, qui travaillait à coté de Wallerant, les avait soigneusement examinés au microscope polarisant. Il en avait conclu que les cristaux de Moissan n'étaient pas du diamant."

The possibility that Sir Charles Parsons had made diamond was far more intriguing. In his Bakerian Lecture to the Royal Society (1919) Sir Charles described various experiments in making diamonds, some of which he claimed were successful, and added: ". . . nothing was obtained of interest with the substances used by Hannay unless, as previously mentioned, some iron was present". This implied that something "of interest" was obtained in the presence of iron. Yet in a letter to *Nature*[6], Lord Rayleigh stated that Sir Charles Parsons had repeatedly conversed with him on the subject of his experiments and had told him that the crystals obtained would not burn in oxygen and therefore were not diamonds.

An article on "The Problems of the Artificial Production of Diamonds" had appeared in *Nature* in 1928[7] signed "C. H. D.", summing up the case for believing that up to that time no diamonds had ever been made in the laboratory. I corresponded with the author, the late Dr. C. H. Desch, and received a letter from him dated November 3, 1942, in which he wrote: "When Sir Charles Parsons had repeated his experiments and had concluded that the crystals which he at first obtained were not diamond he sent me all the notebooks and also his correspondence with the French people who had known Moissan. It seems that Moissan was in bad health in his later years and never made any satisfactory examination of the crystals . . .". Dr. Desch also referred me to Sir Charles Parson's assistant, the late Mr. H. M. Duncan, with whom I had considerable subsequent correspondence. Mr. Duncan gave me permission, in a letter dated March 16, 1944, to mention his name as having supplied me with information about Sir Charles Parsons's experiments on the manufacture of artificial diamonds.

I had been impressed by the fact that Hannay had claimed that his only successful experiments were those in which lithium was included in the mixture. (Lithium and diamond have rather similar lattice constants, 3·509 and 3·567 Å respectively, although Hannay could not possibly have been aware of this fact, which is given by the results of X-ray structure analysis. Lithium is, however, a body-centred cubic structure and diamond a face-centred cubic one.) H. M. Duncan's comment on this (June 17, 1943) was ". . . for many years the late Sir Charles Parsons and I repeated not only Hannay's experiments but also Moissan's over and over again without success, and I can assure you that we very frequently used lithium. . . . When I joined the late Sir Charles Parsons in 1911, he was quite satisfied that he had succeeded in some of his experiments in producing artificial diamond, and naturally I accepted his conclusion and so all our experiments were conducted on the lines which he had thought successful and although at the time of the reading of his paper before the Royal Society (1919) he still held this belief, I was convinced in my own mind that the results which he had obtained before I joined him were very doubtful, more especially since all the experiments in which I took part, although they were conducted with the greatest care and patience, were unsuccessful, and by constantly suggesting to him that he may have been mistaken in the past, he at last decided to test, by burning in oxygen, the characteristic crystals which he had carefully mounted on slides, and it was a bitter blow to him when he found that they would not burn". He went on in a subsequent letter (June 25, 1943) to explain that "the method adopted of burning in oxygen before I joined the late Sir Charles Parsons was very crude and . . . I doubt whether a sufficient temperature was attained to burn the real diamond. . . . It was only after many years of arduous work and continuous doubtings of our results that I tried an experiment of introducing a platinum boat, carefully cleaned, into the furnace with oxygen passing through it and at the required temperature; on the introduction of the boat numerous flashes were observed similar to the ones we had obtained in our experiments. This naturally gave the late Sir Charles Parsons quite a shock and although he said very little at the time he thought it over for many days and at last decided to burn the characteristic crystals which he kept at home . . . on withdrawing the boat and re-examining under the microscope, the characteristic crystal was found untouched". On July 19, 1943, Mr. Duncan wrote: ". . . I remember Sir Charles telling me that he had consulted the President of the Royal Society as to the best means of acknowledging that he was mistaken. I prepared a statement, which was given to Dr. Desch and from which he prepared the letter [this was in fact the article referred to above. K. L.] published in *Nature*. With regard to the characteristic crystals, I can tell you that these consisted of magnesia and alumina. . . .".

It seems worth while to publish the relevant parts of this correspondence because Dr. Desch's first article did not make it really clear that Sir Charles Parsons himself had inspired it, desiring by this means to withdraw his previous claim. In a second article in *Nature*[8], Dr. Desch made this much more explicit, but added the suggestion that the X-ray identification of the Hannay specimens as being diamond might re-open the whole question. The near-certainty that these Hannay specimens are *natural* and not synthetic diamond, however, restores the *status quo* and makes me believe that neither Hannay, Moissan nor Parsons ever, in fact, made diamonds by their respective methods; although whether the claims of the first two were made in good faith throughout (as Parsons's certainly were) is more difficult to judge.

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[1] *Nature*, 151, 334 (1943).

[2] *Mineralog. Mag.*, 26, 315 (1943).

[3] *Proc. Phys. Soc.*, B, 65, 313 (1952).

[4] Milledge, H. J., and Meyer, H. O. A., *Nature*, 195, 171 (1962).

[5] *Nature*, 152, 597 (1943).

[6] *Nature*, 151, 394 (1943).

[7] *Nature*, 121, 799 (1928).

[8] *Nature*, 152, 148 (1943).

EFFECT OF ULTRA-HIGH VACUUM ON *BACILLUS SUBTILIS* VAR. *NIGER*

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THE effects created by exposing organisms to a vacuum have long been a subject of interest to biologists[1]. The use of a vacuum in storing micro-organisms is an essential part of lyophilization preservation technique[2]. In this method, the organisms are frozen at −80° C and dehydrated by a vacuum of 10^{-3} mm mercury. The tubes containing the organisms are sealed off under vacuum and may be stored for several years.

An investigation was carried out by Bakanauskas[3] to determine the effect of a prolonged dynamic vacuum on the viability of selected spore-forming fungi and bacteria. The micro-organisms *Aspergillus niger*, *A. flavus*, *Bacillus globigii* (*B. subtilis*), *B. mycoides*, and *B. cereus* were exposed to pressures ranging from 1×10^{-5} to 5×10^{-7} mm mercury for periods of 2, 4, 8, 16, and 32 days. With the exception of *Bacillus cereus*, all the micro-organisms remained viable after 32 days exposure.

Despite these results, the ability of micro-organisms to withstand ultra-high vacuums of the order of 10^{-8} mm mercury or better has been questioned. Brueschke, Suess and Willard[4] report that exposure of micro-organism spores (*Bacillus subtilis*, *Aspergillus niger*, *A. terreus*, and *Penicillium citrium*) to pressures of $1{\cdot}2 \times 10^{-8}$ mm mercury and lower for a sufficient period of time (greater than 10, but less than 31, days) will cause their destruction. However, recent work by Portner, Spiner, Hoffman, and Phillips[5] failed to show any effects of vacuum on three types of micro-organisms (*Bacillus subtilis*, *Aspergillus fumigatus*, and *Mycobacterium smegmatis*) exposed to a vacuum of less than 10^{-9} mm mercury for 5 days. The work reported here seeks to verify one of these conclusions for the micro-organism *Bacillus subtilis*.

An apparatus was constructed from 'Pyrex' glass to expose simultaneously eight micro-organism samples to a vacuum of 10^{-8} mm mercury or better. It consisted of a round bulb having eight sample tubes (3 cm. × 1·5 cm.) on the bottom, plus a break-seal vent and entry port on the top. An ion gauge (Bayard–Alpert type) was mounted opposite the pump connexion to provide pressure readings at the point of poorest vacuum.

The ultra-high vacuum was obtained with a double-trapped (liquid nitrogen) three-stage mercury diffusion pump. To ensure that mercury would not contaminate the high-vacuum region, a gold plating was so placed that molecular backstreaming from the region of the pump and traps was intercepted and the mercury vapour was held by amalgamation. In order to minimize outgassing from the glassware in the high-vacuum region, it was cleaned with hot nitric acid. After a rinse with distilled water the apparatus was dried and the gold plating was applied by vacuum deposition.

Table 1. RECOVERY OF *Bacillus subtilis*

Inoculum count (micro-organisms)	Bacteriological recovery from controlled room-temperature atmosphere 1	2	3	4	5	6
30	22	49	80	144	53	60
30	14	78	82	82	64	48
300	135	158	150	143	126	120
300	135	156	167	109	139	138
3,000	150×10^1	500	1,410	1,500	1,140	1,080
3,000	138×10^1	1,700	1,580	2,500	1,090	1,910
3×10^{5}*	142×10^{3}*	107×10^{3}*	152×10^{3}*	78×10^{3}*	44×10^{3}*	143×10^{3}*
3×10^{5}*	142×10^{3}*	142×10^{3}*	190×10^{3}*	63×10^{3}*	41×10^{3}*	149×10^{3}*

1, Ultra-high vacuum for 35 days; 5 min of 25-kc/s ultrasonic agitation.
2, Slight vacuum (300μ of air) for 36 days; 5 min of 25-kc/s ultrasonic agitation.
3, Nitrogen at 1 atm. for 38 days, 3 min of 25-kc/s ultrasonic agitation.
4, Argon at 1 atm. for 38 days, 3 min of 25-kc/s ultrasonic agitation.
5, Carbon dioxide at 1 atm for 38 days, 4 min of 25-kc/s ultrasonic agitation.
6, Air at 1 atm for 38 days; 4 min of 25-kc/s ultrasonic agitation
* Average value of 3 counts.

The pressure in the high-vacuum region was monitored by the Bayard–Alpert-type hot-filament ionization gauge. An initial vacuum of 3×10^{-6} mm mercury was obtained. This fell to 1×10^{-8} mm mercury after 15 days, and to a final value of 6×10^{-9} mm mercury after 35 days. The lowest pressure which can be measured directly with this type of gauge is approximately 5×10^{-10} mm mercury.

The samples of *Bacillus subtilis* were prepared, introduced into the vacuum apparatus, and recovered in the following manner. One ml. of a stock culture of an aerobic spore former, *Bacillus subtilis* var. *niger*, was diluted by adding 99 ml. of sterile distilled water. The suspension thus formed was placed in a 60° C water bath for 30 min to kill vegetative organisms. Average control counts of the remaining spores diluted 10^5 and 10^6 were each made in triplicate. Average values of 35×10^6 and 329×10^5 organisms per ml. were found. Further dilutions were so arranged that inocula containing 300, 3,000, 30,000, and 3×10^6 organisms per ml. were obtained.

Each of the eight sample tubes attached to the apparatus was then inoculated with 0·1 ml. of inoculum as delivered through the entry port by a tuberculin syringe and needle. After seal-off of the entry port, a slight vacuum (about 20 mm mercury) was pumped, and the water in the cultures was allowed to evaporate. The sample tubes were immersed in a pan of warm water to prevent freezing of the microorganisms during this drying process. As soon as the water in the cultures had been removed, an ultrahigh vacuum was applied to the spore samples.

The micro-organisms were sealed off from the vacuum system at the end of the 35-day period. The chamber was brought to atmospheric pressure by opening the break seal and allowing air to flow through a 4-in. plug of sterile fibreglass. Two ml. of sterile distilled water were added to each sample tube through the entry port and the whole apparatus was placed in a 25-kc ultrasonic transducer. Vibration for 5 min (with the entry port closed) sufficed to remove the micro-organisms from the glass and disperse them into the distilled water. These suspensions were aseptically transferred into sterile Petri dishes. Warm trypticase soy agar was then added and the mixtures were allowed to solidify into pour plates. All pour plates were incubated at 37° C for 5 days before counting was carried out.

Control samples were prepared from 0·1 ml. of each of the inocula containing 300, 3,000, 30,000, and 3×10^6 organisms per ml. The suspensions were pipetted into test tubes, dried under vacuum in a bell jar, and placed in room-temperature desiccators, each of which contained one of the following atmospheres: (1) air at 300μ; (2) nitrogen at 1 atm.; (3) argon at 1 atm.; (4) carbon dioxide at 1 atm.; (5) air at 1 atm. The control samples were recovered and counted in the same manner as the specimens exposed to ultra-high vacuum.

Table 1 lists the bacteriological recoveries from controlled atmospheres at room temperature. In all cases, experimental as well as control samples, approximately half the micro-organisms were recoverable.

The deviations in tabulated counts are within the range expected for a biological experiment. Spurious results were obtained with the inoculum containing 30 organisms, for more organisms were recovered than were thought to have been in the original sample. However, these counts are not considered significant because they reflect the magnified errors encountered in working with low concentrations of micro-organisms. Samples containing statistically significant numbers of micro-organisms produced consistent data.

The results shown in Table 1 demonstrate that spores of *Bacillus subtilis* var. *niger* will survive exposure to an ultra-high vacuum (10^{-8} mm mercury) for a period of 35 days. The experiment also shows that ultrasonic vibrations and radiation encountered during seal-off of the apparatus have no significant effect.

Since the results of this work completely contradict the conclusions reported in ref. 4, further work on the problem is anticipated. An apparatus is under construction which will produce and directly measure pressures to 10^{-10} mm mercury. Even though a decrease in pressure from 10^{-8} to 10^{-10} mm mercury (which lengthens the average mean free path of a gas molecule from 5×10^5 to 5×10^7 cm) is of little significance in an apparatus having a radius of 12 cm, it will be interesting to expose *Bacillus subtilis* to a vacuum approaching that encountered in outer space. A second experiment will involve exposing a non-spore former and an anaerobic spore former to a vacuum between 10^{-8} and 10^{-9} mm mercury to determine whether the resistance of these forms to ultra-high vacuum is as great as that of aerobic spores.

This work was carried out under contract No. *NAS* 7–100 sponsored by the National Aeronautics and Space Administration.

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NEW RADIOCARBON DATES OF NILE SEDIMENTS

By PROF. RHODES W. FAIRBRIDGE

Columbia University, New York

IN the present Unesco "Save the Nubian Monuments" campaign, it is most fortunate that the Palæolithic works of man along the middle Nile Valley have not been forgotten. The construction of the Saad el Aali, the Aswan High Dam, will drown a 500-km long sector from the First Cataract (Aswan) almost to the Third Cataract (considerably south of Wadi Halfa). This sector contains a unique record of Nile sediments that not only spans the history of man from early Christian and Dynastic times back to the Palæolithic, but also sheds considerable light on the complex problem of climatic change in Africa, indeed, on the world problem of climatic change.

I was fortunate in being invited to lead a Columbia University expedition to Nubia in the winter of 1961–62, jointly organized by the Departments of Anthropology (under Dr. Ralph Solecki) and Geology (myself), and sponsored by the National Science Foundation, Washington. It is a pleasure to record the friendly co-operation of all officials in the Sudan, which helped to bring this mission to a successful conclusion. The geological–palæoclimatical results will be briefly reported here.

Unique Position of the Nile

The Nile occupies a unique place in the geographical world, since its net flow reflects the average precipitation over a large area of equatorial Africa. Running northwards through totally arid Nubia to the temperate Mediterranean, its sediments are not confused by local complications; its middle and lower courses are devoid of flowing tributaries. In the Nubian sector, therefore, the Nile is a sort of 'tide gauge' of tropical rainfall.

The older sediments should also reflect the ancient equatorial climates. If we could date these sediments, we would be in a position to relate tropical climates to events of the late Ice Age. The question was, how to date them. It seemed to me that there were basically three methods available:

(*a*) *Human artefacts*. The cultural relics of Stone Age Man provide a broad basis for dating the fluvial terraces; but they are not precise 'fossils', and there are sources of possible confusion.

(*b*) *Ancient shorelines*. Around the Mediterranean the old beaches of former ocean-levels are approximately dated, not at all well, but at least the rough sequence is established. These shorelines can be related to the river terraces; the latter are 'thalassostatic' in their lower reaches, but above the First Cataract, the oceanic influence disappears.

(*c*) *Radiocarbon dating*. Material for dating might include fossil wood, charcoal from human camp sites, and the calcium carbonate shells of fossil Mollusca. I was very dubious at first about finding adequate shell horizons, but discoveries of these freshwater shells at all the critical levels surpassed my wildest optimism. We now have radiocarbon dates which bring the whole sedimentary régime into mass time perspective.

Theory of Pluvial Climates

Until very recently an unresolved controversy existed as to the causative nature of ancient climatic changes. According to Sir George Simpson, an increase in solar radiation should lead to increased evaporation from the oceans, and thus to increased precipitation, which would become critical in high mountain areas, where it would fall as snow, eventually to develop into great icefields. By various meteorological feed-back mechanisms an ice-age would result. This theory is now generally recognized to rest on completely false premises; to begin with, polar ice-caps are cold deserts, lacking significant precipitation. Ahlmann has shown that it is the mean summer temperature that essentially controls the advance and retreat of glaciers. Precipitation plays a secondary part.

Some critics have further pointed out that the 'solar constant' is indeed rather constant. If it were not so, the continued existence of life on this planet over the past 3×10^9 years would not be possible. However, a number of recent palæoclimatic conferences and symposia have brought to light significant sources of radiative change, notably in the ultraviolet end of the solar spectrum. Furthermore, the much-maligned theory of Milankovitch, based on slight variations of this planet in its attitude and orbit, has been re-examined and found to play a certain part, notably in introducing long-term cycles, of 21,000, 40,000 and 92,000 years[1-3].

The consensus, in brief, at these recent conferences (New York Academy of Sciences, New York; Unesco–World Meteorological Organization, Rome; International Quaternary Association, Warsaw: all in 1961) suggests that climatic change is the product of a number of different mechanisms, varying considerably in orders of magnitude of both period and amplitude. The great long-range control is geological (relative position of poles, degree of mountain building, etc.); the middle-range control is astronomical (the celestial mechanics periodicity recognized by Milankovitch); and only the short-range controls are associated with solar radiation itself (correlation of ultra-violet-ozone production with sunspot variation, etc.).

The geological evidence of climate change in Africa was recognized almost a century ago. Around Cairo, for example, early workers such as Blanckenhorn noticed enormous gravel deposits in deeply scoured wadis where no water flows to-day. The same type of observations were made all across southern Europe and on other continents. It was easy, therefore, to conclude that during the last ice age a large increase in precipitation produced great snow and ice-fields, but that in the warmer latitudes of the Mediterranean and in Africa there was an age of tremendous rains. Indeed, the dry wadi beds have been followed all across the Sahara and old lake deposits have been recognized in East Africa, Australia, central Asia and in the Rocky Mountains of America.

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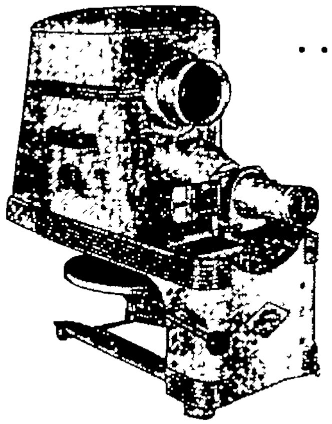

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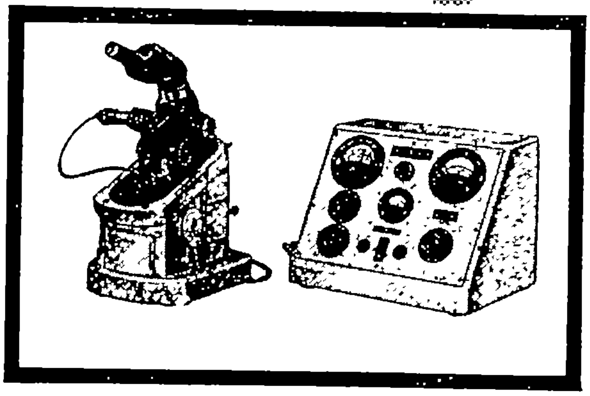

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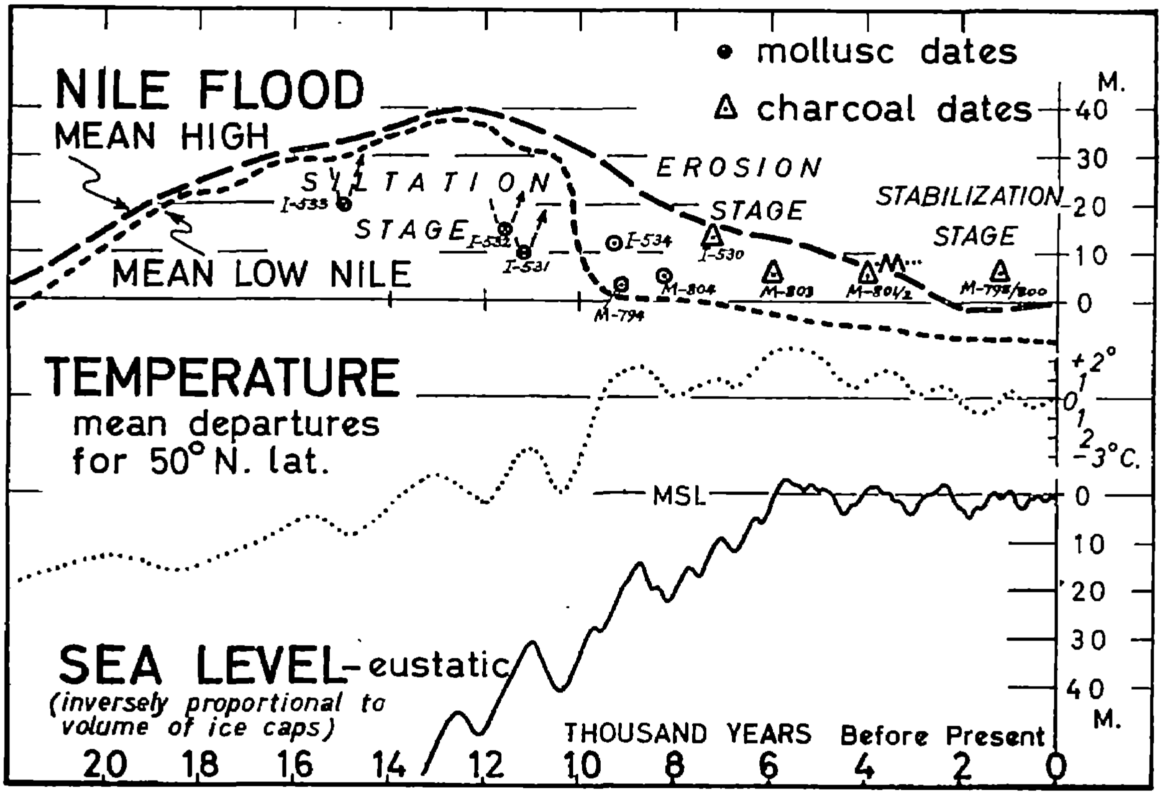

Fig. 1. Variations of middle Nile during past 20,000 years, according to recent radiocarbon dates. Three stages are shown: (*a*) *Siltation Stage* (note: shell samples *I*–531/3 represent temporary dissection of silts, but were immediately covered again—to 40 m depth); (*b*) ***Erosion stage*** (samples show progressive cutting down of the former silt fill of the valley, but were not afterwards covered by more than a few metres of sediment); *M* = the documented 'high Nile' of Amenemhat III, about 3,750 B.P. (*c*) ***Stabilization stage*** (the historical period; detailed oscillations, recorded by archæology and 'nilometers' have been omitted, for simplicity). Below, for comparison, are shown mean curves of temperature (for a typical western European latitude) based on palæobotanical data; and sea-level (eustatic), based on radiocarbon dating of old beach lines (refs. 1, 2). During the low-temperature period (maximum phase of last glaciation), equatorial rains were so reduced that the middle Nile was choked by silt (which is normally carried down to the Delta). Some cold winter rains reached the northern Sudan and produced gravels that interfinger the silts. During the high temperature and rapid glacier melting phase ('climate optimum' of Europe), c. 9,500–4,500 years B.P., sparse summer monsoonal rainfall commonly reached Egypt; to-day this rainfall boundary lies 1,000 km to the south

The evidence for great 'Pluvial Ages', which corresponded to the great Ice Ages, seemed overwhelming (see, for example, Büdel[4]).

Yet, recently, O. Davies (University of Ghana) remarked that the Pluvial Theory hung by a 'gossamer' thread. It has a certain weakness. The synchroneity of pluvial–glacial evidence in Africa was a pure assumption. It was well proved in temperate Europe, where geological relations with the ice margin were easy to work out, but the jump to central Africa was unsupported by intermediate, connective surveys.

On theoretical grounds, Albrecht Penck[5] objected to African pluvial–glacial synchroneity; he argued that the world atmospheric circulation system should remain basically the same, only to be reinforced or weakened from time to time in response to the heat energy that drives it. Thus climatic belts should migrate towards the equator during Ice Ages or vice versa during interglacials. Later[6] he pointed out that great lake developments in temperate latitudes during glacial periods would be the consequence of lower evaporation-rates. Flohn[7] has calculated that evaporation from the tropical oceans would be 20 per cent lower during glacial maxima.

It is apparent, therefore, that in the equatorial regions a lower evaporation-rate should be reflected by lower rainfall, particularly in the monsoonal belts. If Penck is right, the Nile should show a reduced flow during the ice ages and increased flow during interglacials. It should be noted that Emiliani's work[8] on the oxygen-18 isotope-palæotemperatures of tropical oceans shows that the present time is about 25 per cent less than the maximum of an interglacial–glacial cycle.

Radiocarbon Dates

A thick wedge of silts was built up to a height of 40 m above the rocky bed of the Nile in its middle sector during the Upper Pleistocene, thus reflecting a progressive diminution of the Nile discharge. At the present day, similar silts are carried right to the Delta. The middle part of this silt is dated by a bed of *Unio wilcocksi*, a large edible clam, associated with small inedible snails and other bivalves. The sample (Lab. No. *I*–533) is 14,950 ± 300 years B.P. Probably the oldest silts in the valley floor go back to 25,000 or 30,000 B.P.

The upper part of the silt is fixed by some small bivalves, *Corbicula artini* (Lab. No. *I*–532), with 11,650 ± 300 years B.P. Bivalves of this sort mark the middle and upper banks of the river seasonally submerged to-day. The permanently covered bed is better indicated by the Nile 'oyster', ***Etheria elliptica***, which colonizes rocky outcrops in the river bed. A sample from 10 m above present Nile flood-level at Wadi Halfa (*I*–531) is dated 11,200 ± 285 years B.P., and is thus comparable with the *Corbicula* beds (collected farther upstream near Akasha) at 20 m above present flood-level.

Sedimentation built up another 15 or 20 m above the *Corbicula* horizon during the next few hundred years, with a final peak-level about 10,000 years B.P. This must have been about the end of the last ice age desiccation of central Africa, because subsequent history of the middle Nile is one of almost constant cutting down of the river bed. Oscillations of the flow produced a series of terraces, sometimes marked by shell horizons.

One should note that while the maximum sedimentation-levels represent minimum fluvial discharge, in the subsequent dissection and terracing, the terrace deposits may, in contrast, represent abandoned flood-level deposits. A temporary low level (12 m above high Nile, south-east of Wadi Halfa) was marked by the little gastropods *Cleopatra bulimoides* (*I*–534), which dated 9,325 ± 250 years B.P. They were buried by a rising Nile which reached 20 m again. Another negative oscillation, to 13 m above high Nile, was marked by charcoal from a camp-fire site at Ukma (*I*–530), dated 7,300 ± 350 years B.P.

The climatic picture at this time (11,000–7,000 B.P.) in Egypt suggests a considerable incidence of rainfall[9], almost certainly in part summer monsoonal rains, for a rich and varied fauna migrated northwards and are faithfully recorded by artists of the Mesolithic and Neolithic cultures.

At this stage some other radiocarbon dates take up the record (see Fig. 1). Collected by Myers[10,11] from the region of Abka, 20 km south Wadi Halfa in an abandoned section of the Second Cataract, they show how the present level of the Nile was reached about 5,000 B.P.

The Dynastic Period and subsequent times, with man's predilection for building temples, further helps to fill in the story[12-14]. This is not the place for a analysis, but a preliminary study of the record suggests that there have been extended periods c relatively high and low Nile floods. Archæologicall dated 'high water marks' on Egyptian temples an associated Christian constructions near Wadi Half suggest that the last important high Nile periods wer about A.D. 500 and 800.

Conclusions

Comparison of this tentative curve for Nile oscillations with the eustatic curve (of world sea-leve variations), suggests that the 'minor oscillation are in phase, thus implying a common cause, though of course, with differing amplitudes. We feel the this common factor can only be solar radiation.

Much more work is needed, but it is suspected the the tropical lake beds of Africa will also prove to be i phase with solar radiation, while the temperat lake-levels will be out of phase. Further support offered to the concept of long-term climatic cyclicit with its potential for prediction.

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OBITUARIES

Dr. E. W. R. Steacie, O.B.E., F.R.S.

Dr. E. W. R. Steacie, president of the National Research Council (Canada), died on August 28 in Ottawa after a brief illness at the age of sixty-one.

Dr. Steacie had been president of the National Research Council since 1952. He joined the Council in 1939, following a teaching and research career at McGill University. Well known as a physical chemist, he was at the time of his death serving in the three-year presidency of the International Council of Scientific Unions, the chief non-governmental organization for co-ordinating international activity in science.

Edgar William Richard Steacie was born on December 25, 1900, in Westmount, Quebec. He won the Gold Medal of the Westmount High School and the Westmount School Commissioners Scholarship. Enrolling at McGill University, he left to become a corporal in the Canadian Infantry during the First World War. He returned to McGill in 1920, and graduated in 1923 with the degree of B.Sc., with first-class honours in chemical engineering. During this period he won the British Association Medal for highest standing in the undergraduate course and a scholarship of the Imperial Order of the Daughters of the Empire. He obtained his M.Sc. in 1924 and his Ph.D. in 1926, both in physical chemistry from McGill. His postgraduate studies were aided by a Nation Research Council scholarship.

In 1923 he was appointed McGill demonstrator chemistry in McGill University. During 1926– he occupied the Sterry Hunt fellowship in chemistr becoming lecturer in chemistry in 1928 and assista professor of chemistry in 1930. He was on leave absence from McGill in 1934–35, when he did pos doctorate research at Frankfurt, Leipzig, and t University of London on a fellowship from t Royal Society of Canada. In 1937 he was appoint associate professor of chemistry at McGill.

Dr. Steacie joined the National Research Coun in 1939 as director of the Division of Chemistr He became vice-president (scientific) in 1950 an in April 1952, was elected president of the Council.

During the Second World War, Dr. Steacie play a large part in organizing Canadian chemistry f war purposes, especially in the fields of chemic warfare and the development of explosives. Duri 1944–46 he was deputy director, under Sir Jo Cockcroft, of the British–Canadian Atomic Ener Project in Montreal.

For his services during the War, he was appoint an Officer of the Order of the British Empire. He wa Fellow of the Royal Society of Canada and of t Royal Society of London. He was a Foreign Associa of the U.S. National Academy of Sciences of the United States, a Foreign Member of the Academy of Sciences of the U.S.S.R., an Honorary Member of the Polish Chemical Society and the Belgian Chemical Society, and an Honorary Fellow of the Chemical Society of London.

Among other honours, Dr. Steacie was awarded the Gold Medal of the Professional Institute of the Public Service of Canada (1949), the Palladium Medal of the Chemical Institute of Canada (1953), the Tory Medal of the Royal Society of Canada (1955), and eighteen honorary degrees.

One of Dr. Steacie's principal interests was the development of Canada's universities. He was at the time of his death serving as chairman of the Board of Governors of Carleton University, and chairman of the Advisory Committee for Science of the University of Ottawa.

Dr. Steacie's scientific work was in the fields of photochemistry, the kinetics of gas reactions, and free radical reactions, important as the basis of petroleum technology. The work consisted of fundamental research, without immediate application, but making outstanding contributions to basic knowledge. He had published more than two hundred scientific papers, and was the author of three books. One of his books, *Atomic and Free Radical Reactions*, is regarded as the standard text on that subject.

Besides serving as president of the National Research Council, Dr. Steacie was a member of the Defence Research Board and the Atomic Energy Control Board, and a director of Canadian Patents and Development Limited and the Canadian Standards Association.

Dr. J. W. T. Walsh, O.B.E.

By the death of Dr. J. W. T. Walsh on July 17, at the age of seventy, the world has lost one of its leading figures in the field of lighting and photometry. Dr. Walsh was a man of very wide interests and had long been held, both nationally and internationally, in the highest regard and esteem.

Educated at Hampton Grammar School and Merton College, Oxford, he went to the National Physical Laboratory to work under Sir Clifford Paterson (then Mr. C. C. Paterson), and when the latter left the Laboratory in 1919, Dr. Walsh remained to take charge of the work on photometry, until his retirement in 1951. During this period, he was able to exercise, through his position at the Laboratory, a considerable influence on the advances made in photometry, particularly those concerned with the change from visual to physical methods of measurement and with the adoption internationally of a black body radiator as the primary standard of light in place of sets of filament lamps.

His versatile gifts were used to the full on many other aspects of his subject. He served on numerous committees, a specially important one being the Home Office Committee which drew up the first regulations for standards of lighting in factories. He became a founder member of the British Standards Institution Illumination Industry Standards Committee in 1927 and served as its chairman during 1945–55; the subjects in which he was particularly interested were street lighting and nomenclature. He also wrote a number of books, notably his text-book on *Photometry*, the latest of three editions having been published in 1958. In addition, he was the author of numerous papers.

In 1929 he was elected to membership of the Institution of Electrical Engineers, after being an associate member from 1918, and was awarded the Kelvin and Ayrton premiums.

For many years he played a very active part in the Illuminating Engineering Society and he achieved the unique distinction of being elected its president on two separate occasions, in 1929 and 1947.

Dr. Walsh's interest in illumination extended also to the work of the National Illumination Committee, to which he was appointed as the representative of the National Physical Laboratory as far back as 1919. He served as chairman during 1945–55. This work brought him into close touch with the parent International Commission on Illumination, the general secretary of which was provided by the National Physical Laboratory for the period between the ends of the two World Wars. Dr. Walsh himself was the first to hold office in this period (during 1921–28) and was honorary secretary during 1928–31. He attended every meeting of the Commission up to the most recent one in 1959. Throughout this time he steadily built up a reputation as one of the Commission's leading figures and in 1948 was appointed one of its vice-presidents. Later, in 1955, full recognition was given to him by his appointment as president and he duly presided over the next meeting at Brussels in 1959. It is understood that he had recently completed a history of the Commission, and the publication of this will be awaited with much interest.

Recognition of his work was acknowledged in 1951 by his appointment as an Officer of the Order of the British Empire.

During his early time at the National Physical Laboratory Dr. Walsh took a considerable interest in its social activities, but later much of his private life was devoted to youth work, both on behalf of organizations such as the Boy Scouts and in numerous individual cases. He was also interested in education and served on a number of local bodies. He gave a start in life to very many less fortunate than himself.

Although a bachelor with few family connexions, he was by his wide and humane interests a man with innumerable friends in many countries and his passing will be universally mourned.

J. S. Preston
L. H. McDermott

Prof. S. Mangham

Sydney Mangham, who died on July 30 at the age of 76, was the first professor of botany in what was then the University College of Southampton. Sutherland, and before him Cavers, had been professors of biology; but in 1920 the department was divided. Sherriffs was made head of the department of zoology, and Mangham was appointed to the new chair of botany. For the first seven years of his professorship Mangham's staff consisted of one lecturer, Miss F. M. Loader (who remained with him during the whole of his tenure), a temporary and ever-changing student assistant, and a lab-boy; and Mangham taught virtually all branches of botany at all levels, including a course for gardeners. Had he been a man of strong research interests, the task would have been intolerable; but his early interests in analytical techniques and in translocation rapidly succumbed to his genuine love of teaching. Dissatisfied with existing introductory text-books he wrote *A First Biology* and *A Second Biology* with

Sherriffs; then *A Text-Book of Biology* with Hockley; and, lastly, the still-popular *Earth's Green Mantle*—the title of which was borrowed by Tansley, with Mangham's permission, for his own *Britain's Green Mantle*. Mangham was a first-class draughtsman, and his elegant diagrams contributed much to the success of his books.

He enjoyed field excursions, and at 65 could still out-walk many of his students; he knew his plants and was not alone in finding nomenclatural changes exasperating; and he loved—and was justly proud of—his garden. After his retirement in 1951, and after an Australian tour, he returned to the now University of Southampton as curator of grounds, a post which gave him great pleasure and which he filled with distinction until, on the death of his wife in 1957, he moved from the town.

Few of us to-day would be willing to do as much as Mangham did with so little assistance, and to devote thirty years to keeping a small Department going at the cost of our own scientific work and advancement. But his faith in the importance of his subject and in the future of his Department was proof against all inadequacies of staff, finance and space, and he laboured selflessly to ease the path of his successor. The building which he and an engineering colleague personally designed—without the help of an architect—is still in use to-day; and his memory is honoured in the Department, the continued existence of which he made possible. W. T. WILLIAMS

NEWS and VIEWS

Botany at Glasgow : Prof. J. Walton

PROF. JOHN WALTON, regius professor of botany in the University of Glasgow since 1930, who retired on September 30, was educated at Daniel Stewart's College, Edinburgh, and St. John's College, Cambridge (where he was awarded the Hutchinson Research Studentship). He went as botanist on the First Oxford Expedition to Spitsbergen in 1921 and on his return to Cambridge was appointed junior demonstrator. His palæobotanical researches were there begun under the late Sir Albert Seward. From 1923 he held the post of lecturer in botany in the University of Manchester until his appointment to the Glasgow chair. Part of his early work was concerned with new techniques of investigating fossil plants. In 1923 he published an account of his balsam transfer method, whereby plant compressions could be transferred to a transparent base to facilitate microscopic examination. His well-known 'peel' technique, a rapid and easy method of obtaining thin sections of petrified fossil plants, was published in 1928. Another of his early publications was concerned with the factors affecting the form of fossil plants. Most of his work, however, consisted of investigations of Palæozoic plants, particularly those of the Welsh and Scottish Lower Carboniferous. Over the years our knowledge of these strata, which are of special importance in fossil botany, has been greatly increased by Walton and his students. However, the foregoing are only indications, for his interests in both fossil and living plants extended over a wide field.

Among his many distinctions, Walton was awarded the degrees of D.Sc. (Manchester), Sc.D. (Cantab.) and the honorary degrees of Ll.D. of McMaster University and D.-ès.-Sc. of the University of Montpellier. In 1931 he was elected a Fellow of the Royal Society of Edinburgh, on the Council of which he served for some years. During 1949–53 he was a Forestry Commissioner and during the same period he served as a member of the Scottish Committee of the Nature Conservancy. He was elected president of the Botanical Society of Edinburgh, 1962. Among his many other social and scholarly interests and activities, it was perhaps no less than appropriate that he, the son of a distinguished artist, should have served as honorary curator of the very important art collections belonging to the University of Glasgow.

Dr. P. W. Brian, F.R.S.

DR. PERCY WRAGG BRIAN, who succeeds Prof. Walton in the regius chair of botany at Glasgow, has for the past twenty-six years been on the scientific staff of Imperial Chemical Industries, Ltd., from 1936 until 1946 as mycologist at the Jealott's Hill Research Station, Bracknell, Berks., since then as head of the Department of Microbiology, Akers Research Laboratories, Welwyn, Herts. Dr. Brian was educated at King Edward's School, Birminghan, and at King's College, Cambridge. He held the Frank Smart studentship in botany at Cambridge in 1933–34 and spent the next two years as assistant plant pathologist at the Long Ashton Research Station. Dr. Brian's versatility is well known. His personal research work has included notable contributions to both agriculture and industrial microbiology, while he has inspired a team of mycologists, microbiologists and chemists to break much new ground in the field of biologically active substances produced by fungi. Among the numerous antibiotics first characterized by Dr. Brian, mention may be made of griseofulvin, which in addition to its horticultural use as a systemic fungicide has during the past few years become notorious for its use in the oral therapy of ringworm of man and animals. Another notable series of his researches has been concerned with the gibberellins. Dr. Brian gave the Cantor lectures to the Royal Society of Arts in 1953, was elected to the Royal Society in 1958, served the British Mycological Society as president in 1960, and in 1961–62 was similarly honoured by the Association of Applied Biologists. He will bring this wide experience of applied microbiology to the University of Glasgow when next year he takes up his new post.

National Coal Board Research and Development Organization

WITH effect from October 1, the National Coal Board made changes in the departmental responsibilities for research and development. That directly concerned with the production, working and preparation of coal became the responsibility of the production member of the Board, Mr. H. E. Collins, and that concerned with coal processing and combustion became the responsibility of the scientific member of the Board, Mr. A. H. A. Wynn. The main effect of these changes is that the Mining Research Establishment at Isleworth comes under the Production Department. Dr. W. Idris Jones has been appointed director general of research and development (Coal Processing and Combustion) and will be head of the Scientific Department. He will continue to control the Coal Research Establishment at Stoke Orchard, Cheltenham, and extramural research Mr. B. L. Metcalf has been appointed deputy director general of research and development (mining). He will co-ordinate all research and development in the mining field, and will control the Mining Research Establishment as well as the Central Engineering Establishment at Bretby.

Dr. W. Idris Jones, C.B.E.

DR. IDRIS JONES graduated with honours in chemistry at University College, Aberystwyth, in 1921. He was Rhondda and Frank Smart research student of Gonville and Caius College, Cambridge, during 1923–25. On leaving Cambridge, Dr Idris Jones joined the research staff of Synthetic Ammonia and Nitrates, Ltd. (now a subsidiary of Imperial Chemical Industries), and was later appointed a group manager in the Oil Division, where he was concerned with the development of the coal hydrogenation process. In 1933 he was appointed director of research, Powell Duffryn Co , a position which he held until November 1946. Dr. Idris Jones, besides being Fellow or member of many learned societies and institutes, is president, chairman of Council and of the Research Committee of the British Coal Utilization Research Association, and chairman of the British Standards Institution Solid Fuel Industries Standards Committee. He was awarded the C.B.E. in June 1954.

Mr. B. L. Metcalf

BERNARD LESLIE METCALF studied at the University of Glasgow and spent a year on postgraduate work in the mechanical engineering laboratories of the Royal Technical College, Glasgow. In 1923 he began a college apprenticeship course with Metropolitan-Vickers at Trafford Park, Manchester. Later he spent a year at some of the first power stations to be built under Lenin's electrification scheme in the U.S.S.R. Afterwards he was stationed in Japan for the Metrovick Export Co. for five years. Back at Trafford Park, Mr. Metcalf joined the Mining Department, later being appointed engineer-in-charge of that Department, but leaving in 1939 to become chief electrical engineer of Powell Duffryn Co. In 1945 he joined the Brush Electrical Engineering Co. in Loughborough as technical director. He became director of engineering at the National Coal Board in 1948.

Waterston and the Kinetic Theory of Molecules

DR. W. P D. WIGHTMAN, reader in history and philosophy of science in the University of Aberdeen, writes as follows: "In his illuminating article [*Nature*, 195, 744; 1962] on Maxwell's contributions to kinetic theory, Prof. C. A. Coulson states that 'up to this date it had apparently been assumed, by Clausius and others, that as these minute particles flew around in all directions, they maintained always the same uniform speed'. Though most writers (including Prof. Coulson) now give J. J. Waterston the credit for his pioneering memoir on this subject, it does not seem to be generally known that he clearly recognized the essentially statistical nature of the problem. 'Considered separately', he wrote, 'they (*sc.* the molecules) are for ever continually changing the velocity and direction of their individual motions. . . they run rapidly in their zig-zag conflict through every possible mode of concurrence . . .' ('On the Physics of Media that are Composed of Free and Perfectly Elastic Molecules in a State of Motion' (1845). *Collected Scientific Papers*, edited by J. S. Haldane, p. 216. Edinburgh, 1928). In such mathematical elaboration as he attempted he consistently used the terms 'mean velocity' or 'mean square velocity'."

Re-vitalizing School Mathematics

SCHOOL mathematical syllabuses have remained virtually unchanged for many years and have not adequately reflected the great strides that have been made in mathematical theory and practice during the present century. Many countries are now realizing the necessity of re-vitalizing their syllabuses; for example, the United States and the four Scandinavian countries. So far, such work in Britain has been confined to the efforts of a few individual schools, and the School Mathematics Project has been formed to carry such efforts a stage further. It consists of a group of schools containing experienced and vigorous authors and a good cross-section of types of pupil in sufficient numbers to make extensive experiment with a special syllabus feasible and worth while. The aim of the reformed syllabus is not simply the production of more and better professional mathematicians, but is mainly to provide the boy or girl whose mathematical education finishes at 'O' or 'A' level with some insight into the nature of mathematics, into what mathematicians do and into the use of mathematics in the modern world. Inseparable from the problem of what to teach is the problem of how to teach, and research is essential into the ways in which modern mathematics can best be taught. The Project is maintaining the closest contact with the Nuffield Foundation's projects for the reform of the 'O' level science subjects. The Project is based on the University of Southampton and is directed by Prof. B. Thwaites. The University has already appointed in the Department of Mathematics Dr. A. G. Howson, who has special responsibilities towards the Project; it has approved also the establishment of a readership.

MR. M. G. HUGHES, headmaster of Dynevor School, Swansea, on the invitation of the Central Advisory Council for Education (Wales), has prepared a useful report, *Modernising School Mathematics: a Review of New Trends in Mathematical Education at the Secondary Stage*, now sponsored for publication by the Mathematical Association (Pp. 43. London: G. Bell and Sons, Ltd., 1962. 2*s*. 6*d*. net). Mr. Hughes describes what is being done to bring school mathematics up to date, with reasonable brevity in 40 pages, by avoiding unimportant syllabus details. He deals concisely with the case for modernization, gives an account of progress in the United States and of the radical suggestions made at the Royaumont Seminar of the Organization for European Co-operation and Development. The last three sections deal with work in the United Kingdom, such as new diplomas instituted by the Mathematical Association, the pilot scheme under Prof. Thwaites in which the University of Southampton is to co-operate with Charterhouse, Marlborough, Sherborne and Winchester in preparing new syllabuses and producing the relevant text-books, and the proposal by a committee headed by Sir William Hodge that a Federal Council of Mathematics should be set up to co-ordinate all British activities in this field. Teachers of mathematics should read this slim book; they will learn that the difficult and pressing problem is not being neglected, and they will be shown ways in which they may make a contribution to its solution.

European Society for the Study of the Toxicity of Drugs

Scientists employed by the major European pharmaceutical companies who have as their responsibility the safety of a drug met in Zurich on September 20 to discuss their problems. It was decided that every effort should be made to encourage research into methodology and the exchange of information in this field. Towards this end a European Society for the Study of the Toxicity of Drugs was formed, and the following committee was appointed to establish the Society and arrange its meetings: Dr. D. G. Davey, Imperial Chemical Industries, Ltd., Pharmaceuticals Division, England (*chairman*); Dr. P. Koetschet, Rhône-Poulenc, Paris, France; Dr. G. A. Overbeek, N.V. Organon, Oss, Holland; Dr. J. Scholz, Farbwerke Hoechst AG, Frankfurt (M), Germany; Dr. M. Tausk, N.V. Organon, Oss, Holland; Dr. J. Tripod, Ciba A.G., Basle, Switzerland (*secretary*). Membership of this Society is open to all scientists interested, whether employed in industry, universities, Government or international organizations. Further information concerning the organization and application forms for membership can be obtained from the secretary.

The Hampshire South Downs

An area of the Hampshire South Downs, about 151 square miles in extent, is the subject of an order made by the National Parks Commission for protection. The area stretches from the outskirts of Winchester to the Hampshire–Sussex border at a distance of about ten miles inland from the south coast, and includes Petersfield and Selborne. It is a natural extension of the chalk uplands of the South Downs as far west as Old Winchester Hill and the Meon Valley. Further westwards it merges gradually into rolling farmland. In the northern part of the area beechwoods cover the scarp from above Petersfield to Selborne Hangar. The order, which is made under the National Parks and Access to the Countryside Act, 1949, designates the Hampshire downland as an "Area of Outstanding Natural Beauty" and not as a National Park. The responsibility for preserving and enhancing the natural beauty of the landscape rests with the local planning authority, Hampshire County Council. Government grants can be made at the rate of 75 per cent towards the cost of tree planting and preservation, and removing disfigurements. Grants are also available towards expenditure incurred in making agreements or orders for public access to open country and in appointing wardens.

Wild and Domestic Animals

In the August issue of *Man* there is a note by E. S. Higgs on a metrical analysis of some prehistoric bovine and caprini bones from the cave of Haua Fteah in Cyrenaica. Mr. Higgs has been assisting at the excavation of this large site and dealing with the extensive fauna that has been unearthed. The cultures found date from Levallois-Mousterian, Capsian and Oranian times, and pass up into the Neolithic and later periods. It has been possible, therefore, to study the effect of domestication on the bony structure of wild animals, accepting that domestication results in a decrease in size of the animals concerned, and that with domestication a larger number of young animals will be killed for food than would be by the people of a hunting culture. The results are of much interest and the information and charts which appear in the paper are of importance. There is no evidence at Haua Fteah of 'on the spot' domestication of sheep and cattle from indigenous wild stocks, as the domestic animals here seem to have been introduced from elsewhere. The date for the existence of domestic sheep in North Africa is pushed back to about 4800 B.C.

Announcements

Air Commodore D. D. Hatfield has been appointed director of air armament (research and development), Ministry of Aviation, with effect from December 3, 1962.

Dr. Harvey I. Scudder, assistant to the Chief of the Division of Research Grants, has been appointed chief of the Research Training Branch, Division of General Medical Sciences, National Institutes of Health. In this position, Dr. Scudder will supervise the operations of the Research Training Branch providing research fellowships, career awards, career development awards and training grants in the general scientific disciplines basic to medicine and health.

Mr. Olav R. Kåsa, project manager of the Organization for European Co-operation and Development Halden Reactor Project and deputy director of the Institutt for Atomenergi, Kjeller, Norway, has returned to Kjeller, where he has now become director of reactor development. Mr. Emil Jansen, who was previously director of reactor development, has been appointed project manager at Halden, and Mr. Henrik Ager-Hanssen, formerly head of research of the Halden Project, has been appointed deputy project manager.

The 1962 Arthur Stanley Eddington Memorial Lecture, entitled "On Having a Mind", will be given by Prof. W. C. Kneale at the Arts' School Lecture Theatre, Cambridge, on November 9. Further information can be obtained from the Arthur Stanley Eddington Memorial Trust, King's Court, 48 Broadway, London, S.W.1.

An international conference on "The Operating Experience and Future Development of Power Reactors and Radioisotopes", sponsored by the Canadian Nuclear Association, will be held in Montreal during May 27–29, 1963. Further information can be obtained from the General Manager, Canadian Nuclear Association, 19 Richmond Street West, Toronto 1.

A symposium on "High Pressure Measurement", sponsored by the Research Committee on Mechanical Pressure Elements of the American Society of Mechanical Engineers as part of the winter annual meeting of the Society, will be held in New York during November 28–30. Further information can be obtained from the American Society of Mechanical Engineers, 345 East Forty-Seventh Street, New York 17, N.Y.

SCIENTIFIC RESEARCH FOR INDUSTRY IN BRITAIN

*RESEARCH for Industry, 1961**, follows the pattern of recent reports. The bulk of the report—86 pages out of 134—lists the 49 research associations serving particular sections of industry and the three organizations serving industry as a whole, namely, the Commonwealth Mycological Institute, the National Institute of Industrial Psychology and Aslib. For each is given the present addresses of the laboratories and offices, the Chairman of Council and Director of Research and the total income, with a brief indication of the scope of the present work and a list of publications in 1961. This list is preceded by a short review of the principal achievements of eleven research associations the grants of which came up for review in 1961. The retiring chairman of the Industrial Grants Committee, Sir Walter Drummond, contributes some reflexions on research associations in which he stresses the saving in scientific manpower effected by co-operation in this way, and Dr. W. F. Watson writes on research associations and the consumer, indicating some of the benefits which accrue directly to the consumer from the work of the associations.

The major interest of the report as a whole lies in the report of the Industrial Grants Committee itself, which this year provides the comprehensive review of the research associations which the Minister for Science last November said he had asked the then new chairman of the Council for Scientific and Industrial Research, Sir Harold Roxbee Cox, to institute. An accompanying statement indicates that the Research Council has also reviewed the situation in the light of recent discussions and reports and that the analysis and statement of policy contained in this report has its support and also that of the Minister for Science. This analysis and statement is discussed elsewhere (see p. 97 of this issue). In what follows here some of the achievements of the association are selected for note.

The most important single contribution of the British Baking Industries Research Association in recent years has been the devising of the Chorleywood bread process, which cuts the total time required to process bread from more than 4 to less than 2 hours and is estimated to offer bakers savings of about 2*s*. 6*d*. per sack of 280 lb. of flour. Fundamental research includes an investigation of the factors which underlie the elastic properties of wheat proteins, a detailed investigation of the liquids present in flour derived from wheats of different varieties and a study of the swelling and shrinking of starch during and after baking. During the year the Cotton, Silk and Man-made Fibres Research Association made available to members nine new types of equipment based on a previous research, of which the 'separator for hard wastes' is estimated to offer savings of £200,000 a year and the Shirley Pressure-Point System of dust extraction from cards leads to a much clearer atmosphere in mills, and has been recommended to the industry by the Joint Advisory Committee of the Cotton Industry. Work on the application of 'fluid beds' to textile processing continues, and Shirlastain *E* has been developed for the rapid identification of synthetic fibres: in combination with Shirlastain *A*, *D* and *A–D* it permits rapid and conclusive identification of any mixture of fibres. At least fourteen new instruments have been developed during the past five years.

The British Electrical and Allied Industries Research Association reports important scientific advances in explaining the variation of intrinsic strength of thermo-plastics with temperature, and in diagnosing the mechanism of action of traces of moisture in impregnated cellular materials and evaluating its effect on electric strength. A survey of technical detail associated with all lighting on overhead lines in Great Britain during 1956–60 promises to offer a reduction of 10 per cent in the incidence of circuit interruptions due to lightning. The British Food Manufacturing Industries Research Association has studied the public health aspects of the marketing of meat products in partly vacuumized flexible film pouches and has designed an instrument for studying the somewhat complex rheological properties of chocolate and devised a novel method of evaluating the flow properties of chocolate. An instrument developed in collaboration with the National Institute for Research in Dairying for measuring the 'spreadability' of margarine and cooking fats is now in commercial production and is also of interest in the fields of explosives, cosmetics and welding fluxes.

The main effort of the File Research Council has been devoted to the development of new methods and machinery to increase the productivity of member firms, but knowledge gained from investigation of the factors affecting file quality has been used to ensure that new machinery will also give products of uniformly high quality.

During the past five years, the selection of suitable varieties has been carried out by the Fruit and Vegetable Canning and Quick Freezing Research Association, and a selection based on chemical composition, which has had a spectacular success with broad beans for canning, has been adopted for the selection of other materials, notably Bartlett pears and, more recently, rhubarb. A survey has also been made of the quality of frozen peas reaching the consumer, and a series of studies with the antibiotic nisin has shown that it has no effect on the heat resistance of the spores of certain spoilage bacteria in canned foods but that it blocks either the germination or the outgrowth of the spores.

At the British Internal Combustion Engine Research Association Laboratory an engine designed originally for atmospheric charging with a similar overload rating of 97·5 lb./sq. in. bmep has been turbocharged to carry three times that load without excessive stress in any part. Prolonged testing has shown that the Association's Mark I fuel injection system merits serious consideration for engines of the future. The British Non-Ferrous Metals Research Association has devoted considerable effort in the past few years to measuring the emission of smoke and fume from stacks serving a wide variety of processes

* Department of Scientific and Industrial Research. *Research for Industry 1961: A Report on Work done by Industrial Research Associations in the Government Scheme*. Pp. iv + 140 + 12 plates. (London: H.M.S.O., 1962.) 10*s*. net.

in the copper and copper alloy and aluminium industries, and the efficiency of different types of cleaning plant. Corrosion studies continued to occupy a large place in the Association's programme, and, besides development of a thermo-electric gauge for the non-destructive measurement of the thickness of metal coatings, creep-resisting alloys have been developed, including thorium alloys for possible nuclear fuels, titanium alloys for gas-turbine compressor blocking and zirconium alloys for constructional purposes in nuclear plant. Potentiometer studies by the Research Association of British Paint, Colour and Varnish Manufacturers have advanced knowledge of the influence of many anti-corrosive pigments on the electro-chemistry of corrosion processes, and, together with radio-chemical and autoradiographic techniques, have gone far to show how red lead pigment in paint behaves on steel and non-ferrous metals. Studies on materials for the protection of metal-sprayed steel have indicated the value of etch primer pre-treatments and of paint media which do not react with the sprayed metals, and have influenced the choice of treatments designed for the Forth and Tamar road bridges. The development of testing methods to indicate the firing propensity of the class of organic pigments known as toners when treated with resinous materials to enhance colour quality and investigations of the effect of various kinds of resin used have led to a marked decrease of such fires.

The Production Engineering Research Association has developed an improved design of reamer which is estimated to increase production-rates by 2–5 times in most industrial applications and the reamer life 3–8 times. New approaches are being made to the design of structures, and the principles evolved are being increasingly applied by machine-tool makers in the design and manufacture of new machines, while a detailed metallographic study of the mechanism of shear during hole-punching operations has resulted in a new technique for producing precision punched holes in sheet or strip materials, known as finish piercing. A new model of automatic spring-coiling machine being developed by the Spring Manufacturers' Research Association is expected to capture a large part of the export market. The results of investigations of the effect of non-metallic coatings on the corrosion–fatigue strength of springs which have shown that several resinous and plastic coatings possess good adhesion, abrasion resistance and mechanical strength, as well as withstanding corrosion–fatigue conditions for more than 50×10^6 stress reversals are being applied in the industry to protect finished springs against corrosion.

THE MEDICAL RESEARCH COUNCIL

BESIDES accounts of the work of its own research units and that of institutions which it assists with finance, the annual report of the Medical Research Council is of interest to all men of science for the lucidity of a series of articles which have been selected to illustrate some aspects of the Council's work*. Among them are the physiological exploration of physical symptoms of mental and cerebral illness; mental disorders in old age; replacement therapy in hæmophilia and Christmas disease; trachoma and inclusion blennorrhœa; the early lesion of dental caries; the bacterial cell wall and the anthropological significance of blood groups.

Of outstanding value, however, is an important article describing the modern role of a research council in encouraging medical and biological research.

It is now fifty years since, with the creation of the former Medical Research Committee, Great Britain took the lead in setting up central organizations for research. During this period, society, in common with those of other developed countries, has changed profoundly. Fifty years ago appreciation of the part that research would come to play in the machinery of Government was confined to a few far-sighted men. Now it is taken for granted, and the question of how most effectively to organize support for research and development has become a matter of continuing concern to all modern Governments.

In the course of its development a central organization for research passes through a series of stages.

At the outset it is conceived of as a means of satisfying certain special needs which cannot be or are not being met by existing agencies for research—universities on one hand and the practising profession or industry on the other.

It is soon found, however, that these *ad hoc* requirements cannot always be met on the basis of existing knowledge, nor can reliance be placed on the chance that the interests of others will incidentally produce what is required. The second stage is then entered, when such organizations are driven to employ their own staff and find themselves increasingly involved in 'basic' research to fill gaps in knowledge that they have identified. In Great Britain, this stage was reached before the end of the First World War.

The next stage comes with the recognition that it is impossible for any organization to foresee its needs for more than a few steps ahead; that it is no more than enlightened self-interest to support any research that promises additions to knowledge even though its practical utilization cannot immediately be foreseen. In the 1930's, for example, it was impossible to foresee the atom bomb and, consequently, the hazards to health that might follow its use. Had not the Medical Research Council supported as subjects for study, in their own right, research into the effects of ionizing radiations on cells and on the production of mutations, they would not have been in a position, twenty-five years later, to give an assessment to the Government of the effects of fall-out or the hazards of such radiation. Again, looking to the future, the problem of 'population pressures' is looming ahead. Necessarily such a problem cannot be approached directly, and only partially by retrospective observation on human populations. It is evident, however, that if informed judgements are to be made, every fragment of knowledge from ecology, ethology, nutrition and genetics may be required.

The Medical Research Council conceives its function as: to watch over the whole fields of medical and related biological research so as to foresee, to the best of its ability, the needs and opportunities; to give support to any promising research in these fields irrespective of the agent concerned; to work in partnership with the universities and professions on one hand and the various Government departments on the other, so that new knowledge may be made available as the need arises.

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* Committee of Privy Council for Medical Research. Report of the Medical Research Council for the year 1960–1961. Pp. viii+372. (Cmnd. 1783.) (London: H.M.S.O. 1962.) 21s. net.

It is according to this concept that the Council has devised its organization and formulated principles of working. These developments in the concept of the function of a central research organization are set out for two reasons. The first is that it is not yet fully realized that, nowadays, such organizations cannot remain pre-occupied with the institutions and staff for which they are directly responsible, but must pay attention to the development of knowledge as a whole in the field with which they are concerned. The second is that, when viewed in this larger perspective, many of the detailed problems of management disappear.

At the level of management the task of a research council is to reconcile two apparently conflicting requirements. On one hand, it has to try to ensure that the new knowledge required by society through Government is forthcoming and to this end has to distribute its support of research according to its assessment of present and future needs. On the other hand, it has so to devise its conditions of support as to preserve to individual workers that intellectual initiative without which creative work cannot develop. The problem for a research organization is thus to reconcile the implementation of a balanced and comprehensive research policy at the national level with the need for intellectual freedom at the level of the individual or the research team.

The solution of this dilemma is largely a matter of the scale of operation. If resources are restricted, or one thinks only in terms of individual institutions or particular organizations, the dilemma is real, and, in order to discharge its social obligations, a research organization may then be forced to have recourse to control and direction, even at the risk of inhibiting its workers in the creative thought and initiative on which its efficiency ultimately depends. But if the scale of operation is adequate the difficulty need not arise; for if support has been spread widely and with foresight, and if the central organization commands the confidence of scientists in general, then the relevant spontaneous interest can practically always be found. In other words, the solution of the dilemma lies in drawing a distinction between direction of research policy and directing individuals. It is of the essence of policy to have an object; but in relation to creative work the path to that object lies not through prescription to individuals, but through the informed selection of projects to be supported.

The question of what is adequate scale of operation for a central research organization with comprehensive social obligations in its particular field is, therefore, of critical importance. To-day the answer can no longer be in doubt. The demand for new knowledge is such that nothing less than the national scale will suffice, for it is only on this scale that the requisite variety of research work, interests and institutions can be found to allow the necessary freedom of initiative in policy.

There are only two qualitatively different types of scientific investigation: that which is directed to increasing understanding of naturally occurring phenomena—research—and that which aims at bringing knowledge so gained to the solution of human needs—applied research or development. Even these are little more than abstractions at either extreme of the range. Between them lie every shade and combination of activity, and it is this that makes organization in science possible. No research worker is entirely divorced in interest from his fellows or indifferent either to the use that can be made of his work or to its theoretical implications. All range, to some extent, about the centre-point of their interests, provided that they are not prevented by segregation from habitual contact with workers whose interests are contiguous. It is a major task of organization to promote such interplay. Blood transfusion would be hamstrung if separated from relevant investigations in genetics or immunopathology. Research in endocrinology would be grossly handicapped but for the experience available in clinical practice. Laboratory and practical work in nutrition mutually interact. The interdependence of chemistry, biochemistry, chemical pathology and the study of human metabolic diseases is evident. There is a regrouping of disciplines, a prominent feature of which is the disappearance of the previous barriers between the laboratory and practice. It would be undesirable, on either theoretical or practical grounds, to do anything that tended to separate research aimed at the applications of knowledge from the investigation of natural phenomena out of which this knowledge grows.

The remainder of this valuable survey into the role of a research council examines the Medical Research Council's relationship to Government. Its significance as a court of appeal in various aspects of medicine is but one tribute to its well-recognized independence.

THE ELECTRICAL RESEARCH ASSOCIATION 1961

It is significant that the opening paragraph of the report for 1961 of the Electrical Research Association* is headed "Electronics". The paragraph records a decision of the Council to expand the activities of the Association in the electronics field.

When the Association was formed in 1920 the part played by electronics in the electrical industry had scarcely become appreciable. Developments were just beginning whereby telecommunication in all its forms was to be revolutionized and, at a later stage, profound changes were to be effected in many branches of heavy electrical engineering.

The research effort of the Association has been directed primarily towards problems in the heavy current field, but there has been a natural development of interest and activity in the many incidental applications of electronics. It is estimated that about 12 per cent of the Association's present research activities is of direct interest to the electronics branch of the industry and a further 6 per cent of indirect interest. Having regard to the central position which electronics is assuming in the electrical industry as a whole, it is now considered that the research programmes devoted to electronics and related subjects should be expanded until they reach half the total. The Council of the Association therefore proposes to examine the probable response from industry to the development of a programme much more biased towards the electronics side.

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* Electrical Research Association. 41st Annual Report for the year ended 31st December, 1961. Pp. 104. (E.R.A./T. 476.) (Leatherhead Electrical Research Association, 1962.)

Results obtained during the past year are summarized in the Director's Report on research work under six divisional headings. Division 1, that of insulating materials, reports the continuation of the Association's work on solid and on liquid dielectrics both in the laboratories at Leatherhead and in the University Laboratories at Liverpool, Birmingham and London. The Leatherhead Laboratories are now equipped to make dielectric measurements in inert atmospheres at pressures up to 20 atmospheres and temperatures to 500° C. Work on the high-permittivity 'Skanavi' materials has shown these substances to possess two phases, at least one of which is a ferro-electric with an extremely low coercive force.

In an attempt to locate minute leakage paths through paper capacitor tissue a new technique has been devised in which the leakage current electrolyses a thin evaporated aluminium electrode. Small areas of exceptional current density can be detected in this way. The deposition of very thin films of dielectric polymers from the monomer vapour under the action of a high-frequency discharge has been successfully effected. In the field of theoretical physics work on semi-conductors has produced an interesting and potentially important result, namely, that in semiconductors with very low mobility the so-called 'band model' becomes invalid. It has been shown that the band model is valid for mobilities down to the order of $1\ \text{cm}^2\ \text{sec}^{-1}\ \text{V}^{-1}$.

Under Division 2, "Power Plant", it is reported that the new laboratory for the investigation of the creep behaviour of steels is now in full operation. The programme of work which is proceeding includes long-time rupture tests on pipes and superheater tubes, creep and rupture properties of castings for turbine casings and of forgings for turbine rotors, also of steels for turbine blades. The experimental work on the properties of steam is now being extended along the 400° C and 600° C isotherms to a pressure of $9,000\ \text{lb./in.}^2$, the results available up to $5,800\ \text{lb/in.}^2$ and 750° C having been reported during the year.

Of particular interest is a group of experiments designed to obtain data required in the investigation of some of the proposed new methods of energy conversion, namely, thermonuclear fusion, magnetohydrodynamic generation and thermionic emission. Detailed information is required on the electrical conductivity, the thermal conductivity and the distribution of energy as among electrons, ions and atoms in differing forms of high-temperature plasma. A preliminary study has been made of discharge 'temperature' and of departures from the thermal equilibrium in arc discharges under a variety of conditions. Currents up to 200 amp. d.c. and 1,000 amp. peak pulse have been used in various gases at pressures from atmospheric down to 10^{-7} atmosphere and subjected to magnetic fields up to 1,000 gauss. It has been found that spectroscopic measurements of temperature may be misleading if the precise behaviour of the discharge is not taken into account.

Division 3 deals with switchgear, transformers and power system behaviour. An interesting study of the so-called anomalous loss in iron undergoing alternating magnetization has been made at the laboratories of the British Iron and Steel Research Association on behalf of the Electrical Research Association. The problem has been examined theoretically on the basis of domain concepts. and it has been shown that for strip thicknesses down to 0·01 in. calculation predicts satisfactorily the total eddy current loss. Calculation on a classical basis may give results differing from the measured eddy loss by as much as 30 per cent of the total loss. With thinner sheets there is still an anomaly, and the hypothesis that this may be due to the locking of domain boundaries by imperfections is being examined.

It has been found in practice in the impulse testing of transformers that an impulse applied to one winding could induce in other windings voltages much higher than would be expected. This phenomenon has been demonstrated on a model and has been examined on a large production transformer by exploration with a capacitance probe.

Lines and cables for power supply comprise the field of Division 4. The rating of cables and allied problems have again attracted a good deal of attention. Methods of calculation are being reviewed as a contribution towards a programme for the international standardization of the calculation of cable ratings. Experimental work has continued on the short-circuit ratings of a range of paper-insulated, mineral-insulated and polyvinyl chloride-insulated cables.

A good deal of the effort of Division 5, "Non-industrial Applications," has been devoted to off-peak floor heating. Direct observations have been made on the relations between the constructional features of buildings and the temperature distribution with time throughout heated floors, and these have been accompanied by studies made on the Electrical Research Association's network analyser involving a wider range of parameters. These laboratory experiments are being supplemented by records obtained from occupied flats. Several floors in two new blocks of flats in the London area have been modified in accordance with the Association's recommendations and have been fully instrumented for the measurement of heat-flow and temperature distribution.

A related problem, that of the prevention of ice formation on limited areas of road surface, is being studied on the installation at the Mound, Edinburgh. Recordings are being made of the temperature distribution and heat transfer produced under a wide range of weather conditions by the heating mat.

Reductions have been made fairly recently in the bulb sizes of certain electric lamps. As a result, for a given wattage, higher temperatures are encountered, and a preliminary investigation has shown the need for modifications in lampholders and fittings. It has been represented to the British Standards Institution by the Electrical Research Association that there is an urgent need for the production of a standard lampholder of insulating material capable of withstanding the temperatures which are now attained.

In the same section are recorded the Association's activities concerned with rural electrification, farming and horticulture. In collaboration with the National Institute for Research in Dairying laboratory tests have been carried out on a dairy heat-pump, and the plant has now been installed on a farm for trial under practical conditions. A preliminary study has also been made of the application of the heat-pump principle to bulk milk-tanks on farms.

Division 6 of the report deals with industrial applications. Work on flameproof enclosures has continued and it is noted that, for the Ministry of Aviation, a determination of safe gaps for ¼-in. and ⅛-in. flanges has been made for propane as representative of petroleum spirit. Research in the related subject of the intrinsic safety of electric circuits in explosive atmospheres has comprised, along with the extension of the range of gases studied, an investigation of the incendivity of propane–air mixtures for break sparks produced in circuits of low voltage, 0·5–2 V. It is found in such circuits that the ignition energy comes almost wholly from the stored inductive energy, that contributed directly from the supply being negligible.

Work on material transfer in welding arcs has concerned both the stable small droplet, the large globule and the transition region. The possibilities of enforced transfer by electrical means are being investigated for aluminium and for some related materials. The investigation of the long-term stability of fixed resistors under various conditions of operation or of storage continues. The present work is concentrated on standard size metal oxide film and evaporated nichrome film resistors together with miniature oxide film resistors. The behaviour of fuses is being re-examined both experimentally and theoretically in respect of the effect of the pre-heating of the fuse.

An investigation started during the year aims at the collection of data on the occurrence of short-duration transients in low-voltage distribution systems. Such transients are known to cause damage to electronic apparatus and the immediate purpose of the investigation is to collect information as to the frequency of occurrence and the amplitude-level of these disturbances. Preliminary results indicate that pulses of the order of 50 V amplitude happen frequently, but those of 400 V or more only occasionally.

The text of the Director's report concludes with paragraphs on member relations and on the information bureau and library. Then follows a complete list of the research reports issued during the year and of published technical contributions. The structure and composition of the Divisional and Research Advisory Committees are set out in full and—a particularly valuable feature—the scope of each is briefly stated.

J. Greig

SOLID-STATE PHYSICS

A PLEASANTLY informal meeting on several aspects of solid-state physics took place in the pure physics division of the Canadian National Research Council at Ottawa. The meeting occurred during the week of June 18–22. The main purpose was to bring experimentalists and theorists together in an informal atmosphere so that an uninhibited exchange of views and ideas could ensue. Three main areas were explored; electron transport in metals, anharmonic effects in crystals, and basic problems in metallurgy.

The discussions on electron transport theory were opened by Dr. M. Bailyn (Northwestern University, Evanston, Illinois), who outlined a new variational method that could be useful in magneto-resistance problems. This was followed by a discussion between Dr. J. Ziman (University of Cambridge) and himself on the difficulties of obtaining reliable numerical values for low-temperature transport coefficients. From this exchange of views it was evident that both the shape of the Fermi surface and of the phonon spectrum will have to be taken into account before any 'reliable numbers' can be produced from the theory. Dr. Ziman also gave an account of some of the recent developments in the theory of liquid metals. He pointed out that there is a striking correlation between the values of the electron-'phonon' coupling constant obtained from the resistance of liquid metals, and the transition temperatures of superconductors. Dr. G. Chester (University of Birmingham) opened a discussion on the physical interpretation of the present-day theory of superconductivity. Dr. D. K. C. MacDonald (National Research Council of Canada) raised the question as to whether there is any theoretical upper limit to superconducting transition temperatures T_c. The only theoretical answer that was forthcoming was that if the transition was mediated by electron–phonon interactions then T_c will always be very much less than the Debye temperature. Otherwise, no sound theoretical limit could be given.

Dr. Barron introduced the topic of anharmonic effects in crystals. He pointed out that from the point of view of statistical mechanics it is possible to include some part of the anharmonic effects by redefining the phonon frequencies. This idea raises the interesting question as to how far it is possible to carry through this idea of introducing 'effective phonons' to account for anharmonic effects. In other words, to what extent can we talk in terms of effective (or re-normalized) phonons in crystals in the same way as we talk of effective (or quasi) particles in a fermion system? This question has not yet been fully explored theoretically.

Prof. F. R. N. Nabarro (University of the Witwatersrand) reviewed the properties of dislocations and discussed the present state of theories of deformation and strain hardening of crystals. It was concluded that further experimental work is required to determine the relative importance of the various possible obstacles to dislocation motion, namely: (1) the long-range stresses due to groups of dislocations piled-up on the primary slip plane; (2) the stresses due to forest dislocations on intersecting slip planes; (3) the resistance to motion of the jogs on dislocations. The danger of relying too much on observations of surface effects was emphasized. Stereo-pictures shown by Dr. Z. S. Basinski (University of Ottawa) illustrated convincingly the three-dimensional complexity of the dislocation arrangements observed by electron microscopy. Results showing the variation of stacking fault energy with composition in a number of alloy systems were presented by Dr. A. Howie (University of Cambridge) and the relation of this quantity to the mechanical properties and electronic structure of crystals was discussed.

One of the major problems in present-day physics is that of communicating effectively between different groups of physicists. A meeting such as this can provide exactly the right atmosphere to break down the 'language barrier' between experimentalists and theorists. The efforts of Dr. D. K. C. MacDonald and Dr. J. S. Dugdale (University of Ottawa) in organizing the meeting were greatly appreciated by the visitors, all of whom appeared to enjoy both the meeting and their visit to Ottawa.

G. V. Chester
A. Howie

A PHILOSOPHY OF LIBRARIANSHIP

THE Library Association has issued as *Occasional Papers* No. 3 the address "The Creed of a Librarian—No Politics, No Religion, No Morals"—given by D. J. Foskett at a meeting of the North Western Group, Reference, Special and Information Section in Manchester last March*. Mr. Foskett directed attention to the fact that the attitude of the professional man to the body of knowledge and technique that constitutes professional equipment is coloured by a sense of purpose, and the ordering of that knowledge in the professional mind is inspired and directed by the end for which it is acquired. The formulation of a professional creed is bound up with professional education, and it is in the formation of an attitude of mind that we can distinguish between education and mere training. For the basis of a librarian's philosophy, he believed, one must look to the ideas of Mr. Raymond Smith that a library is part of a social organization and that librarianship is a social process inextricably bound up with the life of a community. It was in special libraries that the place of the active information service in the progress of knowledge could be most clearly seen—the library playing its part alongside the other members of the organization, to promote the welfare of the organization by implementing its policy through books.

Librarianship, he pointed out, like all processes, was a dynamic continuum, and the refinement of techniques, such as classification and indexing to provide a better service, was being done mainly by special librarians. The most sustained attempt to work out a philosophy of librarianship was that of Dr. Ranganathan, and his few basic laws expressed in a few short phrases the philosophy of Raymond Smith, that knowledge increases, the people need knowledge, and that the function of the library is to see that they get it. Librarians, Mr. Foskett argues, are the key figures in the process of organizing the flow of information from producer to user, because they deal directly with the reader. However, if they were to provide readers with the sort of reading that they wanted, some close knowledge of readers was necessary, although bringing readers and their material together remained basically a matter of organization. In reference service the librarian should be the reader's *alter ego*, immersed in his politics, his religion, his morals. He must have the ability to participate in the reader's enthusiasms and to devote himself wholly and whole-heartedly to whatever cause the reader has at the time of his inquiry. This involves also looking ahead and trying to correlate the new information pouring daily into the library with the known requirements of its users. Furthermore, one of the most useful functions the librarian could perform to-day is that of taking a broad view, of seeing those factors that are common to many fields, of widening the horizon of the reader, and assisting the cross-fertilization of ideas. A librarian, too, should have strong personal convictions and to be efficient must be dedicated.

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* Library Association . North Western Group. Reference, Special and Information Section *Occasional Papers*, No. 3. *The Creed of a Librarian—No Politics, No Religion, No Morals.* By D. J Foskett. (London The Library Association, 1962.) 3s. 6*d*.

DIET OF THE NEW-BORN HUMAN BEING

BABIES at birth have almost no powers of discrimination in choosing their food. This is perhaps scarcely surprising from the evolutionary point of view; until asepsis was invented, there was only one kind of food that enabled a baby to grow. Now, with many forms of relatively safe infant foods easily available, someone, usually the mother, makes a deliberate choice on behalf of the baby.

This choice is subject to custom, even to fashion, and is often determined by trivial circumstances rather than based on any comprehensive view. This is also not surprising; the comparative merits of human and other kinds of milk are often important for domestic or commercial reasons, but there have been few sound surveys from which to form an opinion on their relative values for the new-born human infant.

Among the notable surveys is one that was carried out in the mid-1950's in two areas of Norrbotten, the northernmost county of Sweden, lying partly within the Arctic Circle and which is described in a recent issue of *The Glaxo Volume* (No. 24). The population is predominantly Swedish with some Finnish and Lapp minorities, and is concentrated in the valleys and coastal areas. The climate is hard, and temperatures of –30° to –40° C are not uncommon in winter. Most of the inhabitants of one area investigated, Kiruna, were workers in a large iron mine. In the other area, Tore-Ranea, most were smallholders. In this county large families are still common, and nearly one-quarter of the babies were fed from the breast for more than six months. The survey has given much careful clinical observation, along with biochemical findings, for comparing the growth and health of infants whose different methods of feeding were recorded.

Frequent examinations by doctors and nurses were made throughout the first year of the babies' lives and continued less frequently up to two and a half years; they were interrupted only by the coldest weather or by the impassability of the roads. The children studied were 402 of the 660 born alive in the area during a period of eighteen months. In the two and a half years of the follow-up, only two died after the neonatal period.

The investigators classified the babies into four groups, according to how long they were fed only from the breast (0–2 weeks; 1–2½ months; 3–6 months; over 6 months). To simplify comparisons, all artificially fed babies received the same milk mixture, consisting of equal volumes of cows' milk and water, with added sugar and a little flour. This provided less protein than is normally received by bottle-fed infants in many parts of the world.

There was no great difference in weight-gain attributable to the type of feeding until about the fifth month, when the babies weaned early from the breast gained faster than the others. Here an old difficulty was encountered in comparing infant foods. The secretion of milk by a mother can be too small for her baby. When a comparison is made between a group of babies continuing to receive human milk and one fed by another regimen, it is inevitably affected by the prevailing view about when a baby should be given supplementary food. Thus it may not measure the simple effect of an unlimited diet of human milk.

Another difficulty is the uncertainty of how quickly a baby ought to grow. To some extent the individual baby, by his demands and by his end-point of satiety, settles how much he will take. This is clear enough when weight curves are drawn for the two sexes. Baby boys gain on average faster than girls and take more milk, even in the first six days of life. Presumably their appetites differ. But within the pattern of individual and sex differences, it is possible to cause some departure from an average gain in weight by altering the food. There appears to come a time when the underfed baby, who after a briefer period on a smaller diet would have voluntarily made up the deficiency, comes to accept small feeds and to grow slowly. There is also the reverse of this, the baby who has always been pressed to take more. It appears that some babies become habituated to extra food and to maintaining a high rate of growth.

In the Norbotten survey the half-strength cow's milk gave an average gain in weight in the first months slightly less than that of the breast-fed babies. In other surveys the bottle-fed have gained weight considerably faster, and this greater gain in weight has been accepted by mothers and pædiatricians alike as an argument in favour of cows' milk. An undercurrent of doubt has now to be taken into account, for it may be that early fast growth brings early maturation, possibly with extra problems and perhaps also early decline, though perhaps also with greater early achievement. These surmises are partly justified by McCance and Widdowson's findings in rats. How far they apply to human development is not known.

Although gain in weight in the first months among the Norrbotten babies differed little, despite the different diets, the babies fed early from the bottle were longer at seven and a half months than those breast-fed; any difference had disappeared by thirty months. Ossification centres in boys did not differ between groups, but in girls significantly more ossification centres had appeared at seven and a half months in those fed early on cows' milk. Girls were known to have more ossification centres than boys at that age, but the relation of this to diet had not been observed before. By thirty months the groups showed no marked differences related to diet or sex.

In Norrbotten, the times at which the deciduous teeth erupted were found to be unaffected by diet. The incidences of caries in breast-fed and bottle-fed infants were ingeniously compared. Its occurrence on the surface of teeth largely mineralized before two months was compared with that of teeth mineralized later in the same child. In this way, although the individual range of caries was wide, it was found that teeth mineralized late were slightly but significantly more susceptible to caries if the baby had been entirely breast-fed up to the time of mineralization. The fluorine content of the water was about 0·1 mg per litre; all children received vitamin D and no radiological evidence of rickets was found.

Although the clinical measurements of calcification varied so little with the kind of milk given, the total intake of calcium and phosphorus was estimated by the Norrbotten workers to be much greater from the diluted cows' milk than from the human milk. The serum calcium was, however, higher among the breast-fed. This reflects, as McCance has pointed out, lower intake of phosphate from human milk limiting the deposition of calcium, rather than differences in the content of calcium itself.

As in other surveys, the incidence of most infections was greater among the babies weaned early. Most of the babies fed from the breast for more than six and a half months came from large families, and the consequent likelihood of greater exposure to infection may have masked other differences. The sedimentation-rate was significantly lower among the babies fed from the breast than among the bottle-fed. The bottle-fed had more pyrexial attacks, but this was due to some babies having several attacks.

Although the amount of upper respiratory infection was not significantly different between the groups, there was more bronchitis and more otitis media among the early weaned, and in one area, more diarrhœa. The number of babies suffering from acute exanthemata was too small, as would be expected, to provide useful statistics. Nevertheless, the trend in the figures conforms with the clear evidence in Douglas's survey that there was less measles among the babies fed longest from the breast.

The antibodies of human colostrum and milk, imprinted as they are with the mother's immunity, may give some protection to the baby, not so much by being absorbed as by coating, and later causing the lysis of ingested organisms. There are some possible mechanisms involved in defence that depend on the diet.

The relative protection against measles conferred by breast feeding would have been expected to be associated with a higher serum γ-globulin, but the Norrbotten survey shows clearly that babies fed for the longer time from the breast had in fact a significantly lower γ-globulin level. Although γ-globulin given by injection can confer immunity to measles, the relatively high γ-globulin induced by feeding with cows' milk does not apparently give this protection. The high γ-globulin content of serum in the group of babies weaned early might be the result of an immune response by the baby to some substances in cows' milk.

This classical Norrbotten survey was undertaken by Prof. Olof Mellander, Prof. Bo Vahlquist and Dr. Tore Melbin, with twenty-three named assistants and many others taking part besides. Between them they made hundreds of thousands of observations. Those who live in other climates must admire their achievement in a region where for a large part of the year darkness heavily predominates.

GUTENBERG'S EARLY *PKP* OBSERVATIONS

By Dr. BRUCE A. BOLT
Department of Applied Mathematics, University of Sydney

IN several important papers, Gutenberg[1-3] has directed attention to seismic waves recorded as first arrivals at epicentral distances, Δ, between about 125° and 140°. At the shorter distances in this range the waves arrive between 15 and 20 sec earlier than the normal *PKIKP* waves. The early onsets are generally of such relatively small amplitude and short period that they "may be missed sometimes in reading the records[4]". Seismograms showing the small onsets and the larger *PKIKP* onsets which follow them are reproduced in Gutenberg's papers[1,3]. In a special study of seismograms from the deep-focus New Zealand shock of December 10, 1958, I have found a number of other clear examples.

These early waves cannot be interpreted solely in terms of reflexion, refraction, diffraction and the present theories of the structure of the Earth's core. Gutenberg's tentative explanation[4] was in terms of wave dispersion in a transition region between a liquid outer core and a solid inner core.

In this communication I present a new explanation of the early waves, which is based on ray theory. By successive approximations, a velocity distribution for the core has been derived which is reasonably consistent, within the limits of the empirical data used, with:

(*a*) a set of travel-times of the early waves measured by Gutenberg ;

(*b*) the Jeffreys–Bullen travel-times for the main *PKP* branches ;

(*c*) seismic ray theory for a spherically symmetrical Earth.

It follows that the relation between the derived velocity distribution and the actual structure of the Earth's core depends on the physical correctness of (*a*), (*b*) and (*c*). The Jeffreys–Bullen tables were taken as a basis for the work since these times are widely adopted as standard for an 'average' Earth.

The Jeffreys–Bullen travel-times for *PKP* are based on the hypothesis[5-7] that an inner core exists having a discontinuous change of velocity at its boundary. The velocity solution[8] which corresponds to the Jeffreys–Bullen times is drawn in Fig. 1. (Hereafter it is called the *J* velocity solution.)

The assumption of a single velocity jump yields a travel-time curve similar to the diagram in the left corner of Fig. 2. The grazing ray at the mantle-core boundary (radius R_c = 3,473 km) is refracted to *A* near 180°. Rays more steeply incident on this boundary give in turn the branches *AB* and *BC* until the ray at *C* grazes the inner core. The branch *BC* is generated by rays reflected from the inner core, and *DEF* by the *PKIKP* rays refracted through the inner core.

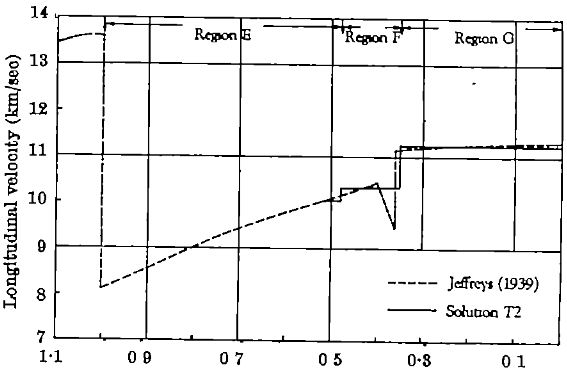

Fig. 1

A caustic curve is located near *B*: for distances slightly beyond *B*, points on the Earth's surface can be reached by two refracted rays with consequent reinforcement of energy. Large *PKP* amplitudes are commonly observed[3] at epicentral distances between 143° and 147°.

Numerous observational studies of the *PKP* phase have been made[6,7,9]. (Some evidence[10,11] indicates that the Jeffreys–Bullen times for *PKIKP* need an increase of about 1·5 sec relative to the *P* times for Δ > 80°). Additional recent data on *PKP* from an East Indies shock of April 16, 1957, have been published by Gutenberg[3]. Stations with 118° < Δ < 140° at which Gutenberg made confident interpretations are listed in Table 1.

The residuals in columns I and IV are the differences (*O–C*) between the observed travel-times of the first and second onsets and the corresponding Jeffreys–Bullen times for the *DE* branch of *PKP*. A focal-depth of 600 km, used by Gutenberg, has been adopted, while Gutenberg's origin-time has been increased by 7 sec to 4 h 04 m 11 s to shift the mean *PKIKP* residual to zero. The early onsets at the 22 stations with

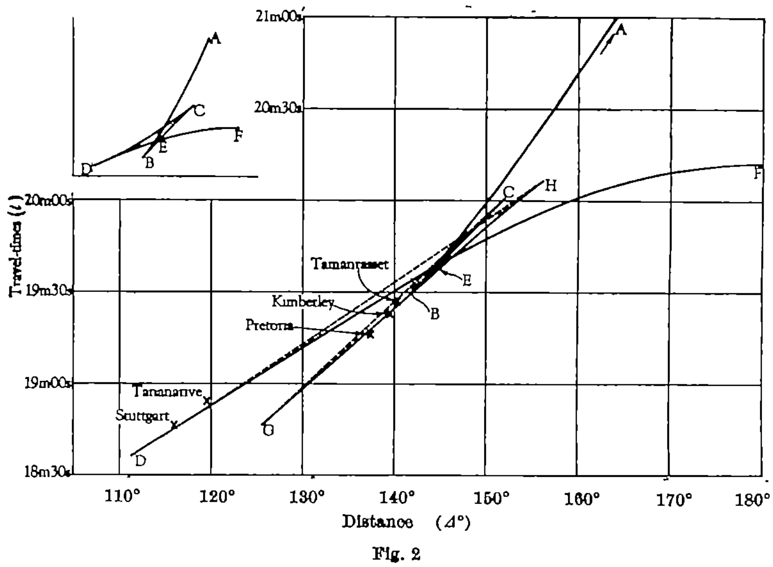

Fig. 2

Table 1

Station	Δ°	Residuals (O–C) (sec)			
		Early onsets			Second onsets
		I J–B *DEF* branch	II *T*1 *GH* branch	III *T*2 *GH* branch	IV J–B *DEF* branch
Seattle	119·5	—	—	—	+2
Berkeley	123·9	—	—	—	0
Santa Clara	124·3	—	—	—	−1
Lick	124·5	−14	+4	+7	0
Tinemaha	127·1	−12	+4	+7	+3
Isabella	127·5	−11	+5	+8	0
Woody	127·8	−20	−5	−2	−2
China Lake	128·1	−17	−2	+1	0
Pasadena	128·4	−15	0	+3	+1
Dalton	128·7	−18	−3	0	0
Riverside	129·1	−21	−6	−3	0
Big Bear	129·3	−15	−1	+2	+2
Schefferville	129·6	−14	0	+3	0
Palomar	129·7	−15	−1	+2	0
Barrett	130·2	−16	−2	+1	−1
Hayfield	130·7	−16	−2	+1	+1
Rapid City	132·2	−17	−4	−1	0
Boulder	133·9	−16	−4	−1	0
Denver	134·2	−15	−5	−2	0
Tucson	134·7	−16	−6	−3	+1
Kirkland Lake	136·1	−14	−4	−1	−1
Seven Falls	137·5	−11	−3	0	+2
Shawinigan Falls	138·1	−12	−4	−1	−1
Montreal	139·1	−10	−4	−1	−1
Ottawa	139·2	−12	−6	−2	−1

J–B, Jeffreys–Bullen

124° < Δ < 140° constitute the set of empirical times mentioned in (*a*). The corresponding residuals range from −21 to −10 sec.

We now consider the hypothesis that there is a double positive jump in longitudinal velocity within the core. In the usual notation, let the two discontinuities at radii R_F, R_G ($< R_F$) divide the core into Region *E*, Region *F* and Region *G* (the inner core).

An initial restriction in accord with (*b*) is that the velocity jumps must not affect the position of the well-observed caustic at *B*. This requires that $0 \cdot 54\, R_c > R_F$, approximately. A possible form of the new *PKP* travel-time curve is sketched in Fig. 2. There are now six branches and five cusps *B*, *C*, *G*, *H* and *D*. The *CG* branch arises from reflexions from the outer boundary of Region *F*; *HD* from reflexions from Region *G*. The effect of the new hypothesis is to introduce a branch *GH* which is earlier than the *DEF* branch and which may be associated with early *PKP* observations with Δ > 120°, approximately.

A considerable amount of numerical analysis was undertaken to obtain a model consistent with (*a*) and (*b*). Two main problems are to reproduce the positions of the cusps *D* and *G*, and also the gradients $dt/d\Delta$ of the observed branches *AB*, *GH*, *DEF*. As a sufficient approximation for the present purposes, the velocities v_F, v_G were taken constant in all trial models.

The *J* velocity distribution (cf. Fig. 1) has a velocity decrease outside the inner core. In order not to exceed the measured travel-time of a diametrical *P* wave when the second velocity jump at R_F is introduced, and at the same time to obtain the required gradients of the branches, the *J* velocity gradient was reduced to zero just above Region *F*. Let the upper boundary of this region be R^*. For $r > R^*$, the *J* velocities were adopted unchanged. As a consequence, in all the trial solutions the times of the branch *AB* and part of *BC* are identical with the corresponding Jeffreys–Bullen times.

The requirements (*a*), (*b*) and (*c*) were found to place strong constraints on the possible ranges of the free variables R^*, R_F, R_G, v_F, v_G. Two solutions, called *T*1 and *T*2, for the models defined in Table 2 are presented here; the differences between them indicate the effect of a change in the variable R_F. Table 2 gives also the times, distances and gradients at the cusps. Times for reflexion at R_F were calculated using:

$$t = 2\int_{R^*}^{R_C} r^{-1}\,\eta^2\,(\eta^2 - p^2)^{-1/2}\,dr$$

where $\eta = r/v$, and $p = dt/d\Delta$. The usual associated integral[13] gave the corresponding distances. Since the integrands become large as $r \to R^*$, Weddle's rule, which requires constant fifth differences only, was used towards R^*. After building up the core times for *K*, these were combined with the Jeffreys–Bullen *PcP* times by selecting points with equal gradient *p*. Various checks suggest that the times and distances in Table 2 are probably accurate to about half a unit.

Table 2

	Solution *T*1				Solution *T*2			
	km			km/sec	km			km/sec
R^*, v^*	1,806	($0 \cdot 52 R_c$),		10·08	1,806	($0 \cdot 52 R_c$),		10·08
R_F, v_F	1,598	($0 \cdot 46 R_c$),		10·32	1,667	($0 \cdot 48 R_c$),		10·31
R_G, v_G	1,216	($0 \cdot 35 R_c$),		11·25	1,216	($0 \cdot 35 R_c$),		11·23
	Time		Δ°	*p*(sec/1°)	Time		Δ°	*p*(sec/1°)
	m	s			m	s		
Cusp *C*	20	00·7	152·1	2·78	19	56·2	151·2	2·90
G	18	47·0	125·6	2·70	18	41·9	124·7	2·82
H	20	6·2	156·2	2·05	20	6·3	156·5	2·05
D	18	36·2	111·3	1·89	18	37·5	111·0	1·89

The branch *DEF* for both *T*1 and *T*2 agrees within a second or two with the Jeffreys–Bullen times for *PKIKP*. The residuals of the first onsets from the East Indies shock against the *GH* branches of *T*1 and *T*2 are listed in columns II and III of Table 1. The 19 observations at the more distant stations give mean residuals of − 3·2 ± 0·4 sec and − 0·2 ± 0·4 sec against *T*1 and *T*2 respectively. A comparison with the remaining observations suggests that the measured onsets at Lick, Tinemaha and Isabella may be late through weakness. Agreement between the predicted and the empirical times appears close enough to warrant further consideration of the new hypothesis. On the data of Table 1, solution *T*2 is to be preferred; its velocity distribution is plotted in Fig. 1.

The values of *p* in Table 2 show that an increase of only 0·7 sec/1° in the ray parameter *p* yields a length of about 30° for the *GH* branch. The corresponding rays therefore leave the focus at nearly the same angle of incidence and relatively little energy is available for the early waves.

It is of interest also to compare the two theoretical solutions with the mean travel-times of first-arrival *PKP* waves from four hydrogen bombs exploded at Bikini[13]. To permit a comparison with the Jeffreys–Bullen tables, the mean residual δ of *P* observations from these explosions was first applied as a correction. From 232 *P* readings, with 34° < Δ < 104°, δ = 1·8 ± 0·6 sec[14]. The five adjusted mean travel-times and the residuals against *T*1 and *T*2 are listed in Table 3 (*n* is the number of observations).

The adjusted times have also been marked in Fig. 1, which shows the complete travel-time curve for *T*1. Comparison indicates that the small onsets at the five stations are in reasonable accord with the predicted travel-times based on the new hypothesis and on (*a*), (*b*) and (*c*).

Bullen and Burke-Gaffney explained the onsets at Pretoria and Kimberley in terms of diffraction near

Table 8

Station	Δ°	n	Adjusted mean travel-times	Residuals (O–C) (sec)	
				$T1$	$T2$
			m s		
Stuttgart	116·2	4	18 46 1 ± 0·6	+1	−1
Tananarive	119·8	2	18 54·5 ± 0·6	+2	0
Pretoria	137·3	3	19 16·4 ± 0·6	−1	+1
Kimberley	139·5	4	19 23·1 ± 1·0	0	+2
Tamanrasset	140·4	4	19 26·9 ± 1·1	+2	+4

the caustic at B. They remark, however, that since the early waves are about 1 sec period, Pretoria lies about 1·6° outside the range where observable diffracted waves might be expected theoretically.

The hypothesis advanced here has consequences which may provide tests crucial to it. The alteration of the J velocities for $r < R^*$ affects the times of all core phases for certain epicentral distances. In particular, for solution $T2$, SKS proper ends at $\Delta \approx 133·5°$ with a travel-time near 26 m 30 s. The corresponding time from the Jeffreys–Bullen tables is 26 m 28 s with a standard error[7] near 2 sec. In another study of recorded SKS, Nelson[15] gives 26 m 32 s at the same distance.

The observational evidence is that no large amplitudes occur near the cusps D and G. Recent theoretical work of Bullen[13] shows that under conditio which resemble those for $T1$ and $T2$, neither D nor are caustics. On the other hand, if the velocit *remained continuous* but the gradient suddenl changed at R_F and R_G these cusps might be associat with large amplitudes.

I propose to publish later a detailed quantitativ discussion of velocity solutions for the core based o the new hypothesis.

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GEOCHEMICAL AND GEOLOGICAL EVIDENCE FOR THE ORIGIN OF DARWIN GLASS

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THE occurrence of a frothy siliceous glass, from the eastern slopes of Mt. Darwin, Western Tasmania, was brought to scientific attention in 1910 by M. Donoghue. It was first mistaken for slag from an abandoned copper smelter. In 1913, H. Conder, State mining engineer, informed the Geological Survey of Tasmania of the occurrence, and a survey was undertaken by Loftus Hills. Samples were sent to Prof. F. E. Suess in Vienna, who named it 'Queenstownite' after the nearby town, and compared it with the moldavites[1]. Later workers have preferred the name 'Darwin glass'. Hills[2] fully described the details of the discovery and location, gave a description of the glass by Suess, and two chemical analyses by Ludwig.

A further description of the occurrence, with two new analyses by Ampt, is given by David *et al.*[3]. All these workers have agreed on the natural origin of the glass, and placed it with the tektites, although recognizing that it was an aberrant type.

Later workers, notably Conder[4], Suess[5] and Spencer[6,7], classified Darwin glass as an impactite, formed by fusion of siliceous sediments by meteorite impact. Preuss[8] reported a single determination of 240 p.p.m. chromium, 314 p.p.m nickel, 1·80 per cent iron, with a nickel–iron ratio of 170 × 10^{-4}. Ehmann[9] found 162 p.p.m. nickel and 1·60 per cent iron, giving a nickel–iron ratio of 100 × 10^{-4}, and he placed Darwin glass among the impact glasses without comment. Baker and Gaskin[10] and Baker[11] considere the glass to be of uncertain origin (no meteorite crate has yet been discovered in the vicinity) and Baker has suggested an origin by fusion of silica in burnin peat horizons. Doubt has also been cast on th authenticity of some specimens, and it seems possibl that some of the smelter slag and also some Tertiar tachylyte have been accidentally distributed a Darwin glass. We therefore decided to undertake geological and chemical examination of the occurrence, and report here the preliminary finding One of us (M. S.) is responsible for the collectio and geological description, and the other (S. R. T for the chemical data and their interpretation.

The glass is very irregular, slaggy, with twiste stalactitic forms, and with rare teardrop and dis like shapes[3]. Numerous bubble pits are present, an the pieces are consequently of very low densit (sp. gr., 1·7–2·2) and have a frothy appearanc well described by the prospectors' term, 'petrifie kelp'. The largest specimen weighed 21 g an measured 6 cm by 2·3 cm. The average weight 330 specimens described by Fenner[12] was 0·57 g; th average of 41 pieces in the University of Queenslan collection was 1·61 g[13]. The density of the powdere glass varies from 2·275 to 2·296 g cm^{-3} and th refractive index from 1·474 to 1·479[3]. Twisted an bent particles of fused silica (lechatelierite) ar present, but later investigators[11,13] have failed to confirm the presence of metallic spherules reported by Conder[4].

The glass is abundant on the east slopes of Ten Mile Hill, which lies 12 miles south-south-east of Queenstown, and occurs sparsely in scattered areas within a zone ½–1 mile wide that extends north from Ten Mile Hill for about 6 miles. Glass probably occurs to the south also but dense vegetation prohibits further observations. The largest (up to 6 cm long) pieces are found at Ten Mile Hill; the average size decreases northwards and eastwards and at the northern limit of the zone of occurrence all the pieces are less than 1 cm long. The glass fragments are scattered in the peaty soil cover, which varies from a few inches to 18 in. in thickness. Many pieces appear broken but there is little evidence of stream-rounding. The shallow depth of occurrence and the lack of abrasion indicate that the glass is no more than a few thousand years old and formed or fell on a surface that has since been only slightly modified.

Within the zone of occurrence there are four rock types, steeply dipping and outcropping in north–south strips (parallel to the zone of distribution). From west to east across the zone these are:

(1) Sheared volcanics, mainly intermediate in composition. Mt. Reade Volcanics (Cambrian).

(2) Well-sorted, coarse-grained quartz sandstone, and conglomerate. Owen Conglomerate (Ordovician).

(3) Dark grey fossiliferous limestone and dark grey shale. Gordon Limestone (Ordovician).

(4) Coarse-grained quartz sandstone. Crotty Quartzite (Silurian).

Darwin glass is found on each of these beds but mainly on the Owen Conglomerate and the Crotty Quartzite. These two formations, together with quartzose rocks from the Silurian and Precambrian, underly more than 50 per cent of this part of the west coast of Tasmania. Glass has been found on the volcanics in only one place (south-west of Darwin) but because it is highly improbable that it has been transported there, this occurrence precludes the hypotheses involving *in situ* origins (for example, fulgurites).

Hills[3] is correct in saying that the glass cannot be slag, because it is within soil that was formed prior to modern occupation and the glass is not at all like the slag from the Crotty smelters (from physical appearance, thin sections and composition). The Mt. Read volcanics do not contain glass.

Table 1. AVERAGE COMPOSITION OF MAJOR CONSTITUENTS IN DARWIN GLASS[2,3] (COL. 1), AOUELLOUL GLASS[13] (COL. 2) AND AUSTRALITES[15] (COL. 3)

	1	2	3
SiO_2	87·98	86·51	73·45
Al_2O_3	7·04	5·76	11·53
Fe_2O_3	0·36	1·30	0·58
FeO	1·54	1·59	4·05
MgO	0·76	0·91	2·05
CaO	0·11	0·73	3·50
Na_2O	0·11	0·14	1·28
K_2O	1·07	2·05	2·28
TiO_2	0·78	0·55	0·70
Σ	99·75	99·54	99·42

A preliminary age determination has been made on a typical sample through the courtesy of Dr. W. Compston. The total rubidium content was 75 p.p.m., total strontium was 15 p.p.m., and the strontium-87/strontium-86 ratio was 0·810. Assuming an initial ratio of 0·710, an age of 450 × 10^6 years is obtained. Until similar work is carried out on possible parent materials the significance of this age is obscure.

Four chemical analyses have been published[2,3] and the average of these is given in Table 1. The average of two analyses[13] of the glass from Aouelloul Crater in the Western Sahara is included in Table 1. Campbell Smith and Hey[13] have directed attention to the remarkable similarity of these two glasses, both in form and composition. The black and white glasses from Wabar[16] are also analogous in composition and the black glass is very similar in appearance to Darwin glass. Further analyses of the major constituents are being carried out by Dr. H. B. Wiik, and a full discussion will be given elsewhere.

Determinations of trace elements, and some major constituents on eight samples have been carried out, and averages of these data are presented here. Full details of the sample preparation and analytical methods will be given elsewhere. Care was taken to ensure that the samples were clean, and to avoid contamination during crushing. The glass was quite friable, and was disintegrated readily in an iron–manganese steel mortar, followed by grinding, to pass 120-mesh bolting cloth, in an agate mortar.

The elements were determined by emission spectrography using the general procedures described by Taylor[14,15]. Analyses for the alkali elements were carried out with a large Hilger glass prism spectrograph, using Kodak *IN* plates, and sodium as a variable internal standard. Sodium and potassium were determined using a Perkin Elmer model 146 flame photometer. The remaining elements were determined using a Jarrell-Ash Ebert grating spectro-

Table 2. ABUNDANCE OF ELEMENTS IN DARWIN GLASS COMPARED WITH DATA FOR AUSTRALITES[15], SANDSTONE[17] AND CHONDRITIC METEORITES[15]. THE ANALYTICAL PRECISION OF THE DARWIN GLASS DETERMINATIONS, EXPRESSED AS A RELATIVE DEVIATION, IS ALSO GIVEN

		Darwin glass						
		Overall average	Group I	Group II	Anal. precision (%)	Australites[15]	Sandstone[16 17]	Chondrite[16]
p.p.m.	Ba	335	350	330	8·1	630	—	3·4
,,	Co	—	24	<3	5·0	15	0·3	800
,,	Cr	116	188	92	5·8	77	9	2200
,,	Cu	9·0	14	7·5	10	8·1	4	90
%	Fe	1·94	2·49	1·76	3·4	3·56	0·38	25·1
,,	K	1·50	1·56	1·48	0·33	1·89	1·07	0·082
p.p.m.	Li	3·7	4·9	3·3	9·1	42	15	2·7
%	Mg	0·46	0·70	0·38	5·9	1·24	0·70	14·4
p.p.m.	Mn	135	250	97	6·4	770	400	1900
,,	Na	370	520	320	1·8	9500	3300	6700
,,	Ni	122	184	100	3·8	29	2·0	13400
,,	Rb	78	105	70	7·9	81	60	4
,,	Sc	4·1	4·3	4·0	14	18	1	9·4
,,	Sr	<10	<10	<10	25	210	20	10
,,	Ti	3600	3640	3590	2·9	3960	1500	790
,,	V	29	34	27	6·6	78	20	39
,,	Y	30	41	26	18	—	40	—
,,	Zr	385	450	365	4·6	400	220	33
	Ni/Fe (× 10^4)	62	74	58	—	8·2	5·3	534
	Cr/Ni	0·95	1·02	0·95	—	2·7	4·5	0·16

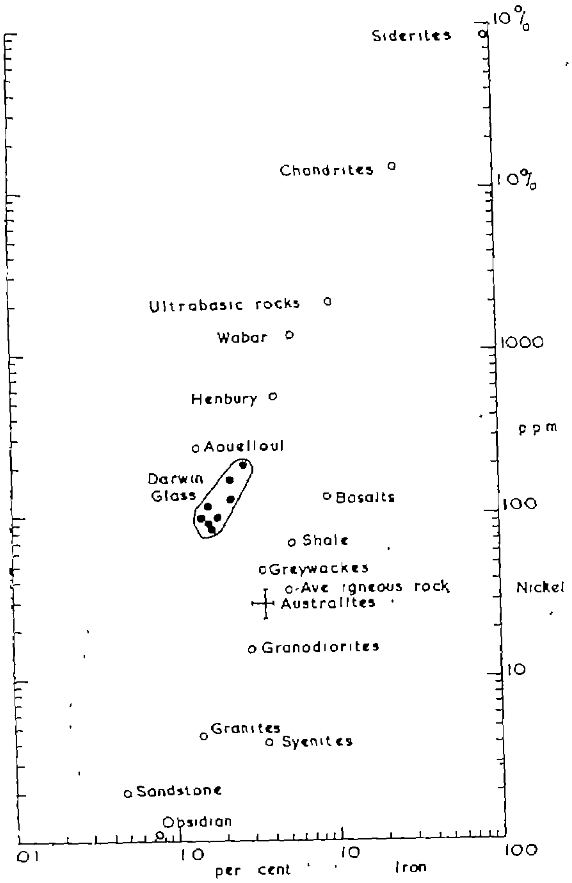

Fig. 1. The relationship between iron and nickel for Darwin glass, meteorites[18], australites[15], impact glasses[9,13 18,19] and common terrestrial rocks[16,17]

graph. The wave-length range from B 2497 to Ba 4934 was recorded on Kodak 103-0 plates, using palladium as an internal standard. The analytical precision of the methods is given in Table 2. Mixtures of the standard granite *G*-1[16], the U.S. Bureau of Standards silica brick 102 and glass sand 165 were used as reference standards.

The results are given in Table 2. The overall average of eight samples is given in the first column, followed by the averages of two distinct groups, the first with two samples and the second with six. The next column gives the analytical precision, expressed as a relative deviation. This is in general satisfactory, but precision falls at low levels, for example, strontium, lithium, scandium, or where substantial corrections for background have to be made, for example, yttrium.

Average compositions for australites[15], sandstones[16,17] and chondritic meteorites[18] are given for comparison. The trace element data show a general similarity with that for sandstones, except that sodium is lower, and iron, and, in particular, nickel and chromium (and cobalt in group I), are significantly higher in the glass. The presence of high concentrations of titanium and zirconium is to be expected in sandstones containing resistant minerals such as rutile and zircon. The high concentrations of iron and nickel are consistent with a meteoritic impact.

The comparison with australites is interesting. Potassium, rubidium, copper, titanium and zirconium have about the same concentration. Iron and barium are about half as abundant, and lithium, sodium, strontium, vanadium, manganese and scandium are depleted by larger factors in Darwin glass. These are the sort of changes to be expected with an increase in silica content of about ten per cent. Nickel, chromium, and, in group I, cobalt, are markedly more abundant in Darwin glass compared with the australites.

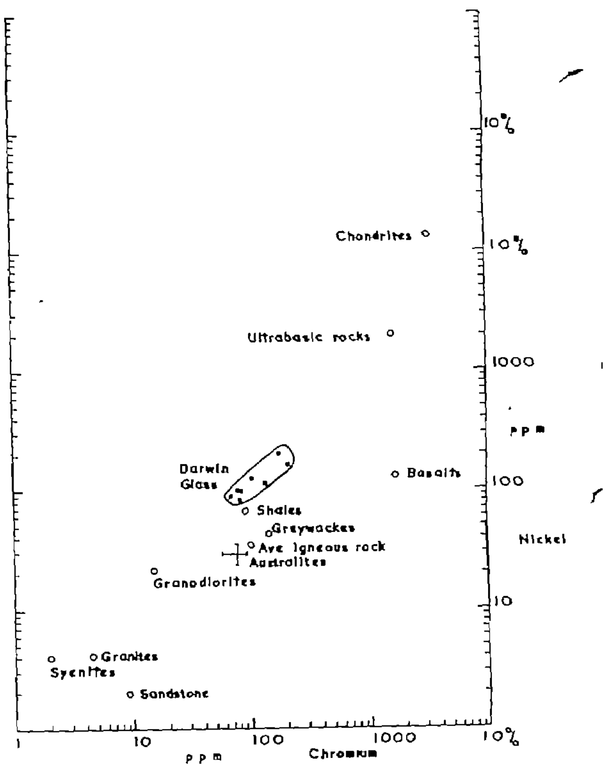

Fig. 2. The relationship between nickel and chromium for Darwin glass, meteorites[18], and common terrestrial rocks[16,17]

The relationships of iron, nickel and chromium in Darwin glass, in australites, meteorites, and a variety of terrestrial rocks are shown in Figs. 1 and 2. These diagrams clearly indicate the distinctive composition of the glass for these elements. The iron–nickel diagram (Fig. 1) illustrates the similarity with the undoubted impact glasses from Henbury and Wabar[19], and the probable impact glass from Aouelloul[9]. Data on chromium and other trace elements are not yet available for these samples.

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MOISTURE EXPANSION OF A CERAMIC BODY AND ITS INTERNAL SURFACE AREA

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NORRIS *et al.*[1] have shown that for an earthenware-type body fired above the temperature of the moisture expansion peak at 1,000° C, moisture expansion and internal surface area follow parallel courses, but below 1,000° C they move in reverse directions. A similar relationship exists for the fired kaolinite-hydrous mica-quartz clay (*A* and *D*, Fig. 1) which I have recently described[2]. Norris *et al.* state that a detailed study of the cause of this relationship would throw valuable light on the mechanism of moisture expansion. It can possibly be explained as set out here, but other problems then arise.

A difficulty in comparing the moisture expansion of a ceramic body with its internal surface area is to differentiate between the internal surface area that is associated with active surfaces and that which is associated with inactive surfaces under the appropriate exposure conditions. Thus the large surface of low-burnt bodies (burnt below 900° C) is not active to water vapour in the air but is active to steam at pressure[3]. For bodies fired to high temperatures the small surface area is not active to air, or to steam initially, but in the autoclave active surfaces are generated by the leaching action of the steam on glassy materials[3]. A peak in the curve of moisture expansion against firing temperature for natural exposure and for steam exposure arises from the formation of an active phase in the bodies on firing at the temperature of the peak. From my results[2,4] this active phase is an amorphous aluminosilicate. Thus the surfaces to be sought are those developed on the formation of this material. They may be found by calculating surface areas from pore size distributions.

The pore size distribution of the kaolinite-hydrous mica-quartz clay recently described by me[2] has been given by Hill[5] (Brick Clay No. 2), and the distributions corrected for ink bottle pores by use of the retention factor as suggested by Hill[5] are plotted against firing temperature in Fig. 2. The corrected pore size distributions are used because Hill has shown that the

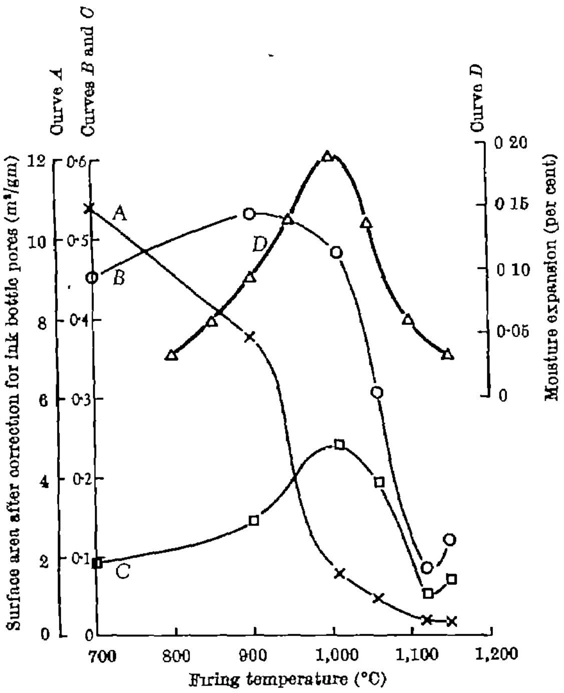

Fig. 1. Surface area and moisture expansion versus firing temperature for a kaolinite-hydrous mica-quartz clay. *A*, surface area calculated from the volume of all pores after correction for ink bottle pores. *B*, surface area calculated from the volume of pores with diameters between 0·77μ and 0·08μ after correction for ink bottle pores. *C*, surface area calculated from the volume of pores with diameters between 0·77μ and 0·2μ after correction for ink bottle pores. *D*, natural moisture expansion after 90 days (undried specimens)

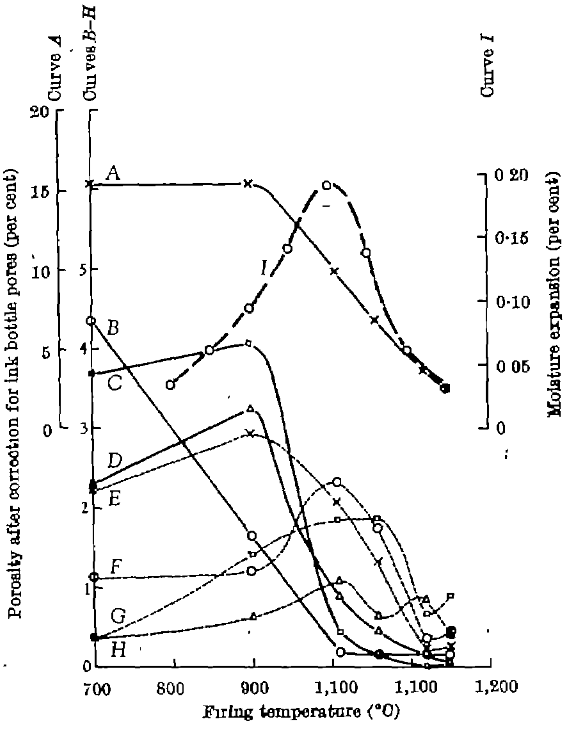

Fig. 2. Porosity (after correction for ink bottle pores) and moisture expansion versus firing temperature for a kaolinite-hydrous mica-quartz clay. *A*, total porosity; *B*, pores with diameters between 0·02μ and 0·01μ; *C*, pores with diameters between 0·04μ and 0·02μ; *D*, pores with diameters between 0·08μ and 0·04μ; *E*, pores with diameters between 0·2μ and 0·08μ; *F*, pores with diameters between 0·4μ and 0·2μ, *G*, pores with diameters between 0·77μ and 0·4μ; *H*, pores with diameters between 1·66μ and 0·77μ; *I*, natural moisture expansion after 90 days (undried specimens)

Table 1. SURFACE AREAS CALCULATED FROM PORE SIZE DISTRIBUTIONS AFTER CORRECTION FOR INK BOTTLE PORES, NUMBER OF POSSIBLE LAYERS OF WATER MOLECULES ON THE SURFACES, AND NATURAL MOISTURE EXPANSION AFTER 90 DAYS FOR A KAOLINITE-HYDROUS MICA-QUARTZ CLAY FIRED FROM 800°–1,100° C.

Firing temperature °C	Total surface area m^2/g	No. of layers of water molecules on total surface		Surface area from pores 0·77μ–0·2μ diameter m^2/g	No. of layers of water molecules on surface of pores 0·77μ–0·2μ diam.		Natural moisture expansion after 90 days	
		Sample undried	Sample dried at 110° C		Sample undried	Sample dried at 110° C	Sample undried per cent	Sample dried at 110° C per cent
800	9·14	5	2	0·10	436	165	0·034	0·016
900	7·50	4	1	0·14	219	79	0·093	0 072
1,000	1·92	12	8	0·24	94	25	0·190	0·136
1,100	0·48	10	4	0·10	48	21	0 062	0 053

total surface areas calculated from them are in good agreement with surface areas obtained by gas sorption methods. If natural moisture expansion is directly related to surface area over the firing temperature-range 800–1,150° C, then the pore volumes most likely involved are those with diameters between 0·77μ and 0·2μ (Fig. 2). Of the other pore volumes those with diameters between 1·66μ and 0·77μ have a complex distribution with firing temperature, but they and larger pores (not plotted) make a negligible contribution to surface area. Pores of diameters less than 0·2μ have the greatest contribution to surface area, but they tend to be a maximum at a firing temperature of about 900° C and are more likely to be associated with the development of metakaolin and mica anhydride than with the breakdown of these phases and the formation of amorphous matter at 1,000° C. However, it is possible that an unknown proportion of the pores with diameters between 0·2μ and 0·08μ, which develop in the body on firing at 1,000° C, is directly related to moisture expansion.

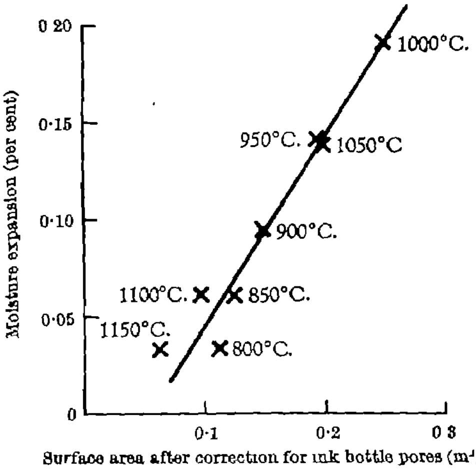

Fig. 3. Natural moisture expansion after 90 days (undried specimens) versus surface area calculated from the volume of pores with diameters between 0·77μ and 0·2μ (after correction for ink bottle pores) for a kaolinite-hydrous mica-quartz clay fired from 800–1,150° C. Firing temperatures are indicated

The results of computing the surface areas from the corrected pore size distributions of the volume of pores with diameters between 0·77μ and 0·2μ, and between 0·77μ and 0·08μ are given in Fig. 1. The surface areas are small but they rise to a peak at about the same temperature as that at which moisture expansion is a maximum, and like it fall to lower values at higher and lower firing temperatures. Best correspondence is found for pores with diameters between 0·77μ and 0·2μ (Fig. 3). In this way a possible explanation can be given to the problem posed by Norris *et al.*[1].

If surface area is related to moisture expansion it should be possible from the increase in weight that accompanies expansion to calculate the number of layers of water molecules on the surface (assuming that the weight increase is produced by water). The results of these calculations, based on one molecule of water covering a surface area of $10·8 Å^2$, are given in Table 1. If the surface area due to pores with diameters between 0·77μ and 0·2μ is related to moisture expansion, then the associated water is more likely to be combined chemically than held by any sorption process (either physical adsorption or chemisorption) on the surface, that is, it has reacted chemically with the body. On the other hand, if it is the total surface area that is related to moisture expansion there is no simple relationship between them. However, the number of layers of water molecules on the surface could arise from the water being physically adsorbed and/or chemisorbed, and is less indicative of a chemical reaction having taken place.

The interpretation given to the mechanism by which water is adsorbed during moisture expansion was suggested by R. D. Hill.

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EROSION OF TIDAL FLATS NEAR GEORGETOWN, BRITISH GUIANA

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THE coastal plain of British Guiana, which is underlain by the Demerara Clay Formation, is a low swampy area at or slightly below mean sea-level. It is mainly an accretional coast which has been built up in recent and present-day times by the accumulation of mud flats. Locally, the deposition of mud by longshore currents and wave action is temporarily interrupted by erosional periods, and this note

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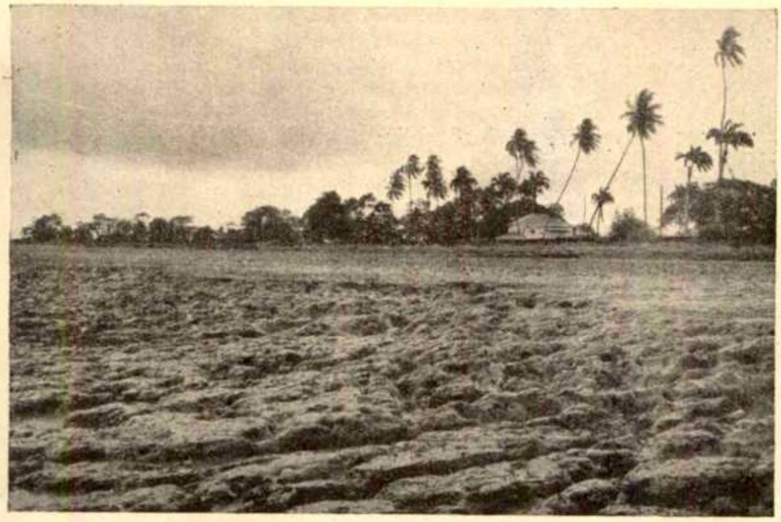

Fig. 1. The zone of intensive potholing and backwash channelling (foreground) in the tidal flat at Georgetown. In the middle distance the low cliff step and the clay platform of the upper tidal flat are seen

summarizes some of the processes of erosion of tidal flats near Georgetown. These processes contrast the commonly described methods of marine erosion in that they are most effective under conditions of calm rather than stormy weather.

A typical shore profile (Fig. 1) consists of a narrow landward zone of salt marsh vegetation, an upper tidal flat which is covered by about one foot of water at high tide, a low cliff step and then a wide mud flat extending to low-water mark. The cliff step, which marks the present limit of erosion, is formed of a stiff bluish clay and varies from one to three feet in height. Constant attack of this step takes place at high-tide by wave action facilitated by shrinkage and cracking of the clay after periods of drying during low-tide. Its retreat is most rapid in the short rainy season (December–mid-February) when the prevailing north-easterly trade winds are accompanied by heavy squalls and storms of short duration. But even in storm conditions the large expanse of mud tends to dissipate the erosive power of the waves before they reach the step. However, during the long periods of tropical calm, active erosion takes place by processes which, although less spectacular, are just as effective in the aggregate. The most potent of these is the drilling of potholes (Fig. 2) immediately beneath the cliff step.

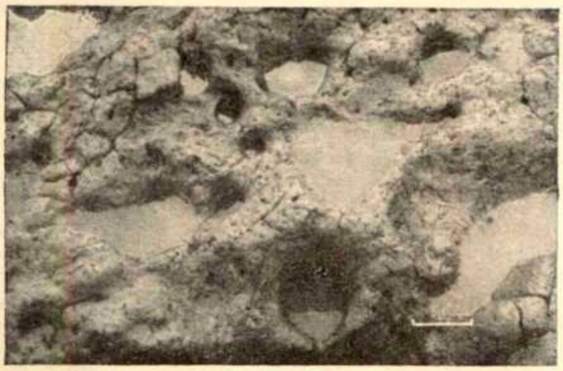

Fig. 2. Shoreline potholes formed in soft mud on the tidal flat at Georgetown. Note mud ball above the scale. Scale represents 12 in.

The formation of these shoreline potholes[1], which are essentially of the same type as river potholes, depends on the presence of a dried or partially dried clay block that is too large to be moved any distance by ordinary waves and breakers. Water swirls around this obstruction and excavates a shallow depression in the underlying soft mud into which the block sinks. It thus becomes securely fixed and can be dislodged only by exceptionally large waves or breakers. The swash and backwash of waves swirling around the obstruction impart a rotational movement to the block and the drilling of a pothole commences. In the initial stages the clay block fits tightly in the hole but later it becomes smaller and rounder (often attaining a high degree of sphericity) while at the same time the pothole becomes wider and deeper. When the pothole is well formed the included mud ball, now considerably smaller and lighter, may be lifted from the hole and thrown on to the upper tidal flat. There it may be rolled to and fro and acquire an armour, which consists mainly of hardened mud flakes. The sediment eroded from the pothole is carried away in suspension by wave-generated longshore and tidal currents, in the present case to be deposited as a mud bank some distance from the shore. Since appreciable changes in the topography of the floor may be observed even within a single 12-h intertidal period it is apparent that the drilling of potholes is a rapid and powerful method of erosion. It reaches its greatest intensity in a strip about fifty feet wide immediately beneath the cliff step. Here there is an abundant supply of fallen blocks and also the abrupt step in the tidal flat which effectively changes oscillatory waves into translation waves to create a zone of turbulence.

A large expanse of the seaward side of the clay platform (upper tidal flat) above the cliff step is strewn with armoured mud balls (Fig. 3), which vary in maximum diameter from one to ten inches. The deposit is poorly sorted and although practically all are rounded or sub-rounded only a few examples approach the perfect spherical form. The mud balls have their origin in clay blocks freshly broken from the cliff step, that are light enough to be carried over the step and transported shorewards by the swash of waves. A few highly spherical balls, as mentioned here, reach the upper tidal flat already rounded by pothole abrasion. Once on the tidal flat the blocks are rolled backwards and forwards by the swash and backwash during high tides. The balls of soft mud acquire their armour from clay flakes formed by the desiccation of the upper tidal flat at low tide.

Although they are formed in a similar manner, these mud balls differ from those described by Bell[2] in that they occur in a littoral marine environment and have an armour composed of the same material as the core. A few which are located near a shell deposit may have an outer rim partly composed of shells but the majority are perfectly homogeneous in composition and seldom contain a single foreign fragment in their armour. Further, Bell has concluded that armoured mud balls are most effective as vehicles for the transportation of bedload. In contrast to this

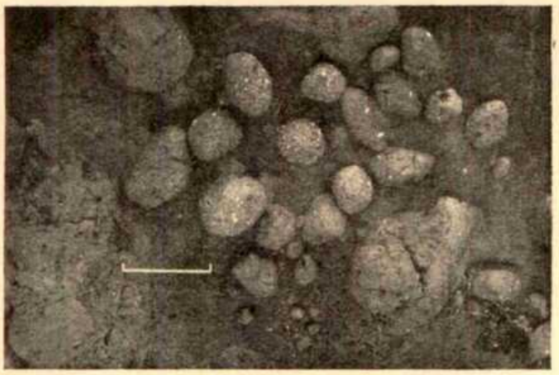

Fig. 3. Armoured mud balls. Although in the photograph shells are conspicuous these foreign fragments are subordinate to flakes of dried clay. Usually the armoured mud ball is perfectly homogeneous in composition. Scale represents 12 in.

the mud balls under discussion are powerful agents of erosion rather than transportation. Most of the armoured balls are stranded by the ebb tide and under favourable drying conditions they harden and develop an appreciable abrasive power. While in motion during high tide, especially when dragged seaward by the backwash, they tend to follow and accentuate furrows and channels in the clay platform. Some are rolled down the cliff step, where they fall into pre-existing potholes or are thrown back up again by later waves. Others, with the additional weight of their acquired armour, may attain the critical size to prevent them being carried back to the shore and will initiate new potholes.

The armoured mud balls will be preserved in the geological column only if they are completely buried by a sudden influx and deposition of sediment. This is not uncommon on tidal flats where rapid changes in the sites of erosion and deposition do occur. Th origin of the resulting deposit may not be immediatel obvious since the clay balls or nodules may b enclosed in a clay matrix of identical composition This communication is believed to be the first to sho the association of mud balls and the erosion of potholes; nevertheless mud balls from similar environments in the Mississippi Delta[3] and especially on th coast of Surinam[4] have recently been described. O the tidal flats near Georgetown clay beds which hav been deposited in historic times contain armoure mud balls. It need scarcely be added that any attemp to estimate the distance of the source materia utilizing measurements of roundness[5] or sphericity would, in the case of mud balls formed during th erosion of potholes, lead to very misleading results.

It should be stressed that the formation of shorelin potholes and mud balls is effective only in periods o calm weather since large waves and breakers ar capable of carrying the clay debris away. In tropica and sub-tropical regions where long periods of cal prevail, the spectacular methods of erosion which ar characteristic of the stormy temperate regions ma be subordinate to calm weather erosion. Certainly o the tidal flats which have been described it is apparen that the drilling of potholes is a major, if not th predominant, process of erosion. It is probable tha calm weather erosion, especially of estuarine an littoral clays, is of more importance in these region than has hitherto been realized.

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CONFIGURATION OF ALGINIC ACID IN MARINE BROWN ALGAE

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DURING the investigations proceeding in this laboratory into the variability of the structural polysaccharides of marine algae, we have encountered species among the Phaeophyceae containing crystalline compounds in addition to the cellulose which might be expected to form the basic skeletal polysaccharide[1]. It is well recognized that cellulose forms quantitatively only a minor component of brown algae[2]. Of the other known major constituents of these plants three can be considered in attempting to identify the additional crystalline components: (*a*) the alginates (salts of polyuronic acids); (*b*) laminarin (a β-1,3-linked glucan); (*c*) fucoidin (a polysaccharide sulphate ester) all present in variable amounts and proportions according to species, season of the year, habitat, etc.[3], but often in great excess over the cellulose. Of these, only the first two, namely, the alginates and laminarin, are known to be crystalline when precipitated from solution. They then yield X-ray diagrams containing reflexions which we also find, identical in disposition and relative intensities, in the diagrams given by t plants themselves. These plants, therefore, can co tain molecular chains of alginic acid and of laminar in orderly arrangement, and the occurrence, distrib tion and order of each of these compounds may examined by standard techniques. The wo described here is confined to the alginates, and t main technique utilized has been X-ray diffracti analysis, using copper *K*α radiation collimated to beam 0·25-mm diameter in a flat-film camera.

Alginates and alginic acid have been subjects intense investigation, largely on account of th commercial value. The chemical constitution of the polyuronides is still not completely known. In t middle 1940's, when Astbury[4] was obtaining crystallographic data, the main constituent w thought to be poly-*d*-mannuronic acid, a β-1,4-link chain polymer[5], and the X-ray diagram was attribut to this substance. In 1955 Fischer and Dörfel[6] fi proved the presence, often in large quantity, another polyuronic acid, poly-*l*-guluronic aci together with small amounts of polyglucuronic acid. They found that the two major polyuronic acids vary in proportion from 1 : 2 to 2 : 1 among the 22 species of brown algae they examined. The presence of polyguluronic acid in alginic acid has since been confirmed by other investigators[7], and the most recent work shows this acid also to be 1,4-linked[8].

Our own interest in the alginates lies chiefly in their organization within the plants and it is from this point of view that we are examining their properties. We have, however, of necessity become involved in considerations of the crystal structure of extracted alginic acid and these have yielded some unexpected results to which we shall direct attention.

Chemically untreated stipes of *Himanthalia* yield X-ray diagrams with very diffuse reflexions which are difficult to evaluate. The most intense reflexion takes the form of a broad ring or arc ranging in spacing from about 6·6 Å to about 8·3 Å (Fig. 1*a*), often with a sharper reflexion at about 8·9 Å. After washing the material in dilute hydrochloric acid a different diagram is obtained with much sharper reflexions, corresponding to markedly different spacings (Fig. 1*b*), closely similar to the diagram reported in the literature as arising from alginic acid and clearly referring to this substance. If the acid-treated material is now subjected to prolonged washing in hard tap water, or is treated with calcium hydroxide solution, the original diffuse diagram is again obtained and the material can be taken back and forth from one condition to the other by repeating these treatments in turn. On the other hand, prolonged treatment either of the original or of the acid-washed material with dilute alkali or sodium carbonate solution merely causes the corresponding diagram to disappear, leaving revealed the hitherto masked diagram of the cellulose. We conclude that the crystalline material in untreated specimens of *Himanthalia* stipes is a salt of alginic acid containing one or more divalent cations, among which Ca^{++} probably predominates. This recalls the statement made by Thiele and Andersen[9] (based on somewhat obscure arguments from the optical properties of sections) that the alginates in the Fucales and the Laminariales are ordered in the optical sense and are salts of predominately multivalent cations. Our treatment with acid and with calcium hydroxide must then convert the calcium salt to alginic acid and back again, in harmony with the change in swelling behaviour of brown algal tissues reported by Wasserman[10]. The presence of crystalline calcium alginate in these plants is further supported by comparison of the X-ray diagrams of untreated stipes of *Alaria* (Fig. 2*a*) with that of commercial calcium alginate fibres (Fig. 2*b*). Fig. 2*a* illustrates the common condition in brown algae that arcs attributable both to the alginate and cellulose (particularly the meridional arc at 5·25 Å $(10\bar{1})$) are present in the diagram. Calcium alginate is reputedly badly crystalline, as exemplified in Fig. 2, and it is not therefore surprising that our untreated material gives such diffuse diagrams. The faint possibility that the alginate may have become crystalline on drying need not be considered since the diagram of Fig. 2*a* was obtained from a fresh specimen which had never been allowed to dry before examination and which was maintained in an atmosphere of 98 per cent relative humidity during exposure to the X-ray beam.

In order to determine how widespread crystalline alginates may be as a constituent of brown algae, we have examined a number of species spread over various orders. As shown in Table 1, the great majority of these plants yield a diagram containing reflexions which may be referred to calcium alginate, with intensities relative to those of the cellulose reflexions varying presumably according to the content and degree of crystallinity of the alginate. Most of the plants were collected during the summer from various localities along the coast of Britain, at a time when the alginate content is known to be minimal. It should, however, be noted that the members of the Laminariales and the Fucales, normally so rich in alginates as to be used commercially, are the plants yielding the strongest alginate diagram.

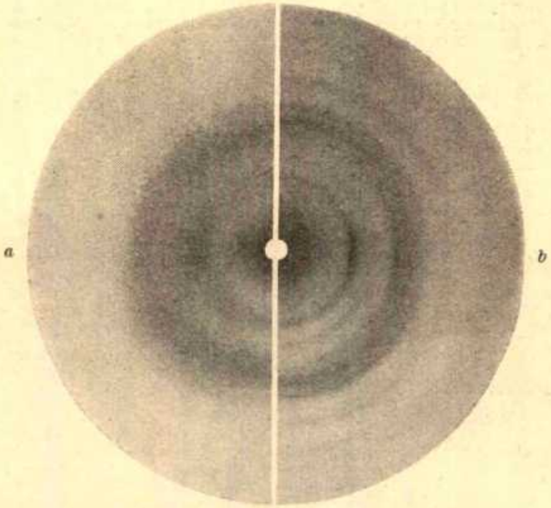

Fig. 1. X-ray diagrams of air-dried *Himanthalia elongata* stipe. Stipe axis vertical, *a*, rinsed with water; *b*, washed with hot 2 per cent hydrochloric acid

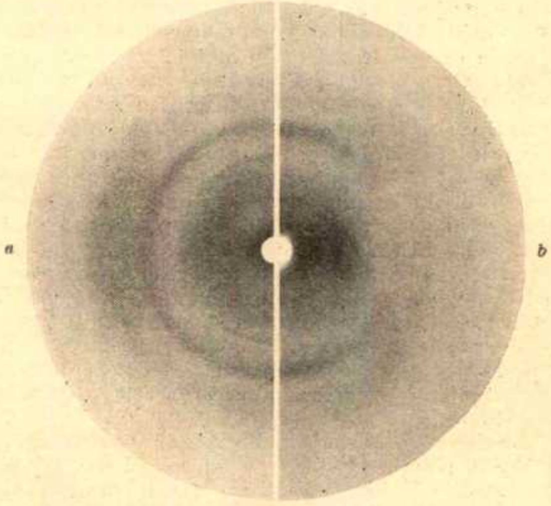

Fig. 2a, X-ray diagram of fresh *Alaria aesculenta* stipe, stipe axis vertical, maintained during exposure in an atmosphere at 98 per cent relative humidity; *b*, X-ray diagram of a bundle of commercial calcium alginate fibres, axis vertical, maintained during exposure at 98 per cent relative humidity

The treatment usually applied in the fractionation of wall polysaccharides (hot water for 12 h followed by chlorination for 4 h, to produce the so-called holocellulose) does not as a rule change the diagram of the material though the cellulose reflexions are enhanced since the background scatter is reduced. Considerable amounts of alginic acid are nevertheless

Table 1. RELATIVE INTENSITIES OF THE X-RAY DIAGRAMS OF ALGINATE AND ALGINIC ACID RESPECTIVELY IN UNTREATED AND ACID-WASHED MATERIAL OF THE BROWN ALGAE INVESTIGATED

		Untreated (alginate)	Acid-washed (alginic acid)
Ectocarpales	*Pylaiella littoralis* (L.) Kjellm.	0	0
	Elachista fucicola (Vell.) Aresch.	0	0
	Chordaria flagelliformis (Müll.) Ag.	weak	medium
Scytosiphonales	*Scytosiphon lomentaria* (Lyngb.) Endl.	weak	medium
Cutleriales	*Cutleria multifida* (Sm.) Grev.	weak	medium
Laminariales	*Chorda filum* (L.) Stackh.	strong	strong
	Laminaria digitata (Huds.) Lamour. Holdfast	medium	strong
	Stipe	strong	strong
	Frond	weak	medium
	Alaria esculenta (L.) Grev. Stipe and midrib	strong	strong
	Lamina	weak	medium
Sphacelariales	*Cladostephus spongiosus* (Huds.) Ag.	0	?
Dictyotales	*Dictyota dichotoma* (Huds.) Lamour.	weak	medium
Fucales	*Fucus serratus* L.	variable	variable
	Pelvetia canaliculata (L.) Done. et Thur.	strong	strong
	Himanthalia elongata (L.) S. F. Gray.	strong	strong

extracted both by the water and by chlorination as revealed by paper partition chromatography of the acid hydrolysates. The holocellulose still reacts to dilute acid as did the original material and the effect is again reversible. As an example we illustrate in Fig. 3 the relevant X-ray diagrams of *Chorda*. Extraction of the alginates from the holocellulose of *Chorda*, or of any of the plants listed in Table 1, using the standard method[11], gives a residue with an X-ray diagram of cellulose only (Fig. 4*d*), and acidification of the extract invariably gives a precipitate which yields again the X-ray diagram of alginic acid (Fig. 4*c*). Strangely enough, the alginic acid recovered from extracts of previously untreated plants yields a whole range of new diagrams different in detail among the species even though with a strong family resemblance. In attempting to resolve this apparent paradox, the nature of the alginates removed by water and by chlorination is clearly of critical importance.

The alginic acids recoverable from the water extract and the chlorite extract (exemplified here with *Himanthalia* receptacles) yield two different X-ray diagrams (Figs. 4*a* and *b*) which may be compared with Fig. 4*c*, the classic alginic acid diagram. If, for the moment, we consider the material yielding the diagrams in Figs. 4*a* and *c* as two different pure substances, then the material giving the diagram of Fig. 4*b* could be interpreted as a mixture of them. This has been verified by showing that, when equal amounts of the two putative pure substances are mixed as powders, then the X-ray diagram of the mixture is indistinguishable from that presented in Fig. 4*b*. When, however, the mixture is dissolved as sodium alginate and the alginic acid is reprecipitated, the precipitate now gives a diagram in which only the inner strong ring of Fig. 4*c* can be clearly recognized. Only after a brief heating in water, or dilute hydrochloric acid, at 90° is the diagram shown in Fig. 4*b* regained. Mixtures of the two compounds when precipitated from solution therefore tend to give a diagram which is essentially that of Fig. 4*c*.

All our many different X-ray diagrams of alginic acid extracted from fresh plants can be explained if the materials yielding them are mixtures, in various proportions, of two compounds which individually yield the diagrams represented by Figs. 4*a* and *c*. Since the diagram illustrated in Fig. 4*c* (and Fig. 1*b*) is closely similar to that regarded as standard for alginic acid and referred to polymannuronic acid, with spacings very near to those listed by Warwicker[12] and to those calculable from the unit cell of Astbury[4], then it seems reasonable to assume that the material giving our second diagram, illustrated in Fig. 4*a*, should be polyguluronic acid, the second major component of alginic acid. Paper partition chromatography of the hydrolysates of our alginate fractions by the methods of Fischer and Dörfel[13] do not, however, confirm this view. It does verify the fractioning we believe we have achieved but, while the material of Fig. 4*b* gives spots approximately equal in size and density indicating the presence of two uronic acids, the material of Fig. 4*a* gives a prominent spot for mannuronic acid, with only weak indications of the presence of guluronic acid, and that of Fig. 4*c* yields guluronic acid with only a little mannuronic

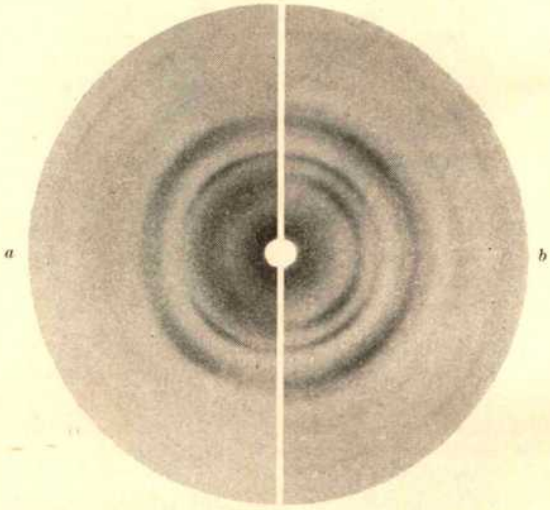

Fig. 3. X-ray diagrams of a bundle of air-dried cortical tissue from *Chorda filum*; cell-length vertical. *a*, After treatment with hot water and chlorination (holocellulose); *b*, holocellulose after washing in cold 2 per cent hydrochloric acid

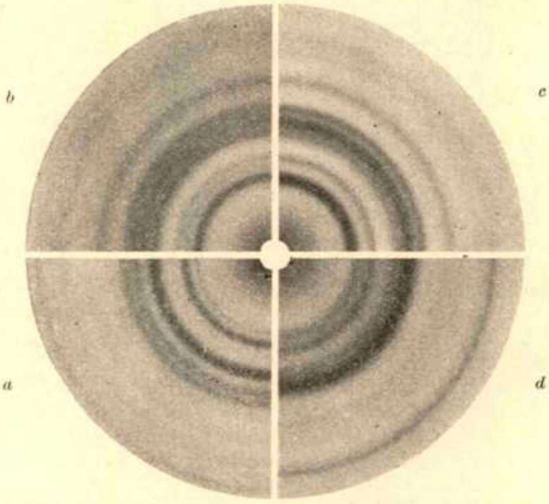

Fig. 4. X-ray diagrams of pellets of *Himanthalia elongata* materials, air-dried. *a*, Alginic acid extracted by hot water; *b*, alginic acid extracted by chlorination of water-extracted material; *c*, alginic acid extracted with sodium carbonate solutions from halocellulose; *d*, residue after complete extraction of alginic acid (cellulose)

acid. Chromatographic separation of the lactones of these three alginic acid fractions reveals, in addition, the presence in all of them of very small amounts of glucuronic acid, which in the chromatograms of the uronic acids runs with the guluronic acid.

We are aware that a quantitative interpretation of these chromatograms must be approached with caution since the considerable losses of uronic acid which occur during hydrolysis may not necessarily be proportional to the concentrations of the various polyuronic acids initially present. There seems, nevertheless, no doubt that the diagram up to now accepted as that of poly-*d*-mannuronic acid is actually that of poly-*l*-guluronic acid. As a further check on this point a commercial preparation of alginic acid giving a mixed X-ray diagram like Fig. 4*b* was fractioned by the method of Haug[14]. According to Haug, the sodium salt of polymannuronic acid is preferentially precipitated from a 0·3 per cent solution of sodium alginate by mixing it in equal volume with a saturated solution of potassium chloride and standing for 1 h. We find that the alginic acid of such a precipitate, which must be polymannuronic acid, gives a diagram like that illustrated in Fig. 4*a*.

We are faced, therefore, with a situation in which a known substance, polymannuronic acid, has a hitherto unrecognized X-ray diagram and a known X-ray diagram refers to an hitherto unrecognized crystalline substance, polyguluronic acid. This confusion is clearly associated with the fact that, in mixtures, crystallization of polyguluronic acid tends to suppress that of polymannuronic acid. The interplanar spacings of these two acids, as we now conceive them, are listed in Table 2 together with those of the cellulose typical of brown algae.

Table 2. X-RAY SPACINGS OF POWDERS OF THE TWO MAJOR COMPONENTS IN ALGINIC ACID IN AN ATMOSPHERE OF 98 PER CENT RELATIVE HUMIDITY AND THE PROMINENT SPACINGS OF CELLULOSE, ALL FROM *Himanthalia elongata*

Polymannuronic acid *	Polyguluronic acid *	Cellulose
6·53 *W*	6·65 *VS*	
		6·10 *M*
5·66 *S*		
	5·26 *M*	5·25 *M*
5·07 *VW*		
4·32 *VS*	4·27 *MW*	4·32 *W*
	3·94 *S*	3·90 *S*
3·81 *M*	3·84 *S*	
	3·61 *M*	
3·56 *M*		
3·33 *VW*		
	3·09 *M*	
2·96 *W*		
2·72 *W*	2·75 *W*	
	2·64 *W*	
2·56 *VW*		2·58 *M*
2·47 *M*		
2·36 *W*	2·39 *VW*	
	2·16 *VW*	

VS, very strong, *S*, strong, *M*, medium, *MW*, medium weak *W*, weak, *VW*, very weak, rings on diagram.
* Some of the spacings vary with water content.

The crystalline alginate found in untreated plants persists as a rule in the holocellulose in which the only polyuronic acid detected is polyguluronic acid. The naturally occurring crystalline alginate must therefore be a salt of polyguluronic acid with multivalent cations. This could not have been decided by X-ray analysis alone since the calcium salts of polymannuronic acid and polyguluronic acid (and indeed of polygalacturonic acid) give diagrams which are very much alike.

The question remains of the location of the polyuronic acids in the plant; are they preferentially in the wall, are they rather in the intercellular mucilage, which in the central tissue (medulla) of the fleshy weeds of the Laminariales and the Fucales is often extensive, or are they, separately or together, spread between the two? The evidence in the literature concerning both *Laminaria* and *Fucus*, based on staining reactions and taking into account the maceration of the tissues when sections are treated with sodium carbonate, is that none[9] or only minor amounts[15] of alginic acid occur in the cell wall, the bulk of it being situated in the intercellular mucilage (regarded[9] as highly swollen primary walls). If this is so, then it is difficult to understand why the cell walls swell appreciably during extraction with sodium carbonate. Our evidence is that the cell walls also contain alginic acid. This evidence comes from several sources.

When two contact X-ray microradiographs are compared of transverse sections of a *Laminaria* stipe, one treated with acid only and one with acid followed by copper acetate solution to form the copper salt of alginic acid, it is very clear that not only the mucilage but also the cell wall is very much more opaque to X-rays in the latter case than in the former. Further, when one of the above copper-treated sections is ashed on a microscope slide, the section, now in the form of a spodogram, appears unchanged under a light microscope, the cell wall being still distinct. Under these conditions a section which has been merely washed in acid leaves no residue. Since suspensions of material from which alginic acid has been removed retain only small amounts of cations, it seems reasonable to assume that the metallic ions were in either case attached to the polyuronic acids in the material and that their distribution therefore parallels that of alginic acid. Finally, thick sections of stipes, or small fragments of fronds, tend to macerate when treated with hot water and sodium chlorite, yet the resulting holocellulose, which must in the main be cell wall material, still contains appreciable amounts of alginic acid as already emphasized. This gives us a pointer toward the relative distribution of polymannuronic acid and polyguluronic acid since the polymannuronic acid is almost quantitatively removed from the material by the treatment. By and large, therefore, polymannuronic acid may be characteristic of the intercellular material, and polyguluronic acid of the wall, at least in the more complex brown algae. This concept is supported by an electron microscope examination of one particular species, *Chorda filum*, the results of which will be reported elsewhere.

Finally, it is of interest to look into the structural interrelationships of polyguluronic acid and cellulose in the walls. The X-ray diagrams of *Chorda* are particularly revealing. According to Pringsheim[16] *Chorda* is unique among the brown algae in that the walls of the cortical cells show spiral striations and spirally oriented slit pits with a strong resemblance to the condition found in conifer tracheids. The X-ray diagram of a flattened bundle of carefully aligned pieces of cortex gives a four-point diagram of cellulose as do some tracheids[17] and fibres[18], confirming the expected similarity of structure between the cells of plants with such remote relationships. The only important differences are that in the *Chorda* diagram the cellulose shows uniplanar orientation, so that the arc at 6·1 Å is faint or missing when the X-ray beam passes normally through the flattened bundle, and that a clear alginate diagram is superposed on that of the cellulose. This is evident in the holocellulose both before (Fig. 3*a*) and after (Fig. 3*b*) washing in dilute mineral acid. The arcs referring to the alginate (Fig. 3*a*) or alginic acid, specifically polyguluronic acid (Fig. 3*b*), diffuse in the former, are aligned with those of the cellulose, showing that the molecular chains of the alginate lie in identically the same helical arrangement as do those of the cellulose. This is not the first time that the polyuronides have been found crystalline and oriented in cell walls, for Roelofsen and Kreger[19] showed some years ago that the pectic compounds in the collenchyma cell walls of *Petasites* are (weakly) crystalline and with marked axial orientation. We have not, however, observed specific orientation either of cellulose or alginate in any other brown alga. We do nevertheless commonly find that the molecular chains of cellulose and alginate lie flat in the wall, parallel to wall surface, so that X-ray diagrams with the beam parallel to wall surface show pseudo-orientation of both, and Figs. 1 and 2*a* are examples of diagrams demonstrating this point.

It is quite obvious that there are here many problems still awaiting solution, including those associated with the quantitative relationships between the polyuronic acids in these plants and with the crystal structure both of polymannuronic and of polyguluronic acid. We intend to continue work along these lines.

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DIRECTIONAL EFFECTS OF ALKALI METAL IONS ON ADENOSINE TRIPHOSPHATE HYDROLYSIS IN ERYTHROCYTE GHOSTS

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ONE of the basic questions in the field of active transport is how the energy of adenosine triphosphate (ATP) is utilized to move ions against concentration gradients. In the analogous problem of muscular contraction, the contractile element is an adenosine triphosphatase, and it is therefore significant that nerve membranes have been shown to contain adenosine triphosphatases which are stimulated by sodium plus potassium and affected by substances that influence transport of sodium and potassium[1]. Similar enzymes are present in many disrupted cells and tissues, and in the presence of sodium plus potassium they are inhibited by glycosides, such as ouabain, which inhibit active ion movements in intact cells[2].

In applying results from suspensions of enzymes to intact cells, regard must be paid to the loss of the different ionic compositions normally found on the two sides of the membrane, for perhaps the most characteristic feature of transport of sodium and potassium by cells derives from this uneven ionic distribution. Thus, membranes select potassium for inward movement from a solution rich in sodium, and sodium for outward movement from an intracellular fluid rich in potassium; these unidirectional ion movements show that membranes are spatially asymmetrical in their affinity for these ions. This asymmetry is preserved in work with reconstituted erythrocyte ghosts, which allow measurements of adenosine triphosphatase activity to be made with different ionic concentrations in ghosts and medium. It has been shown that sodium stimulates from inside and potassium from outside, an effect of one ion being seen only in the presence of the other ion on the opposite side of the membrane[3,4]. Sodium and potassium therefore stimulate intracellular ATP hydrolysis synergically even though they are spatially separated by the membrane; furthermore, they stimulate at the sides of the membrane from which they are transported either inwards (potassium) or outwards (sodium). ATP hydrolysis catalysed by erythrocyte membranes has now been measured in ghosts containing various alkali metal ions in order to study further the dependence of enzymatic activity on the location of the activating cations.

ATP and one of the alkali metal chlorides were introduced into ghosts of human erythrocytes, and a low permeability was regained by allowing the membranes to be reconstituted after hæmolysis[5]. Ghosts containing one of the cations, lithium, sodium, potassium, rubidium or cæsium, were washed in saline media with a variety of ionic compositions, thus allowing the cation composition of ghosts and medium to be varied independently. The suspensions of ghosts (about 0·2 ml. packed ghosts/ml.) were incubated for 1 h at 37° in the presence and absence of ouabain (0·1 mM), and the liberation of orthophosphate from ATP was measured[3].

To find the effect of the internal cation, ghosts were incubated in a sodium–saline medium either potassium-free or containing 10 mM potassium.

The results in Table 1 show that in potassium-free medium, the rate of liberation of orthophosphate (0·99–1·46 µmoles/ml. packed ghosts/h) and the decrease caused by ouabain (0·44–0·61 µmoles/ml. packed ghosts/h) were largely unaffected by the kind of internal alkali metal. In contrast, in medium containing 10 mM potassium, more orthophosphate (in µmoles/ml. packed ghosts/h) was produced than in potassium-free medium in ghosts loaded with lithium (1·99) or sodium (2·32), although in ghosts containing potassium (1·67), rubidium (1·23) or cæsium (1·10) the orthophosphate production was not significantly increased. In the presence of 10 mM potassium in the medium, the part of the orthophosphate production sensitive to ouabain was also greater with lithium (1·44) or sodium (1·61) than with potassium (0·87), rubidium (0·75) or cæsium (0·71) as the predominant internal cation. Rubidium and cæsium therefore qualitatively resemble internal potassium in not stimulating ATP hydrolysis. Internal lithium, however, is like sodium in stimulating ATP hydrolysis when 10 mM potassium was present in the medium. The synergic stimulation due to internal lithium and external potassium was inhibited by ouabain in the same way as that due to internal sodium and external potassium.

The influence of alkali metal ions in the medium was investigated by examining their effect on ATP hydrolysis in ghosts loaded with sodium which were incubated in sodium media containing 10, 38, and 75 mM of lithium, potassium, rubidium or cæsium in place of an equivalent amount of sodium. Control incubations were made in sodium medium not containing other alkali metals. Fig. 1 shows that an increase in the rate of orthophosphate production above the controls was found in the presence of each of the ions, lithium, potassium, rubidium and cæsium, the percentage increases with concentrations of 75 mM being 66 for lithium, 84 for potassium, 53 for rubidium and 65 for cæsium. Although lithium, rubidium and cæsium were not so effective as potassium, they none the less caused a significant stimulation of ATP hydrolysis. Ouabain abolished the increased adenosine triphosphatase activity due to external lithium, potassium, rubidium and cæsium (Fig. 1) just as it did the increased activity due to internal lithium and sodium (Table 1). The replacement of external potassium by rubidium and cæsium is not surprising, for these ions are pumped into human erythrocytes like potassium[6,7], but the stimulation by external lithium is unexpected because lithium does not appear to be actively transported[7]. The finding of stimulation, however, suggests that lithium can replace external potassium as well as internal sodium.

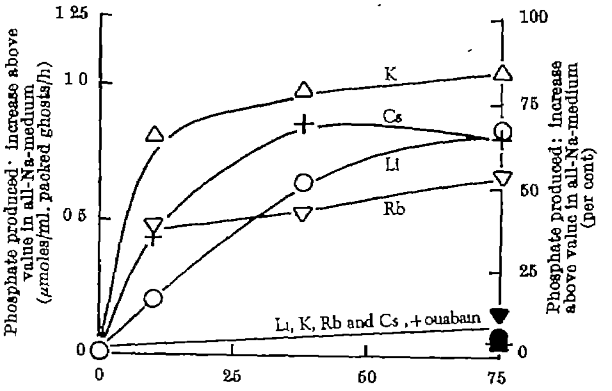

Fig. 1. Stimulation of the membrane adenosine triphosphatase by ions in the medium. A hæmolysate was made by adding 1 vol. of erythrocytes which had been previously washed with 0·15 M choline chloride, to 5 volumes of a solution of 4 mM sodium ATP and 4 mM $MgCl_2$. Sufficient 3 M NaCl was added to restore iso-osmolarity, the hæmolysate was incubated for 30 min at 37° and the ghosts were sedimented by centrifugation. They were washed once in a medium containing 150 mM NaCl, 2 mM $MgCl_2$ and 10 mM *tris* buffer, *p*H 7·6, and then suspended in otherwise similar media containing various concentrations of lithium, potassium, rubidium and cæsium, with sodium making up the alkali-metal ion concentration to 150 mM. After incubation for 1 h at 37° in the presence and absence of 0·1 mM ouabain, orthophosphate was estimated, and the graph shows the extra orthophosphate liberated in ghosts suspended in media containing lithium, potassium, rubidium or cæsium compared with that in ghosts in medium containing sodium as the only alkali metal ion (1·24 ± 0·08 (24) µmoles orthophosphate/ml. packed ghosts/h)

Table 1. THE SYNERGIC STIMULATION OF THE MEMBRANE ADENOSINE TRIPHOSPHATASE BY INTERNAL LITHIUM OR SODIUM AND EXTERNAL POTASSIUM

Orthophosphate produced from ATP (µmoles/ml. packed ghosts/h)

Alkali metal ion in ghosts	K-free medium		10 mM K in medium	
	Control	Decrease in presence of ouabain	Control	Decrease in presence of ouabain
Li	1·11 ± 0·07 (12)	0·61 ± 0·11 (6)	1·99 ± 0·18 (11)	1·44 ± 0·11 (10)
Na	1·24 ± 0·08 (24)	0·59 ± 0·07 (15)	2·32 ± 0·18 (18)	1·61 ± 0·09 (14)
K	1·46 (3)	0·61 (3)	1·67 ± 0·12 (8)	0·87 ± 0·10 (8)
Rb	1·25 (4)	0·60 (2)	1·23 (4)	0·75 (4)
Cs	0·99 (3)	0·44 (1)	1·10 (4)	0·71 (4)

A hæmolysate was made by adding 1 volume of erythrocytes to 5 volumes of a solution of 4 mM sodium ATP and 4 mM $MgCl_2$. Solutions (3 M) of either LiCl, NaCl, KCl, RbCl or CsCl were then added to restore iso-osmolarity. After 30-min incubation at 37°, the ghosts were centrifuged, washed in medium containing 150 mM NaCl, 2 mM $MgCl_2$ and 10 mM *tris*, *p*H 7·6 and suspended in the same medium. In one lot of suspension, M KCl was added to give a final concentration of 10 mM. The suspensions were incubated for 1 h in the presence and absence of 0·1 mM ouabain, and orthophosphate then estimated. *S.E.* are given and the numbers of observations are in parentheses.

In order to test this possibility, ghosts containing either sodium or lithium were incubated in a saline medium containing 150 mM lithium, either potassium-free or containing 10 mM potassium, to see whether addition of external potassium would still stimulate in all lithium medium as it does in all sodium medium. After incubation in potassium-free lithium medium, as much orthophosphate was produced in ghosts containing lithium (2·27) as in sodium ghosts (2·28), and the addition of 10 mM potassium to the medium did not cause a significant increase either with lithium (2·50) or sodium (2·47) ghosts. Further, the part of the rate inhibited by ouabain was about the same for lithium (1·15 and 1·40) and sodium (1·39 and 1·46) ghosts, both in the absence and presence of 10 mM potassium respectively. This result shows first, that the addition of 10 mM potassium in lithium medium did not further stimulate adenosine triphosphatase activity, proving that 150 mM lithium in the medium alone stimulated, secondly, that the same enzymatic activity was observed with sodium and lithium ghosts, and thirdly, that the ouabain-sensitive adenosine triphophatase activity was roughly the same in each condition. It thus appears that internal sodium and lithium stimulate adenosine triphosphatase synergically with external lithium, and that lithium is unlike the other alkali metals in stimulating from both sides of the erythrocyte membrane.

These results show that stimulation of the ouabain-sensitive ATP hydrolysis by alkali metal ions is synergic, and that the stimulation depends on the location of the cations. Thus, internal lithium and sodium stimulate but not internal potassium, rubidium and cæsium; external lithium, potassium, rubidium and cæsium stimulate but not external sodium. The striking fact that the enzymatic stimulation depends on the location of the stimulating alkali metal and that it is inhibited by ouabain, supports the suggestion that the ouabain-sensitive ATP hydrolysis is linked to the movements of the ions across the membrane[1-4]. If such a kind of asymmetrically stimulated ATP hydrolysis is indeed obligatory for active sodium and potassium movements, then unidirectional ion movements could be regarded as steric effects associated with an enzymatic reaction. The details of the possible chemical changes cannot be pursued further until the enzyme or enzymes responsible here have been purified, because the hydrolysis could equally well be due to the action of a phosphokinase and a phosphatase as to a single adenosine triphosphatase[8,9].

Whatever the detailed nature of the reaction, the finding of asymmetrical directional effects of alkali metal ions on intracellular ATP hydrolysis by membranes seems to be of some general significance with regard to the control of metabolic-rates. The rates of glycolysis and respiration are partly determined by the concentration of intracellular ADP, which is controlled in turn by the rates of the reactions hydrolysing ATP[10], one of which is the ouabain-sensitive adenosine triphosphatase of the cell membrane. Active transport by its requirement for ATP would appear to be one of the factors regulating the ADP concentration and hence to exert a feed-back control of the metabolic rates. Indeed, evidence with brain and kidney cortex suggests that some 40 per cent of the energy from respiration is utilized for active sodium and potassium transport[11]. It therefore seems that directional effects of ions regulate the rate of ATP hydrolysis by the endergonic process of active transport and that the latter exerts a feed-back control on the rate of the supply of energy from cell metabolism.

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CHARACTERIZATION OF LIPOAMINO-ACIDS AS O-AMINO-ACID ESTERS OF PHOSPHATIDYL-GLYCEROL

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THE formation of lipoamino-acid complexes has been reported in a number of preparations from tissues and micro-organisms, for example, hen oviduct mince[1,2], *Penicillium chrysogenum*[3], ascites tumour cells[4], liver slices[5], protoplasts of *Bacillus megaterium*[6,7]. In general, this type of complex has been demonstrated after the addition of a radioactive amino-acid to the cellular preparation, in a medium supporting metabolism, by the measurement of radioactivity in a crude or purified fraction extracted with lipid solvents and the subsequent recovery of the unchanged amino-acid after hydrolysis of the lipid fraction. Control experiments with killed preparations or with addition of the amino-acid to the lipid extract showed that the complex formation was not an artefact. Several amino-acids have been shown separately to form such complexes, and it has been suggested from various experimental findings, such as the metabolic lability and the sensitivity to chloramphenicol, that the complexes are concerned in protein synthesis[2,3,7].

Although in one sense therefore the existence of lipoamino-acids is well documented, their chemical constitution has not been elucidated, apart from the facts that the complexes are usually found in the phospholipid fraction and that the amino-acid can be recovered after acid or alkaline hydrolysis. On the other hand, the ready formation of artefacts has been emphasized[8], and investigations of lipids of cells[9,10] and micro-organisms[11] have not shown unequivocally the presence of lipoamino-acids, as distinct from lipo-peptides, proteolipids, etc.; in particular, the phospholipid fraction of the protoplast membrane of a strain of *B. megaterium*[12] and of *Micrococcus lysodeikticus*[13] were virtually free from nitrogen; in the latter organism[14] and in *Staphylococcus aureus*[15] the principal phospholipids isolated were diphosphatidylglycerol (cardiolipin type[16]) or monophosphatidylglycerol (G-P-G lipid).

Recently, however, it has been found that the phospholipid in a batch of *Clostridium welchii* cells was almost entirely bound to amino-acids, and evidence for the constitution of a lipoamino-acid as the *O*-amino-acid ester of phosphatidylglycerol is here presented.

A batch of *Cl. welchii*, type *A*, was prepared by Dr. D. E. Dolby in 30 l. of a non-toxigenic peptone–glucose medium (3 per cent peptone, 0·5 per cent glucose, 0·45 per cent NaCl). The bacteria, for some reason not determined, were not satisfactorily separated by centrifugation or filtration, but settled rapidly on acidification of the medium to about pH 3; the supernatant was siphoned off, and the bacteria were washed with water three times by centrifuging. (This apparently trivial manœuvre of acidification probably accounted for the high yield of lipoamino-acids by the arrest of enzymatic processes in presence of medium.) The packed bacteria (about 40 g dry wt., 600 ml. vol.) were extracted first with 1·6 litres of methanol, and then with 1 l. chloroform/methanol, 1:1 (v/v) by stirring at 20°. The methanol extract was concentrated and the residue dissolved in the second extract, which was then washed twice with 200 ml. 0·3 per cent sodium chloride. The chloroform layer was dried with sodium sulphate, filtered and evaporated *in vacuo*; wt. 620 mg, phosphorus, 1·8 per cent; yield of lipid phosphorus about 280 μg/g dry wt. bacteria. The methyl esters of the mixed fatty acids prepared from a portion of the lipid were identified by Dr. G. M. Gray by gas chromatography as mainly those of even-numbered *n*-saturated acids from C_{10} to C_{20}, predominantly C_{14} (24 per cent) and C_{20} (30 per cent).

The remaining lipid (8·0 mg phosphorus) in light petroleum was fractionated on a column of Mallinckrodt silicic acid (12 g), from which 140 mg of lipids free from phosphorus were eluted with benzene and chloroform. The phospholipids were then eluted with increasing concentrations of methanol in chloroform, collecting 10 ml. fractions. Eluates 9–50, eluted with 2–6 per cent methanol (v/v), were refractionated on magnesium trisilicate to give Fraction *A*, about 1 mg phosphorus, tentatively identified as a mixture of phosphatidylglycerols and phosphatidic acids, and Fraction *B*, a glycolipid (12 mg sugar) giving mannose and a trace of glucose on hydrolysis. Eluates Nos. 51–57 (3·9 mg phosphorus) eluted with 10–15 per cent methanol, Nos. 78–105 (3·0 mg phosphorus), eluted with 20–40 per cent methanol, and Nos. 106–118 (0·3 mg phosphorus), eluted with 50–80 per cent methanol, were pooled as Fractions *C*, *D* and *E* respectively, on the basis of their behaviour on silicic acid paper in diisobutylketone/acetic acid/water, 40 : 20 : 3 by volumes; all the fractions showed a lipid spot approximately in the position of phosphatidylglycerol, the one from Fraction *C* being also ninhydrin-positive, while Fraction *D* showed a separate slower ninhydrin-positive spot.

Fraction *C* was soluble in ether, chloroform and ethanol, sparingly soluble in methanol and light petroleum, insoluble in acetone; atomic ratio phosphorus/amino nitrogen[17] (ethanolamine standard)/total nitrogen, 1 : 1·03 : 1·01; phosphorus 3·5 per cent on dry wt. (calc. for the *O*-alanyl ester of distearoylglycerylphosphorylglycerol phosphorus = 3·6 per cent). On mild alkaline saponification[18] in 0·1 N methanolic sodium hydroxide/chloroform (1:1 v/v) the water-soluble product gave on paper chromatography in butanol/acetic acid/water (5 : 3 : 1 by volumes) or phenol–ammonia a single phosphorus spot corresponding to glycerylphosphorylglycerol (G-P-G) and several separate ninhydrin spots, the main spot corresponding to alanine; after acid hydrolysis, the water-soluble product gave a single phosphorus spot corresponding to glycerophosphate, and on estimation of ethanolamine and serine by treatment with fluorodinitrobenzene[19] the dinitrophenol compounds appeared in the 'serine,' that is, the amino-acid fraction. On treatment of the intact lipid with periodic acid in chloroform/95 per cent acetic acid, 1 : 1 (v/v) at 20°, with batyl alcohol as standard, only 0·1 mole of formaldehyde per mole phosphorus was produced compared with one mole for a sample of phosphatidylglycerol[14]. On thin-layer silicic acid plates developed with chloroform/methanol/water, 85 : 16 : 2, the lipid was resolved into four conjoined phosphorus spots, one very prominent, and corresponding ninhydrin spots, with a fifth ninhydrin spot visible (cf. Hunter and Goodsall[7].)

Fraction *D* was less soluble than Fraction *C* except in chloroform; the ratio phosphorus/amino nitrogen/total nitrogen was 1 : 1·1 : 2·1; phosphorus 3·4 per cent on dry wt. It was resolved similarly into four conjoined slower-moving spots on a thin-layer plate and after saponification gave on paper several ninhydrin spots distinct from those of Fraction *C*, the position of the two main spots approximating to those of lysine and ornithine. Tests for hexosamines were negative.

These facts indicated that Fraction *C* and Fraction *D* consisted of a series of *O*-amino-acid esters of phosphatidylglycerol of the general structure I, in which the ester linkage is arbitrarily assigned to the α^1 carbon of the unacylated glycerol. It was presumed that this linkage, like that of the alanyl ester in teichoic acids[20], would be very labile to alkali. Confirmation of the structure was sought by identifying the products of alkaline digestion, by the examination of infra-red spectra and by the preparation of dinitrophenyl (DNP) lipids.

$$CH_2O.OCR_1.CHO.OCR_2.CH_2O.P{:}O(OH).CH_2O.$$
$$CHOH.CH_2O.OC.CHNH_2.CH_2R_3$$
(I)

R_1 and R_2 = fatty acid residues, R_3 = amino-acid residue

After shaking an ethereal solution of Fraction *C* briefly with cold 0·1 N aqueous hydrochloric acid, the phosphorus and nitrogen were recovered in the ether phase; but on treatment of the lipid with aqueous alkali, followed by acidification and extraction with ether, the nitrogen became water-soluble, the phosphorus remained ether-soluble and, concomitantly with the release of the amino-acid, the lipid became oxidizable by periodic acid with formation of formaldehyde. A slower hydrolysis of the fatty acid residues and possibly of the phosphoric di-ester linkages also occurred, and in time all the phosphorus became water-soluble. It will be seen from the course of some alkaline digestions (Table 1) that in the ether-soluble lipid the ratio of formaldehyde produced on oxidation to the total phosphorus was at the best about 0·75–0·8; a selective decomposition could presumably be achieved enzymatically.

Table 1. DECOMPOSITION OF PHOSPHATIDYLGLYCEROL AMINO-ACID ESTERS IN ALKALI

Exp.	Treatment	Time	Ether phase (μmoles) P	Amino-N	H.CHO*
C 1	N NH_4OH	0	1·61	1·6	0·14
	20°	2 h	1 26	0 2	0·93
C 2	0 05 N NaOH	0	2 06	2·07	0·16
	40°	5 min	2·03	1·14	0 98
		15 min	2 00	0·64	1·22
		30 min	1·58	0·43	1·06
C 3	2·0 ml. ether	0	7·70	7 70	0·50
	1·0 ml. 0·1 N NaOH	10 min	7·10	1·83	5 60
	20°, shaken	30 min	7·20	0·34	4·40
D 1	as *C* 3	0	8·0	7·6	0·92
		30 min	7 4	0 6	5 60

* Formed on oxidation with 0·04 M periodic acid in chloroform—95 per cent acetic acid; estimated colorimetrically with chromotropic acid.

The infra-red spectra in chloroform of Fraction *C* and *D* differ from that of a sample of phosphatidylglycerol isolated from *M. lysodeikticus*[14] (Fig. 1) in the regions 3,100, 2,100 and 1,550 cm^{-1}, indicative of amino-acids, and in the region 1,000–1,120 cm^{-1} (primary and secondary alcohols); the data are not sufficient to assign the free hydroxyl group, especially as migration may have occurred on the silicic acid column.

The DNP derivatives of Fractions *C* and *D* were formed[21] by treating the lipid (4 μmoles) in 2·0 ml. benzene with about 20 μmoles fluorodinitrobenzene and 140 μmoles triethylamine at 30° for 2 h. After evaporation of the solvent, the product was applied in chloroform along the starting line of a thin-layer silicic acid plate which was developed in chloroform.

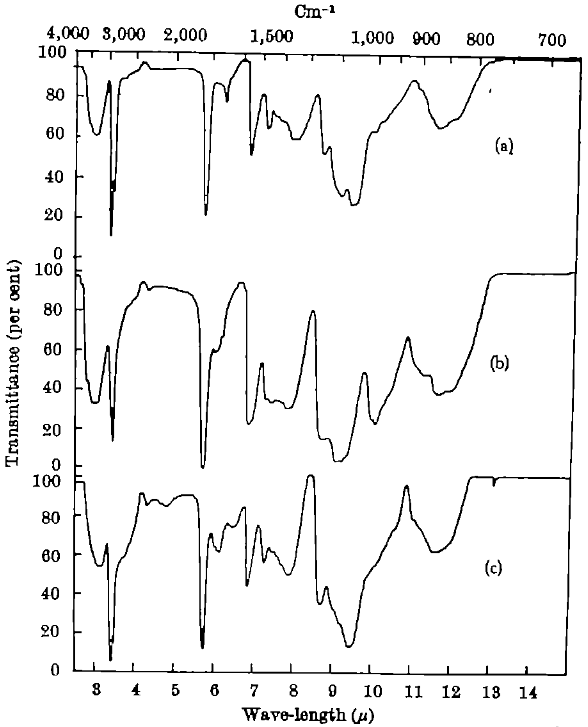

Fig. 1. Infra-red spectra on Perkin–Elmer Infracord 137. Sodium chloride cells, path-length 0·5 mm. Lipids dissolved in chloroform (*a*) phosphatidylglycerol, *M. lysodeikticus*, (*b*) diphosphatidylglycerol, ox heart; (*c*) amino-acid compound, *Cl. welchii*, Fraction *D*

The excess reagent ran with the solvent, and the yellow band at the origin was scraped off and eluted with 10 per cent methanol in chloroform. Fraction *C* DNP-lipid contained 90 per cent of the original phosphorus; ratio phosphorus/nitrogen, 1 : 2·9; λ_{max} in ethanol 342 mμ; ε(calc. on phosphorus content) $16{\cdot}7 \times 10^3$. The DNP-lipid from Fraction *D* contained 86 per cent of the original phosphorus; ratio phosphorus/nitrogen, 1 : 3·7; λ_{max} 345 mμ; ε, $24{\cdot}4 \times 10^3$ (cf. DNP-kephalin[11], λ_{max} 345 mμ, ε, $15{\cdot}5 \times 10^3$). On shaking a chloroform solution of the DNP-lipid with 2 per cent potassium carbonate solution at 20° the colour remained in the organic phase, but after warming the alkaline mixture for a few minutes the colour passed into the aqueous phase and was only re-extracted into chloroform after acidification; there is therefore little doubt that the amino-acid ester bond was intact in the DNP compounds isolated. Attempts to resolve the DNP-lipids into separate components were not successful.

The amino-acids present in the *Cl. welchii* lipids were examined by Dr. Elizabeth Work. The main acid found in Fraction *C* was alanine, and in Fraction *D* alanine, glutamic, aspartate, lysine and possibly arginine or histidine were present. No diaminopimelic acid was found.

A phospholipid fraction from *Staphylococcus aureus*, previously reported[15] as phosphatidylglycerol mixed with a nitrogenous component, was found on re-examination to contain about 60 per cent of amino-acid esters. The earlier failure[14] to find these esters in *M. lysodeikticus* was possibly due to their enzymatic decomposition during washing of the bacteria or protoplasts.

The chemical and chromatographic properties of the amino-acid esters of phosphatidylglycerol are consistent with those of the lipoamino-acids described by Hendler[1] and by Hunter and Goodsall[7], and it seems probable that their formation is a general phenomenon. The existence of phosphatidylglycerol was first recognized by Benson and Maruo[22] in plant lipids; it has also been identified in mammalian lipids[23]. In *M. lysodeikticus* most of the phospholipid of the cell is present in the protoplast membrane, either as phosphatidylglycerol or diphosphatidylglycerol; in mammalian tissues diphosphatidylglycerol is concentrated in mitochondria[24,25]. Both compounds show a rapid uptake of phosphorus-32 in metabolic conditions[23,26,27]; the route of biosynthesis is not known.

The identification of lipoamino-acids as defined compounds of a metabolically active phospholipid present in cell membranes offers some support for the suggestions[2,3,7] that they play some part in transport and, directly or indirectly, in the synthesis of protein; the incorporation of amino-acids into protein by cell fractions by a mechanism distinguishable, by the effect of chloramphenicol or fractionation procedures, from the amino acyl–ATP activating systems has been demonstrated[28,29]. It seems possible that the 'incorporation factor' for the assimilation of amino-acids by bacterial preparations, which can be derived from glycerol[30], might be concerned in such a mechanism through the intervention of a cytidinediphosphate-diglyceride[31].

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AN EXPERIMENTAL EVALUATION OF THE ASSOCIATION OF PREGNANCY AND ACUTE LEUKÆMIA

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THE problem of leukæmia associated with pregnancy has been observed clinically by many investigators in human beings, but has been ignored experimentally except by Burchenal[1], who concerned himself with the problems of leukæmic transmission from the mother to the fœtus via the placenta in mice.

Although the clinical cases are rather isolated, there are several conclusions that have been made by the clinician which seem to be substantiated by a review of the literature. The presence of a placental barrier to the leukæmic factor is well established by the experimental works of Burchenal[1], and through the autopsies and observations made by a number of investigators[2–8]. The facts that no leukæmic mother has delivered a *leukæmic child and that* several cases of congenital leukæmia have been delivered by non-leukæmic mothers[3,12] greatly substantiate this view.

It is felt that leukæmic women do not conceive easily, due to poor physical condition[6], and to the presence of anæmia and amenorrhœa that naturally accompanies the disease, and not due to the actual deleterious involvement of the reproductive system[6]. Although there may be infiltration of the reproductive system[2,10–12], it does not seem to have any great inhibitory effect on conception as evidenced by the occurrence of women who do not have amenorrhœa and who become pregnant some months after contracting leukæmia[2–5,10,13,15], and by the occurrence of the multipara cases[2,8,9,16].

The effect of pregnancy on the course of leukæmia is one of controversy. Many clinicians feel that pregnancy causes exacerbation of the disease[13,15], while *others feel that there is little or no change* when pregnancy and leukæmia are associated[2,5,7,8,10–12,14,16]. Even a third idea is postulated by Gillim[9] that pregnancy may even have an ameliorating effect on the course of leukæmia. With these conflicting ideas in mind we have set out to study experimentally the effect of pregnancy on the course of acute myeloid leukæmia.

The animals used in this study were *C57 Bl/6J* male and female mice. The male mice were used for the transplantable tumour colony and for control animals. The females were used for controls and as the experimental subjects. The tumour used was myeloid leukæmia *C*1498, which is transmissible into the *C*57 black mouse.

In order to maintain a constant supply of transplantable tumour material, a cyclic tumour colony was established. On arrival of an initial supply of the tumour, a group of animals was inoculated (12 animals were required to initiate the cyclic transplantable colony and a number of extra inoculated animals were kept in reserve to assure that a sufficient number of animals would survive for transplanting since the average survival time for the tumour-bearing mouse is between nine and fifteen days). The cyclic colony was initiated 7 days later (the time required to allow the tumour to reach a transferable age) by killing two animals of the group of animals and inoculating two more. This procedure reoccurred each day for six days of the week. The following week the transfers were made from animals inoculated just 7 days previously. In this manner, there was tumour available on six days of the week. In addition, an auxiliary colony of 3–5 animals was carried to be used on the seventh day of the week, or in case the tumour in the cyclic colony was lost through failure of the tumour transplant.

The spleen and thymus of the tumour carriers were the organs utilized for the tumour transfer. The organs were weighed, and homogenized in a Kontes tissue grinder with sufficient 0·9 per cent sterile saline to make a 10 per cent suspension. 0·2 c.c. of the suspension was inoculated either intraperitoneally or subcutaneously into animals in which transmission of the leukæmia was desired. This was sufficient inoculum to assure 100 per cent fatality in control animals.

The results from this investigation are presented in Tables 1 and 2.

Table 1 is a presentation of the survival times of experimental animals as compared with that of the control group for those animals. Of the 41 experimental animals, 27 animals delivered viable healthy young. The remaining animals either failed to live long enough to deliver, delivered stillborn young, or on autopsy showed evidence of fœtal reabsorption. Those animals which delivered living young were inoculated during the last four days of gestation. The remainder were inoculated prior to the 14th day of gestation.

Of those animals that delivered young, either viable or non-viable, there was no indication of prematurity of birth. The normal gestation period for these mice is 19·5 days. The animals usually delivered overnight between the 19th and 20th day after conception.

The mean survival time for three sets of animals, inoculated either intraperitoneally or subcutaneously, is presented in Table 2. The mean survival time for both male and female controls, when inoculated intraperitoneally, was found to be 14·03 days and 13·10 days, respectively, with an average survival of 13·35 days. The mean survival time for those receiving subcutaneous injections was 17·86 days for the males, and 17·35 days for the females, with an average survival of 17·47 days. The mean survival time for the experimental animals was found to be 14·21 days and 17·50 days when injected via the intraperitoneal or subcutaneous route, respectively.

In an examination of the effects of pregnancy on the longevity of the mother with leukæmia, it is worth while to review the values obtained from the literature concerned with human patients.

A study of longevity forces one to divide leukæmia into acute and chronic forms. Acute leukæmia seems to follow a different course when complicated by pregnancy, or when it is contracted during pregnancy. Gillim[3] presents data that show the mean survival of 149 autopsied, leukæmic, non-pregnant adult patients to be 3·82 months[7]. This data is in close accord with work presented by Tivey[17], who found the mean survival in a study of 179 adults to be 3·3 months. The average value for the 328 patients would then be a mean survival of 3·54 months. From our review of the literature, it is found that the mean survival of those pregnant patients who have acute leukæmia is 5·54 months. This is a 36 per cent increase in longevity and would indicate that pregnancy is having an ameliorating effect on these leukæmic patients.

A comparison of the results as presented in Table 1 shows a close relationship between the survival time of the experimental animal and its control group. There is a variation (± 0–3·5 days) between the survival time of the experimental animal and its control group (except for those inoculated on the 18th day of gestation) which can be expected, since the animals usually die during the six-day period following the initial onset of death in a given group of inoculated animals due to individual vigour and resistance to disease.

By cursory examination of Table 1, it would seem that the animals inoculated during the last four days of gestation have a longer survival-rate of leukæmia. Closer examination shows that the controls for these animals lived a correspondingly longer time, thus indicating a variation in virulence of the inoculant. This is to be expected and is the reason for comparing each group with its control.

Table 1. RESULTS OF THE EFFECT OF PREGNANCY ON EXPERIMENTAL MYELOID LEUKÆMIA

Day of gestation represented	Survival time of experimental animals after inoculation (average)	Survival time of male controls after inoculation (average)	Survival time of female controls after inoculation (average)	Results of young
4 days prior*	23 (1)	15·5 (2)	20·7 (3)	Stillborn
0–8	12·0 (5)	13·2 (4)	12·2 (34)	Reabsorption or undelivered
8	16·0 (2)	16·0 (5)	14·6 (5)	Stillborn or undelivered
9	11·5 (2)	11·5 (2)	12·0 (5)	Undelivered
12	12·0 (1)	14·0 (2)		Stillborn
13	9·0 (1)	12·0 (2)		Stillborn
14	11·0 (1)		12·8 (6)	Living
15	12·0 (1)		17·0 (4)	Living
16	14·5 (6)	18·0 (4)	16·6 (19)	Stillborn (one case) all others living
17	15·7 (4)	18·0 (4)	17·0 (8)	Living
18	12·7 (3)†	18·0 (4)		Stillborn (one case) all others living
19	14·7 (7)	12·5 (2)	15·9 (29)	Living
20	17·0 (6)		61·1 (21)	Living‡

The number in the bracket represents the number of animals averaged in that survival time.

* Prior to conception.

† A fourth animal appeared to be in good health two months following inoculation. Blood samples inoculated in three animals subcutaneously and two animals intraperitoneally. All animals died of myeloid leukæmia, including mother.

‡ The young were delivered before inoculation of the maternal animal.

Table 2. SUMMARY OF RESULTS

No. of animals	Route of injection	Group	Mean survival time after inoculation (days)	Average survival time of male and female (days)
31	i.p.	Male controls	14·03	13·35
84	i.p.	Female controls	13·10	
19	i.p.	Experimental	14·21	
7	s.c.	Male controls	17·86	17·47
23	s.c.	Female controls	17·35	
19	s.c.	Experimental	17·50	

It is interesting to note that those animals which received the inoculation as late as the thirteenth day of gestation either delivered non-viable young, failed to survive sufficiently long to deliver, or showed evidence of reabsorption on autopsy. This presents a period (6–7 days) before parturition during which the tumour may have had sufficient time to cause a state of maternal nutritional deficiency which culminated in death of the fœtuses.

As shown in Table 2, the mean survival time between the experimental animals and controls is practically negligible. The survival time of the experimental animals was compared with that of the male and female controls. The averages presented show little or no difference between sexes as to duration of the disease. The mean survival time of the experimental animals inoculated intraperitoneally was 14·21 days as compared with the control survival time of 13·35 days, and those inoculated subcutaneously show a mean survival time for the experimental animals of 17·50 days as compared with the control value of 17·47 days. No significant difference in the mean survival of the two groups is shown either in those inoculated intraperitoneally or subcutaneously. Thus it would seem that the presence of pregnancy during the course of leukæmia has little or no effect on the duration of the disease in *C*57 black mice.

The findings of the first experimental work concerned with the survival time of mice, where pregnancy and leukæmia are associated, are presented. Experimental results indicate that pregnancy probably has little or no effect on the survival time of *C*57 black mice which have acute myeloid leukæmia. Experimental animals inoculated early in gestation either deliver non-viable young, fail to survive long enough to deliver, or show evidence of fœtal reabsorption. Experimental animals inoculated late in gestation (last four days) deliver viable healthy young.

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ENTEROHEPATIC CIRCULATION OF UNCONJUGATED BILIRUBIN IN MAN

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WHETHER or not bilirubin is reabsorbed from the intestine has been a matter of controversy for more than half a century. The failure to observe significant increase of urobilin in the fæces after tube-feeding of solutions of crystalline bilirubin to subjects with common bile duct obstruction led Fromholdt in 1912 to speculate that absorption of bilirubin had occurred[1]. Several later investigations offered direct and indirect evidence to support the concept that bilirubin is reabsorbed[2-5]. Yet other works failed entirely to support such a concept[6-10].

In accordance with earlier work[11-13] our own experiments have failed to account for tube-fed free bilirubin either as urobilinogen or bilirubin in the fæces although the similar administration of conjugated bilirubin has regularly been followed by highly significant increases of fæcal urobilinogen often approximating the amount to be anticipated. These investigations are described elsewhere in detail[14]. The disappearance of the administered free bilirubin suggested either absorption from the intestine or conversion to unrecognized substances by the fæcal flora.

The recent work of Lester, Ostrow and Schmid, using bilirubin labelled with carbon-14, clearly demonstrated that bilirubin is absorbed from the intestine of the rat and reappears in the bile[15]. Recovery of labelled bilirubin in the bile was accomplished whether labelled bilirubin was fed as the conjugated or unconjugated form, although absorption of the latter was more rapid and quantitatively greater. It was not determined whether conjugated bilirubin was absorbed as such, or whether first hydrolysed with absorption of the resulting free bilirubin. The present work was undertaken to determine whether free bilirubin is absorbed from the intestine of man.

Crystalline bilirubin labelled with nitrogen-15, 100 mg, was dissolved in 12 ml. 0·1 N sodium hydroxide and this was adjusted to pH 8·25 with 0·3 N hydrochloric acid. The bilirubin contained 1·68 atom per cent excess nitrogen-15 and was part of a large amount previously isolated from the fæces of a patient with hæmolytic jaundice who was receiving tetracycline and who was given glycine labelled with nitrogen-15. The details of this isolation were described in an earlier paper[16].

The subject of study was a 79-year-old male patient who had recently undergone cholecystectomy and choledochostomy for common duct stones and cholangitis. The latter recurred and eventually caused the patient's death. On the second post-operative day the T-tube drainage of bile was collected for 14 h as a control sample. The aforementioned solution of ^{15}N-bilirubin at pH 8·25 was administered through an inlying duodenal tube, after fluoroscopic verification of its position, on the third post-operative day. Gentle suction at 10–12 cm water pressure was applied to the T-tube for the next 48 h to divert most of the bile to the outside in accordance with the method of Layne and Bergh[17]. The bile was collected for 48 h in six samples, each of 6–12 h.

Total bilirubin content was determined on each sample by the method of Malloy and Evelyn[18]. Using the method of Ostrow, Hammaker and Schmid[19] bilirubin was crystallized from each sample for nitrogen-15 analysis. It was noted that the crystals from the final methanol were frequently accompanied by a fatty material which was readily removed by washing with petroleum ether, after which the bilirubin was recrystallized. Purity was established by determination of the nitrogen percentage (carried out in the Microanalytical Laboratory, Division of Organic Chemistry, University of Minnesota) and measure of absorptivity[20]; the nitrogen was 9·36 ± 0·3 per cent and the molar extinction coefficient 58,913. The nitrogen-15 percentages were determined in the mass spectrometer (carried out in the Department of Physics, University of Minnesota, through the courtesy of Prof. A. O. Nier) following preliminary digestion according to Rittenberg[21].

The period of collection, total bilirubin content of the corresponding sample and amount of bilirubin crystallized from each sample, are given in Table 1. Nitrogen-15 atom per cent excess of the bilirubin from each is plotted in Fig. 1. It is seen that the concentration reached a maximum within the first 18 h, then steadily diminished, although bilirubin containing nitrogen-15 in concentration greater than the control was isolated even at 48 h.

Table 1. BILIRUBIN CONTENT OF BILE AFTER INTRADUODENAL ADMINISTRATION OF NITROGEN-15 CRYSTALLINE BILIRUBIN

Bile sample No	Time period of collection after ^{15}N bilirubin feeding (h)	Bilirubin in total sample (mg)	Bilirubin crystallized for ^{15}N analysis (mg)
1	Control (14 h)	160·0	7·8
2	0–6	17·1	6·8
3	6–18	58·7	10·8
4	18–24	22·0	6·7
5	24–36	29·0	10·5
6	36–42	29·5	7·9
7	42–48	21·0	6·0

Several factors made it impossible to estimate the proportion of fed bilirubin absorbed from the intestine in the present study. Stool collections were difficult and inaccurate. *It is unlikely that the bile collection* from the T-tube was complete; some fraction of the bile probably gained access to the duodenum. Finally, the progressive hepatic dysfunction made it unlikely that the liver excreted all the absorbed bilirubin.

In the work recorded here, conclusive evidence of the enterohepatic circulation of unconjugated bilirubin in man is presented. Isotopically labelled bilirubin, fed via duodenal tube, was absorbed from the intestine and re-excreted in the bile. The

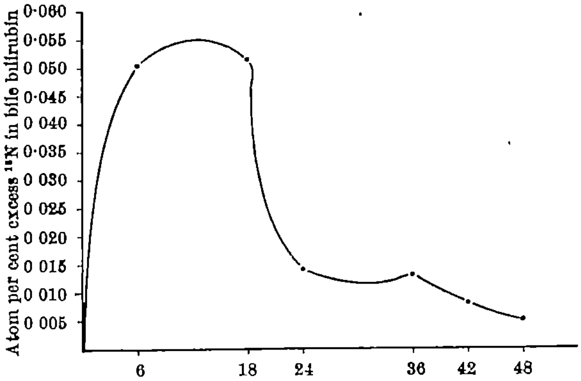

Fig. 1. Nitrogen-15 concentration in bile bilirubin after intraduodenal feeding of crystalline bilirubin labelled with nitrogen-15. The control sample described in Table 1 contained 0 000 atom per cent excess nitrogen-15

findings of Lester, Ostrow and Schmid in the rat[15] have thus been shown to be true in the human species as well. It should be noted that in the latter as well as the present investigation, the 'free' bilirubin was actually given as the sodium salt and it is not at all certain that this is formed in the intestine assuming that hydrolysis of some of the conjugated bilirubin, may occur.

The failure, in previous work, to account for tube-fed crystalline bilirubin as such or as known derivatives in the fæces of subjects with common bile duct obstruction[1,11-14] may be explained at least in part by intestinal absorption. Whether conjugated bilirubin is also absorbed as such is not yet established. In view of previous studies in this laboratory showing good recovery of tube-fed bilirubin glucuronide as urobilinogen[11], it seems unlikely that any large fraction of the conjugated bilirubin is absorbed in man, either as such or following hydrolysis.

Brief consideration should also be given to the part that intestinal absorption of bilirubin might play in explaining the frequently observed discrepancies between the amount of bilirubin formed from hæmoglobin destruction and the amounts of urobilinogen and bilirubin which can be recovered from the fæces. In order to account for these discrepancies by intestinal absorption of bilirubin, it becomes necessary to postulate that a variable fraction of the absorbed bilirubin is altered by the liver in a manner as yet unknown. We thank Prof. C. J. Watson for advice.

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MUSCLE ENZYME ACTIVITY AND CHANGES IN WEIGHT IN AGEING WHITE RATS

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SEVERAL earlier papers reported specific age-related biochemical changes in invertebrate and mammalian structures which exhibit gross functional and anatomical changes reflecting senescence[1-3]. Moreover, such changes can be related to cessation of growth in accordance with reports by others[4,5]. This article brings up to date the findings of a long-range investigation on changes, with age, in the metabolism of organophosphorous compounds by the gastrocnemius muscle, correlated with changes in total body weight and in total muscle mass. The animals studied were male white rats from the Sprague–Dawley [(Hormone Assay Laboratories) and from the *CFN* (specific-pathogen-free (Carworth Farms)] strains, maintained at 25° C on a standardized commercial pelletized diet. Procedures used in this work have been described elsewhere[3,6].

Fig. 1 shows the results obtained for the right and left gastrocnemius muscles of animals for each of three major age-groups (based on observable changes in weight representing: (1) a juvenile period of steady growth; (2) a mature phase (400–600 days) in which the body weight remains unchanged; (3) a period of senescence during which weight loss is steady and inexorable[6]). The median values shown for the adenosine triphosphatase (ATP-ase) (magnesium-activated, *p*H 7·4) decline markedly from young to senile old age, especially during the senile period. As can be seen from both Fig. 1 and Table 1 this represents a loss in enzyme activity on a muscle mass basis of approximately 66⅔ per cent of the original enzyme activity from young, growing to old senile animals. On the other hand, changes in muscle mass on a total weight basis closely parallel those of the

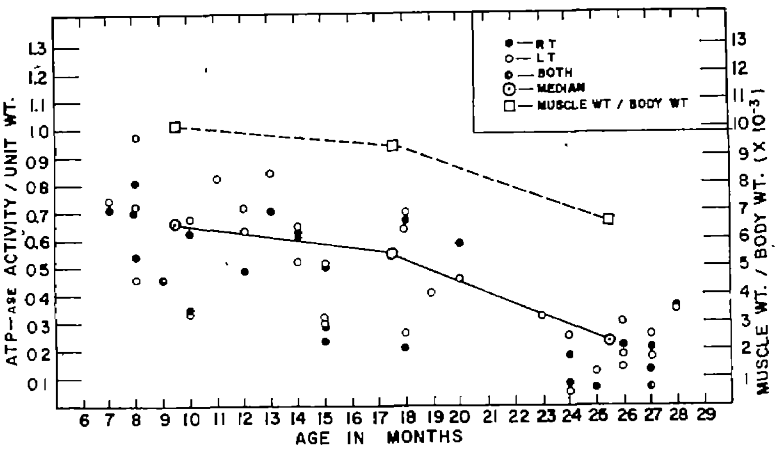

Fig. 1. Changes in magnesium-activated ATP-ase activity in the gastrocnemius muscles of ageing male Sprague–Dawley rats. Enzyme activity is expressed in µg of inorganic phosphorus released in 15 min. at 37° C per mg of fresh muscle mass

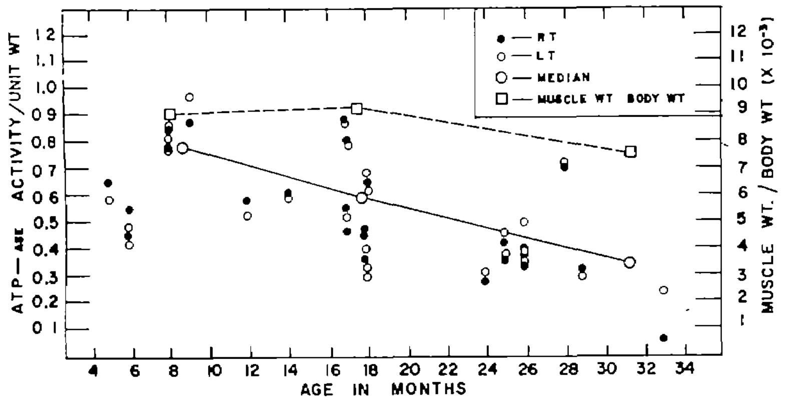

Fig. 2. Changes in magnesium-activated ATP-ase activity in the gastrocnemius muscles of male *CFN* rats. Enzyme activity is expressed in µg of inorganic phosphorus released in 15 min at 37° C, per mg of fresh muscle mass

enzyme on a muscle mass basis; this means that on a body-weight basis, enzyme activity falls even more markedly (cf. Table 1). Thus, the amount of enzyme available to move the entire body mass of an old animal is only one-fifth of that available to move that of a young growing Sprague–Dawley male white rat. It is of interest to note, in this connexion, that old male Sprague–Dawley rats exhibit a muscular dystrophy-like syndrome of the hind limbs[6,7]. The longer-lived *CFN* animals, with a much longer middle age (300–800 days) (and showing no detectable signs of muscular failure of the hind limbs at an advanced age), show only about a 50 per cent loss in enzyme activity from young to senile old age, on a muscle mass basis (Fig. 2). On a total body-weight basis, moreover, the loss in enzyme activity is only about 33⅓ per cent from young to old *CFN* animals. Comparison of Figs. 1 and 2 also shows that *CFN* animals appear to age biochemically in a more regular fashion, whereas, in the Sprague–Dawley strain, the marked decline occurs chiefly during the last third of life, and in an abrupt, precipitous fashion. Comparable results for loss in body weight of senile males in these two strains are also seen in Table 1. No significant age-related changes in the calcium-activated (actomyosin) ATP-ase were found for either strain.

Table 1. WEIGHT AND ATP-ASE ACTIVITY CHANGES IN AGEING MALE RAT SKELETAL MUSCLE

Median age (months)	Median muscle weight (g)	Median body weight (g)	Enzyme/ muscle weight	Enzyme/ body weight (× 10⁻³)
		Sprague–Dawley strain		
10	2·3	483	0·69	4 1
17	2·3	491	0·51	2 5
26	1 2	357	0·24	0 8
		CFN strain		
8	2·0	429	0 79	3 4
17	2·4	525	0·58	3·1
26	2·2	521	0·37	2·0

It is interesting that the enzyme, generally thought to be primarily concerned with contraction, itself (the myosin ATP-ase) is unaltered with age in failing skeletal muscle. Although the precise role of the magnesium-activated enzyme in the metabolism of muscle still remains a matter of speculation, the work of a number of biochemists[8,9] suggests its importance in intermediary metabolic processes intimately concerned with synthesis and dismutation of naturally occurring high-energy organic substances. Moreover, the work of Potter *et al.*[10], Moog[11] and de Villafranca[12] clearly points to a significant participation by magnesium-activated ATP-ase in the development of muscular function, that is, its activity increases with the development of the fibrillar substance in the immediately post-partum and very young chick and rat. In recent work on the giant mitochondria in the flight muscle of ageing male house flies, magnesium-activated ATP-ase activity shows a precipitous decline with the failure of flying ability in senescent male house flies, which drop is culminated in the complete loss of wings in aged males. Similar results being obtained from present work on other enzymes from such flight muscle indicate that this decline is part of a complex pattern of biochemical senescence, just as the development of flight ability is associated with the increased activity of such enzymes in post-emergent flies.

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MODIFIED EQUIPMENT FOR MEASURING CHANGES IN FROZEN FISH BY LOVE'S CELL FRAGILITY METHOD

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IN 1958 R. M. Love described a new method for determining changes in frozen fish fillets based on the fragility of individual muscle cells when subjected to high-speed homogenization for a short time[1]. The equipment used in his later work[2-4,6] has consisted of a modified Marsh–Snow homogenizer[7]; however, the basic homogenizer is not available commercially, and it appears that much of the trouble that various other workers have experienced with the method[5,6] has been caused by the difficulty in accurately reproducing the equipment. For this reason, an investigation of the application of standard type equipment, with or without modification, to the testing of cell fragility was carried out.

A ¼-h.p. Servall Omnimixer homogenizer (1) manufactured by Ivan Sorvall, Inc., Norwalk, Connecticut, was found to be quite suitable when 50-ml. Servall cylindrical stainless-steel mixing chambers were used (6). These chambers have four 2·75-in. long grooves which start at the base of the cup and provide proper turbulence for good mixing. The complete homogenizer, including the modifications described here, is illustrated in Fig. 1.

A 0·375-in. diameter hole was drilled through the aluminium coupling adapter (2) so that the speed of the motor could be determined with a stroboscope rather than with a tuning fork, as was done by Love and Mackay[4].

The shaft tube of a 4-in. Servall rotor knife blade assembly was shortened and refitted with the bottom oilite bearing so that the overall length was 2·795 in. (4). The rotor shaft was shortened by cutting 1·180 in. off the top and 0·125 in. off the bottom and then the bottom thread was extended to the shoulder (5). A 1·375-in. long stainless-steel paddle 0·090 in. thick and 0·550 in. wide with rounded edges was made from a rod 1·812 in. long and 0·750 in. in diameter and the diameter of the top 0·437 in. of the rod was reduced to 0·375 in. (11). A socket was tapped along the axis of the top of this rod to hold the end of the rotor shaft and the top 0·125 in. of this socket was increased to the diameter of the rest of the shaft above the shoulder to allow for a true fit. The shaft tube was fitted with a close tolerance stationary chamber seal (7) which is described here.

A stainless-steel, externally threaded bushing, having an outside diameter of 0·625 in. and an inside diameter of 0·474 in. with a bottom shoulder 0·750 in. in outside diameter and 0·125 in. thick was made and fitted with a 'Teflon' washer (7*b*) placed between one 1·215-in. diameter upper and one 1·00-in. diameter lower stainless-steel washer. These washers were assembled on the bushing and secured against the shoulder by a stainless-steel lock-nut. This chamber seal was locked on to the shaft tube by means of an Allen set-screw (7*a*) passing perpendicularly through the threaded bushing. The 'Teflon' washer was made to fit very snugly against the inside wall of the mixing chamber so as to exclude air from the homogenate.

Each mixing chamber was fitted with an external, 3 in. diameter, concentric stainless-steel ring (8) by three evenly spaced, soldered stainless-steel connectors (9). A 1-pint polyethylene bottle was cut and the bottom (10) (4·0 in. deep) was attached to the outside of the bottom half of the ring by several aluminium rivets. A stationary, permanent ice and water bath was thus provided for each chamber. A cover for each ice bath was made from the upper portions of a 1-pint polyethylene bottle (3) to allow the mixing chamber to be emptied and re-used without loss of ice water. This fitted snugly around the upper half of the outside ring and the top was cut to fit just below the threaded portion of the mixing chamber. The bottom portion of each ice bath was fitted into a separate piece of 'Styrofoam' insulation (12) made by boring a cylindrical hole 3·0 in. in diameter and 3·5 in. deep into a piece of 'Styrofoam' with a 5·0 in. square cross-section and

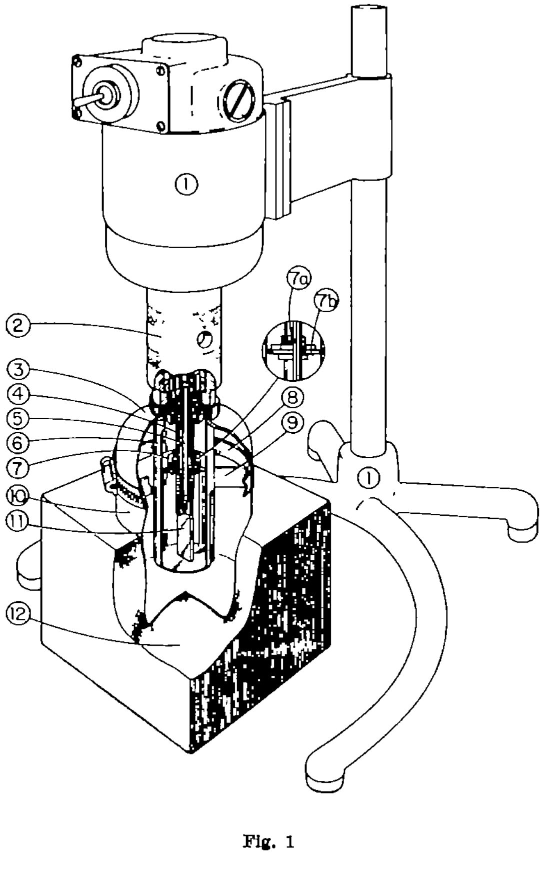

Fig. 1

a 4·0 in. depth. This insulation is capable of maintaining 32° F in the baths for 2·5 h with a room temperature of 85° F.

Only one mixing blade is necessary for several mixing chambers. The position of the chamber seal may be adjusted by means of the Allen set-screw to a minimum volume of 53·5 ml. in a Servall 50-ml. mixing chamber. With 535 ± 5 mg of fish added to this volume of the formaldehyde solution (10 mg/ml.)[6] it is possible to homogenize with an ice bath for 40 sec at 9,000 r.p.m. without beating any air into the mixture. The use of boiled, cooled distilled water for preparing the formaldehyde solution seems to help minimize the chances of foaming. The formaldehyde solution should be stored at room temperature since it has been found that refrigerated storage induces polymerization of the formaldehyde and promotes subsequent foaming of the homogenate. The latter has been confirmed by Love[5].

We wish to acknowledge Gorton's of Gloucester for their financial support of this project.

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IN VITRO STIMULATION OF AMINO-ACID INCORPORATION INTO PROTEIN BY LIVER NUCLEAR RNA

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It has been postulated that 'messenger RNA' (that is, the RNA which specifies amino-acid sequence during protein synthesis) is synthesized on a DNA template. One might, therefore, expect that significant quantities of this RNA would be found in the cell nucleus. Indeed, Sibatani *et al.*[1] have recently reported the isolation from calf thymus nuclei of an RNA fraction which has a base composition almost identical to calf thymus DNA, and which may be 'messenger RNA'. Although ribosomal RNA was at one time believed to contain the information required for genetic control of protein synthesis, studies in bacteria have tended to negate this concept[2].

The relative 'messenger activity' of nuclear and ribosomal RNA from mammals has heretofore been difficult to measure directly. Recently, however, Nirenberg and Matthaei[3] have developed a stable DNase-treated *E. coli* extract in which amino-acid incorporation into protein is stimulated by several types of RNA. This system, furthermore, synthesizes a protein resembling tobacco mosaic virus protein when tobacco mosaic virus RNA is added[4]. Since the *E. coli* extract can, in the presence of tobacco mosaic virus, make a protein similar to that made in the infected tobacco leaf, rigid species specificity of 'messenger RNA' seems to be precluded. It therefore might be assumed that the *E. coli* system would be stimulated by mammalian 'messenger RNA' and that it could be used to assay the relative 'messenger activity' of mammalian nuclear and microsomal RNA. The present report describes a comparison of the ability of rat liver nuclear, microsomal, and soluble RNA to stimulate incorporation of amino-acids into protein by this extract.

The rat liver nuclear and microsomal RNA's used in this work were isolated from adult male albino rats by a slight modification of methods which are fully described elsewhere[5]. In brief, liver nuclei were obtained by gently homogenizing the chilled, minced tissue in 0·32 M sucrose containing 0·001 M magnesium chloride and 0·0008 M potassium phosphate, *p*H 6·7–6·8. The crude nuclear sediment obtained by centrifugation of the homogenate at 600*g* for 8 min was washed three times with the homogenizing medium and then centrifuged in the Spinco *SW* 25 rotor at 50,000*g* (av.) for 2 h in 2·1 M sucrose containing 0·001 M magnesium chloride, 0·0035 M dipotassium hydrogen phosphate, and 0·0007 M ATP disodium salt, *p*H 6·7–6·8. The resulting nuclear pellet was treated with 0·10 per cent sodium lauryl sulphate in 0·001 *M* potassium phosphate, *p*H 7·0 for 5 min at room temperature and then extracted with an equal volume of redistilled phenol. The aqueous phase obtained from the phenol extraction was made 0·2 M in potassium acetate, treated twice more with 0·5 vol. of phenol, and then precipitated with 2·5 vol. of cold ethanol. The precipitated RNA was then dialysed for two days, first against 0·02 M and then against 0·005 M potassium acetate. The isolated nuclear RNA was found to be contaminated with less than 1 per cent DNA. Microsomes were obtained by first centrifuging the tissue homogenate at 34,000*g* (av.) for 8 min in the Spinco *SW* 25 rotor and then centrifuging the resulting supernatant at 105,000*g* (av.) for 60 min in the Spinco 40 rotor. RNA from the microsomal pellets was obtained in a manner identical to the isolation of RNA from nuclear pellets, except that the final aqueous phase obtained from the phenol extraction was centrifuged at 105,000*g* for 1 h to remove glycogen. The physical properties of nuclear and microsomal RNA have been found to differ markedly[5]. The use of sodium lauryl sulphate during the isolation of RNA was found to be essential for the complete liberation of nuclear RNA by phenol. The observation of Sibatani *et al.*[1] that the DNA-like RNA found in calf thymus nuclei was not liberated by conventional phenol treatment may be a consequence of excluding detergents from the extraction procedure. Recent work by Hiatt[6] appears to validate this interpretation.

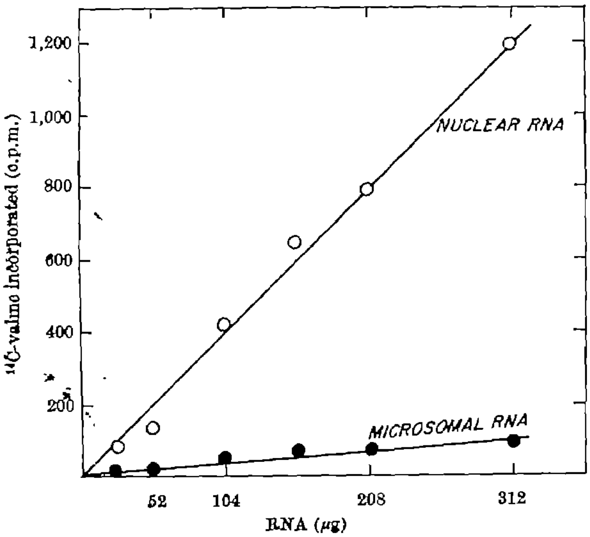

Fig. 1. Stimulation of ^{14}C-valine incorporation into protein by rat liver nuclear and microsomal RNA

Each reaction mixture contained the following in a total volume of 0·4 ml.: 40 µmoles *tris*-hydrochloric acid *p*H 7·8, 4 µmoles magnesium acetate, 20 µmoles potassium chloride, 2 4 µmoles β-mercaptoethanol, 0·4 µmoles adenosine triphosphate, 2·0 µmoles potassium phosphoenolpyruvate, 8 µgm. crystalline phosphoenolpyruvate kinase, 0·03 µmoles guanosine triphosphate, 0 02 µmoles each of 20 different L-amino-acids (as used by Nirenberg and Matthaei[2]) with 84,000 c.p.m. in L-valine, and extensively dialysed *E. coli* extract containing 1·6 mg protein and 0·5 µg crystalline DNase. No uridine triphosphate or cytidine triphosphate were added. The indicated amounts of RNA were added and the mixture was incubated for 90 min at 35° C. 10 per cent TCA was then added and the precipitate was treated by a modification of the method of Siekevitz[5]. Three tubes containing no added RNA were found to incorporate an average of 60 c.p.m. into protein and these counts were subtracted from the observed counts in the RNA-containing tubes and the resulting figures plotted

Nuclear RNA was fractionated by layering the RNA over a 4–20 per cent linear sucrose gradient containing 0·1 M potassium acetate at *p*H 6·0, and centrifuging at 25,000 r.p.m. for 16–17 h at 4° C in the Spinco *SW* 25 rotor. The optical density at 260 mµ was determined on 1-ml. fractions, collected by puncturing the tube bottom, and selected fractions were pooled and dialysed against 0·02 M potassium acetate, to remove all sucrose. These pooled RNA fractions were then concentrated by precipitation with 0·01 M magnesium acetate (final concentration) and 2·5 vol. of ethanol, and the precipitates were dried in an evacuated desiccator prior to assay of their ability to stimulate amino-acid incorporation. Rat liver cytoplasmic soluble RNA was prepared by homogenizing the tissue in 0·32 M sucrose, 0·0008 M potassium phosphate, *p*H 6·7–6·8, and centrifuging at 105,000*g* for 3 h. The RNA was then isolated from the supernatant by the procedure described here for nuclear RNA and fractionated by sucrose gradient centrifugation in a manner identical to that used for nuclear RNA. Material sedimenting between 0·5 and 9·0 $S_{20,w}$ with a peak of 5·0 *S*, was pooled for assay. Calculation of sedimentation constants was made by the method of Martin and Ames[7].

Preparation of the *E. coli* protein-synthesizing extract was carried out by the method of Nirenberg and Matthaei[2]. An 'incubated *S*-30' preparation[2], which had been dialysed against 3 changes of 0·01 M magnesium acetate, 0·06 M potassium chloride, 0·01 M *tris*-hydrochloric acid *p*H 7·8, and 0·006 M β-mercaptoethanol for a total of 18 h, was used throughout. This preparation of ribosomes and soluble materials contained crystalline Worthington DNase(3 µg/ml.), and had been pre-incubated to reduce amino-acid incorporation by the extract in the absence of exogenous 'messenger RNA'. The reaction mixtures utilized for assay of amino-acid incorporation into protein are described under Fig. 1. Supplementation with *E. coli* soluble-RNA (1 mg/ml. of reaction mixture) did not stimulate baseline incorporation by the system and did not alter the stimulation produced by liver nuclear and ribosomal RNA. Reactions were terminated by addition of 5 ml. of cold 10 per cent trichloroacetic acid, and the precipitates were washed and counted by a modification of the method of Siekevitz[5].

It may be seen in Fig. 1 that addition of liver nuclear RNA to the *E. coli* extract results in marked stimulation of ^{14}C-valine incorporation into protein. Microsomal RNA, by contrast, has only 1/10 the activity of nuclear RNA (Fig. 1). Preincubation of nuclear RNA, 800 µg/ml. with 12 µg/ml. of crystalline deoxyribonuclease for 30 min at 25° C in 0·07 M potassium acetate, *p*H 6·0, produced only slight (15 per cent) inhibition of the activity of this material. Soluble RNA prepared from rat liver cytoplasm produced no detectable stimulation of amino-acid incorporation (when 131 µg of soluble RNA were added to a 0·4-ml. reaction mixture and incubated for 60 min, 34 c.p.m. of ^{14}C-valine were incorporated, whereas the control preparation incorporated 27 c.p.m.). The time course of the reaction with nuclear RNA is shown in Fig. 2 and resembles that seen when TMV–RNA[4] and polyuridylic acid[3] are used in this system.

In an attempt to characterize further the nuclear RNA responsible for this stimulation of amino-acid incorporation, the specific activity (in terms of counts per minute of incorporated ^{14}C-valine per mg RNA) of each of four fractions obtained by zone centrifugation in a sucrose gradient was determined. These results

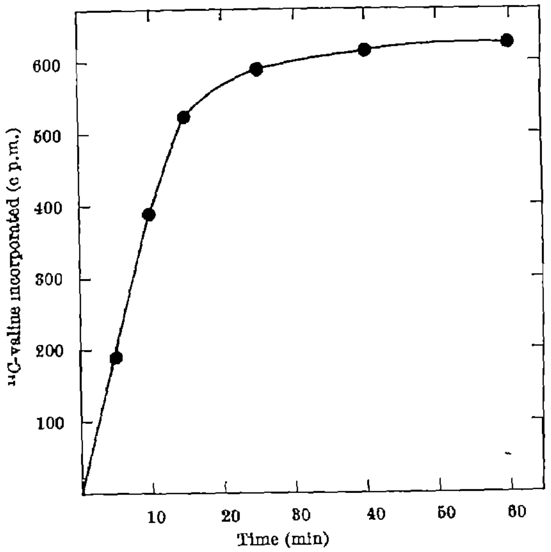

Fig. 2. Time course of stimulation of ^{14}C-valine incorporation by rat liver nuclear RNA

A reaction mixture of 2 63 ml. containing a total of 1,014 µg of rat nuclear RNA was prepared. Other reagents were present in the same concentrations as indicated under Fig 1. 0 4-ml. aliquots were removed at the indicated times and treated as indicated under Fig. 1. An identical mixture containing no added RNA was also incubated, and 0 4-ml. aliquots were removed at the same time-intervals as those indicated here and the observed c p.m. in the control samples were subtracted from the RNA containing samples before the results were plotted. After 60 min of incubation 83 c.p m. of ^{14}C-valine had been incorporated into protein in the control preparation

Table 1. STIMULATION OF AMINO-ACID INCORPORATION BY NUCLEAR RNA FRACTIONS

Fraction	$S_{20,w}$* value of fraction peak	Range of $S_{20,w}$* values pooled in each fraction	μg RNA used in assay†	Stimulation of amino-acid incorporation, c.p.m./mg RNA
1	6·4 ± 1·3‡	2·4—10 3	96	960
2	19·4 ± 0·9	16·0—24·3	45	3,300
3	32·9 ± 0 9	29·6—37·6	100	2,600
4	>40	---	125	4,900

* Calculated by the method of Martin and Ames (ref. 7).

† The assay was conducted as described under Fig. 1. Incubation was for 60 min. After the c.p.m incorporated into protein in a control tube containing no RNA were subtracted from the observed incorporation with the various RNA fractions, the data were converted to c.p.m./mg RNA. Although different amounts of the different RNA samples were used, this comparison was considered valid since stimulation with whole nuclear RNA varies linearly with the amount of RNA added throughout the range of amounts used in this experiment (Fig. 1).

‡ Values given are the mean ± the *S.D.*

are reported in Table 1, together with the sedimentation velocity constants for each fraction. It may be noted that the stimulating activity is heterogeneous with respect to sedimentation velocity, with most of the activity migrating faster than 10 *S*.

It may be concluded that RNA from mammalian liver can stimulate amino-acid incorporation into protein in an *E. coli* extract and that nuclear RNA contains far more 'messenger activity' than microsomal RNA. Although the amount of protein synthesized in the foregoing experiments was too small to characterize, one might speculate that proteins resembling mammalian proteins were synthesized. The finding of a comparatively small amount of 'messenger activity' in the microsomal RNA does not necessarily indicate that ribosomal RNA *per se* may function as 'messenger' but may be the result of contamination with 'messenger RNA', which has been shown to associate with ribosomes both *in vivo*[9] and *in vitro*[10].

We thank Dr. Marshall W. Nirenberg and Dr. Donald B. Tower for their help. Dr. Fred H. Bergmann kindly supplied the *E. coli* *s*–RNA used in these experiments.

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ARTEFACTS OF ORNITHINE AND α-γ-DIAMINOBUTYRIC ACID FOUND DURING CHROMATOGRAPHIC INVESTIGATION OF RUMEN LIQUID

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WHILE examining amino-acids in the liquid from the rumen of cattle by means of electrophoresis at *p*H 4·5 and afterwards chromatography in the solvent system butanol–acetic acid–water (4 : 1 : 5) as shown earlier[1] a new compound, *X* (Fig. 1), was detected. From its position on the diagram it follows that *X* must have basic properties; with ninhydrin it shows a yellow colour turning to grey-blue after some time.

It was evident that *X* is formed from arginine after its conversion into ornithine. As it was not possible to get any further information about *X* with many spray reagents, the compound was isolated. After freeze-drying of the ammonia eluate on a 'Dowex 50*W*' column the amino-acids are separated by electrophoresis on cellulose powder drenched in the buffer of *p*H 4·5 as described by Montant *et al.*[2]. After freeze-drying of the aqueous eluate of *X* a separation of *X* from basic amino-acids is obtained by chromatography on 3*MM* paper in butanol–acetic acid–water. The aqueous eluate of *X* is freeze-dried, and the residue is extracted with absolute alcohol. The alcohol is evaporated and this process is repeated until no more precipitate is formed on the addition of alcohol. Finally, the brown residue is dissolved in water and treated with 'Norit'. The clear solution is freeze-dried and the residue can be purified by sublimation *in vacuo* at 80°–100° C against a surface cooled at –40° C.

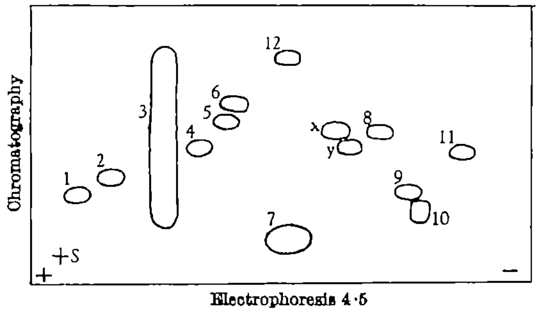
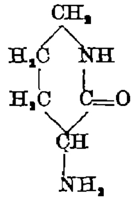


Fig. 1. 1, Aspartic acid; 2, glutamic acid; 3, neutral amino-acids; 4, β-alanine; 5, γ-aminobutyric acid; 6, δ-aminovaleric acid; 7, basic amino-acids; 8, colamine; 9, histamine; 10, cadaverine and putrescine, 11, unidentified, 12, tyramine; *X*, β-aminopiperidon, *Y*, β-aminopyrrolidon

Compound *X* is a white powder which after some days turns yellowish-brown. It melts at 115°–117° C under decomposition. It is soluble in water and in alcohol. Chemical analysis (carried out by the Micro-analytical Section of the Laboratory of Organic Chemistry of the University of Amsterdam) gave the formula $C_7H_{14}N_2O_3$ and indicated the presence of one acetyl group on 2 nitrogen atoms. When *X* is gently warmed with 0·1 N sodium hydroxide it decomposes into ornithine and sodium acetate.

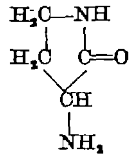
From these results it seemed probable that a cyclic compound of acetylornithine had been formed. However, when ornithine was warmed with water or with 0·1 N sodium hydroxide it appeared that ornithine was partly converted into a compound (named *Z*) which gave as *X* a yellow colour with ninhydrin. *Z* was isolated in the same way as mentioned here for *X*. It gave an infra-red spectrum completely similar to that of *X*. So *Z* is identical with *X* and must contain acetic acid. In order to avoid acetic acid, the separation of the compound formed by ornithine and ornithine itself was carried out by chromatography in propanol–ammonia. Now it was not possible to sublimate the base, but the same properties were found as in *X* in matters of electrophoresis and chromatography and decomposition by 0·1 N sodium hydroxide. So it was concluded that *Z* and *X* must be the acetic acid salt of the lactam of ornithine (β-aminopiperidon).



In 1909, E. Fischer and G. Zemplen[3] prepared β-aminopiperidon from monobenzoylornithine. They found that the melting point of the chloroplatinate was 200°–205° C under decomposition into a brown liquid. The chloroplatinate of *X* was prepared in the same way as given by E. Fischer *et al.*, and after recrystallization the melting point appeared to be 198° C and a dark brown liquid remained.

From this it is certain that ornithine, by warming with water or ammonia or 0·1 N sodium hydroxide, is partially converted into β-aminopiperidon. There seems to be a kind of equilibrium between them, because by warming β-aminopiperidon with 0·1 N sodium hydroxide the greater part of it is converted into ornithine again within 5 min. With 2 N sodium hydroxide all is converted into ornithine. The reaction may easily be followed by one-dimensional chromatography in butanol–acetic acid–water. As ornithine already is converted partially at 38° C, it is possible that in the rumen of cattle a small quantity of β-aminopiperidon is formed, but the greater part of the spot found on the diagram must certainly be attributed to decomposition of the ornithine present during the experiments.

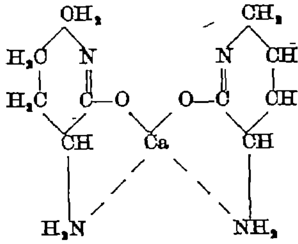
As it seemed likely that other compounds would give similar reactions, especially in cases with a possibility of formation of a five-ring, α-γ-diaminobutyric acid was examined in the same way. A yellow spot *Y* (Fig. 1) was found on the electrophoresis–chromatography diagram. *Y* was isolated in the same way as *X* with the exception of the sublimation. It reacts with 0·1 N sodium hydroxide in the same manner as *X* during formation of α-γ-diaminobutyric acid. From this it follows that *Y* must be β-aminopyrrolidon.



Just like ornithine, α-γ-diaminobutyric acid is partly converted into a cyclic form when heated with ammonia or 0·1 N sodium hydroxide.

As the compounds lysine, γ-aminobutyric acid and ε-aminocaproic acid may be present in rather large quantities in rumen liquid their reaction with ammonia has been examined. Lysine only gives a very weak spot on the chromatogram after heating with ammonia or 0·1 N sodium hydroxide, but the monoamino-acids do not form lactams in these circumstances. By gentle dry heating of these acids lactams were formed. They did not react with ninhydrin but could be detected with the reaction of Rydon *et al.*[4]. The R_F value of caprolactam is in accordance with Czerepko's results[5]. After dry heating of lysine a rather strong spot on the chromatogram was obtained in the same place where the artefact with ammonia had been found. Possibly this is also a cyclic compound.

The fact that lysine by heating with ammonia forms much smaller amounts of cyclic compounds than ornithine and α-γ-diaminobutyric acid may easily be understood as five and six rings are formed sooner than seven rings, but it is not clear why γ-aminobutyric acid does not give a cyclic compound in the same circumstances as do the diamino-acids. There are indications that the diamino-acids form chelates with calcium and magnesium; for example, when isolating *X* from rumen liquid it always contains calcium and/or magnesium. Only after sublimating *X* calcium and magnesium remain. A possible formula may be:



Of course, monoamino-acids cannot form similar chelates, but it is just a hypothesis. R_F values of the investigated compounds are given in Table 1. On electrophoresis at *p*H 1·9 the spots of β-aminopiperidon and β-aminopyrrolidon are found to the left above the spots of the corresponding amino-acids; this contrasts with electrophoresis at *p*H 4·5, when they are found on the right-hand side of the amino-acids.

Table 1. R_F VALUES OF THE COMPOUNDS INVESTIGATED

	Butanol/ acetic acid/water (4:1:5)	Propanol/ 0 2 N ammonia (3:1)
Ornithine	0·14	0·13
β-Aminopiperidon	0·38	0·58
α-γ-Diaminobutyric acid	0·12	0·26
β-Aminopyrrolidon	0·35	0·46
Lysine	0·18	-
Artefact of lysine	0·40	
γ-Aminobutyric acid	0·42	
Butyrolactam	0·70	
ε-Aminocaproic acid	0·54	
Caprolactam	0·80	

I thank Dr. A. H. Holtz for discussions and Drs. J. H. van der Maas under whose direction the infra-red spectra were obtained in the Laboratory of Analytical Chemistry of the University of Utrecht.

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EGG-LAYING HABITS OF *SIMULIUM DAMNOSUM* THEOBALD AND *SIMULIUM MEDUSAEFORME* FORM *HARGREVESI* GIBBINS IN NORTHERN NIGERIA

By JOHN B. DAVIES

Sandfly Research Unit, Ministry of Health, Jamaica

DURING February–April, 1960, *Simulium damnosum* Theobald was observed ovipositing at its breeding sites on the rivers Kaduna, near Kaduna (10° 30′ N., and 07° 25′ E.); Tapa and Iku near Abuja (09° 11′ N. and 07° 11′ E.); and Mimi at Lokoja (07° 48′ N. and 06° 44′ E.). At this time, during the late dry season, the rivers concerned were very much reduced, but while they seldom dried up completely there was usually sufficient flow to support a limited population of *S. damnosum*.

The breeding site on the Tapa at the Tapa Falls near Abuja was typical of all the sites observed and will therefore be described in detail.

The river at the Tapa Falls where most observations were made was about 60 yd. wide. At this time of the year the river itself was split into three main channels which flowed swiftly between scattered boulders, rocks, clumps of grass and small thickets of *Mimosa* spp., to reach the top of the falls where it cascaded down over a much-scoured rock face, scattered with clumps of grass, to a pool some 80 ft. below. In the quarter of a mile of river above the falls only three *S. damnosum* oviposition sites were found after a careful search, and of these one was more popular than the other two. This site consisted of a 'run' of smooth, very swift, unbroken water some 10 ft. long and 6 ft. wide which flowed over the face of a rock in such a way that the surface of the water lay at about 30° with the horizontal. Below this the 'run' entered a small pool forming an area of broken foaming water.

The bank adjacent to this site was covered with large dense clumps of grass (*Chloris* spp.) overhanging the water so that a few stems and leaves trailed on the surface. It was on these trailing grass stems that large masses of eggs, sometimes exceeding ½ in. in thickness were found. Adult flies found entangled among the eggs, and later taken in the act of laying on these sites, proved to be female *S. damnosum*.

Oviposition was observed on many occasions at Abuja and usually took place about half an hour after sunset, when it was almost too dark to see and so observations were made with the aid of a torch. At Kaduna, further north, egg-laying usually began a little earlier when the Sun was just sinking below the horizon, and on one occasion, in late March, oviposition was completed half an hour before sunset. This time, however, the western horizon had been obscured by the heavy clouds of an approaching storm. This supports Crisp's[1] suggestion that the presence of an approaching storm may encourage oviposition.

As a result of these observations the stages in behaviour of *S. damnosum* prior to the act of oviposition can be described as follows:

(1) About one hour before sunset, when the humidity by the river begins to rise, adult male and gravid female flies can be observed resting on the tips of tall grasses by the river bank often as high as 6 ft. above ground-level (the inflorescence of *Chloris* spp. is a favourite site). As the light intensity decreases the females tend to move closer to the water's edge and rest lower down on grass stems and leaves. At this stage they can be easily collected by means of an aspirator if required for experimental purposes.

(2) About half an hour after sunset the females leave the foliage and are apparently attracted by the whiteness of the broken water below the laying site.

(3) From there they fly upstream about 1 in. above the surface of the water, following a zig-zag course until they reach a suitable site on which to alight. Should they fail in this after flying for about 6 ft. upstream, they will return to the broken water and approach again.

(4) On finding a suitable site (usually a grass stem or leaf trailing in the water) they will alight 1–2 in. above the water-level facing upstream, turn about, walk down the leaf to the water-level, turn about again and, facing up the leaf, will lay immediately either on the part of the leaf directly wetted by the water or in the splash zone.

(5) The eggs emerge from the abdomen in a single string which is laid down haphazardly on the substrate as the fly moves about. The eggs tend to lie side-by-side in the string, and not end-to-end as might be expected. Freshly laid eggs are white in colour changing to golden brown before morning. Once laying has started it is usually completed within a minute, while the period over which oviposition takes place lasts about half an hour.

Oviposition by up to 50 flies at a time has been observed on a single grass stalk, but since oviposition by single flies has also been observed at the same sites it is probable that mass egg-laying is due to a shortage of available sites rather than social behaviour as suggested by Muirhead-Thomson[2]. It has been observed, however, that on some days only one or two flies will lay while on other apparently similar days (in the absence of meteorological data) flies will be abundant. Marr's[3] suggestion that laying may be triggered by a drop in humidity is probably correct, although in view of Crisp's[1] observations atmospheric pressure may have some effect.

In no cases were flies observed to penetrate the surface of the water in agreement with the observations of Wanson and Henrad[4] and Marr[3]. It has been noted, however, that flies often deposit their eggs in the trough of air which forms below the water-level on the downstream side of a stalk or similar object, due to the flow lines of the water around the obstruction. Viewed from the side this could appear as if the flies were actually penetrating the surface of the water.

During oviposition, female *S. damnosum* are particularly prone to predation by the Muscids *Xenoymia oxycera* Emden which gather round the breeding sites toward evening and attack the flies as they prepare to lay[5].

While mass-laying was taking place the weight of the egg-masses caused the trailing stems to sink lower in the water, submerging the earlier eggs. It is significant, however, that the stem and egg-mass together acted as a float, keeping the eggs near the surface of the water where they would be warmed by the Sun, regardless of fluctuations in water-level.

Similar egg-laying has been observed with *S. medusaeforme* form *hargrevesi* Gibbins at the Pottery Rapids on the River Iku at Abuja between April and June 1961. In this case adult flies of both sexes could be found in large numbers in the evening on the grass bordering the rapids. On one occasion one catcher collected 72 flies in a single evening; of these about a tenth were males. The behaviour of *S. medusaeforme* was very similar to that of *S. damnosum* except that water-splashed rock surfaces formed the chief oviposition site, agreeing in general with Meeser's[6] description of laying by *S. elgonense* Gibbins (now considered as synonymous with *S. medusaeforme* form *hargrevesi*), but at Abuja the flies have never been observed to penetrate the water surface.

Adults of *S. adersi* Pomeroy have been taken from grasses along the River Mimi in the evening at Lokoja, but oviposition in this species has not been observed in Nigeria. Marr states that their laying sites are somewhat similar to those of *S. damnosum* in Ghana.

It is significant that all the *S. damnosum* and *S. medusaeforme* oviposition sites so far discovered lie upstream of any sizeable waterfall or rapid, even though comparable sites exist in the rapids or waterfalls themselves. It would seem that this is a mechanism to ensure the maximum chance of survival for the young larvæ, which once they have been swept away from their eggs, thereby stand a greater chance of finding a suitable attachment site.

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TRYPTOPHAN METABOLISM IN *PSEUDOMONAS*

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IN 1949, two groups of investigators, using the technique of sequential induction, showed the existence of two pathways for the dissimilation of tryptophan in strains of *Pseudomonas*[1-2]. Stanier and Hayaishi[3] extended and generalized these findings and indeed found that of 25 new strains examined 20 degraded tryptophan via the route discovered by Suda *et al.*[1] (through anthranilic acid (V) (Fig. 1) the aromatic pathway) and 5 via the route described by Stanier and Tsuchida[2] (through kynurenic acid (XV), the quinoline pathway). A complete scheme for the transformation of L-tryptophan (I*a*) via the aromatic pathway, pyrocatechol (VI), and β-ketoadipic acid (XIV) to Krebs cycle intermediates has been known for some years[4]. Recently, however, Dagley *et al.* have demonstrated a new type of fission of aromatic molecules[5]; an alternative metabolic branch thus exists at the level of pyrocatechol (VI). Nishizuka *et al.*[6] have worked out the metabolic steps lying between α-hydroxymuconic semialdehyde (VII) (the product of the alternative cleavage of pyrocatechol) and pyruvate and acetate (VIII–X).

There existed two major gaps in our knowledge of tryptophan metabolism in *Pseudomonas*: both were concerned with the quinoline pathway. The first of these was the pathway for D-tryptophan (I*b*) and the second, the pathway for kynurenic acid (XV). Recent work has narrowed these gaps and, further, a strain which possesses some additional enzymatic capacities has been discovered.

Transformations of Kynurenic Acid. The major experimental block in uncovering the steps in kynurenic acid metabolism arose from the difficulty of preparing cell-free extracts capable of degrading kynurenic acid (XV). Investigations revealed a requirement for DPNH in the first step (XV→XVI)[7]; it was presumably this requirement that was not easily satisfied in unsupplemented preparations. (Hayaishi *et al.*[8] have recently discovered that certain oxygenases, including the kynurenic acid hydroxylase, can be maintained in an active form by sodium borohydride reduction.) Once this difficulty was overcome, progress was rapid, especially following the identification of the next stable intermediate, 7,8-dihydroxykynurenic acid (XVI)[7,9]. Hayaishi *et al.*[10,11] have undertaken the further elucidation of this pathway the next few steps of which are shown in Fig. 1. It should be noted that the cleavage of 7,8-dihydroxykynurenic acid (XVI) is of the type discovered by Dagley *et al.*,[5] that is, α to two adjacent hydroxyl groups rather than between them.

Metabolism of D-tryptophan. Tashiro *et al.*[12] have recently clarified the pathway for D-tryptophan (I*b*) catabolism in quinoline pathway organisms. The initial hypothesis of Stanier and Tsuchida was confirmed[8]. Their later finding[13] of the non-utilization of formyl-D-kynurenine (II*b*) was probably due to impermeability. The pathway of D-tryptophan in these organisms is a separate one: D-tryptophan is converted to D-kynurenine (III*b*); the mechanism is presumably via a D-specific pyrrolase and formyl-D-kynurenine (II*b*). D-Kynurenine is then, remarkably, oxidatively deaminated; spontaneous ring closure of IV to kynurenic acid follows. It will be recalled that this same organism transaminates the L-isomer of kynurenine.

Since, of the strains known at that time, only quinoline-pathway organisms oxidized the D-isomer

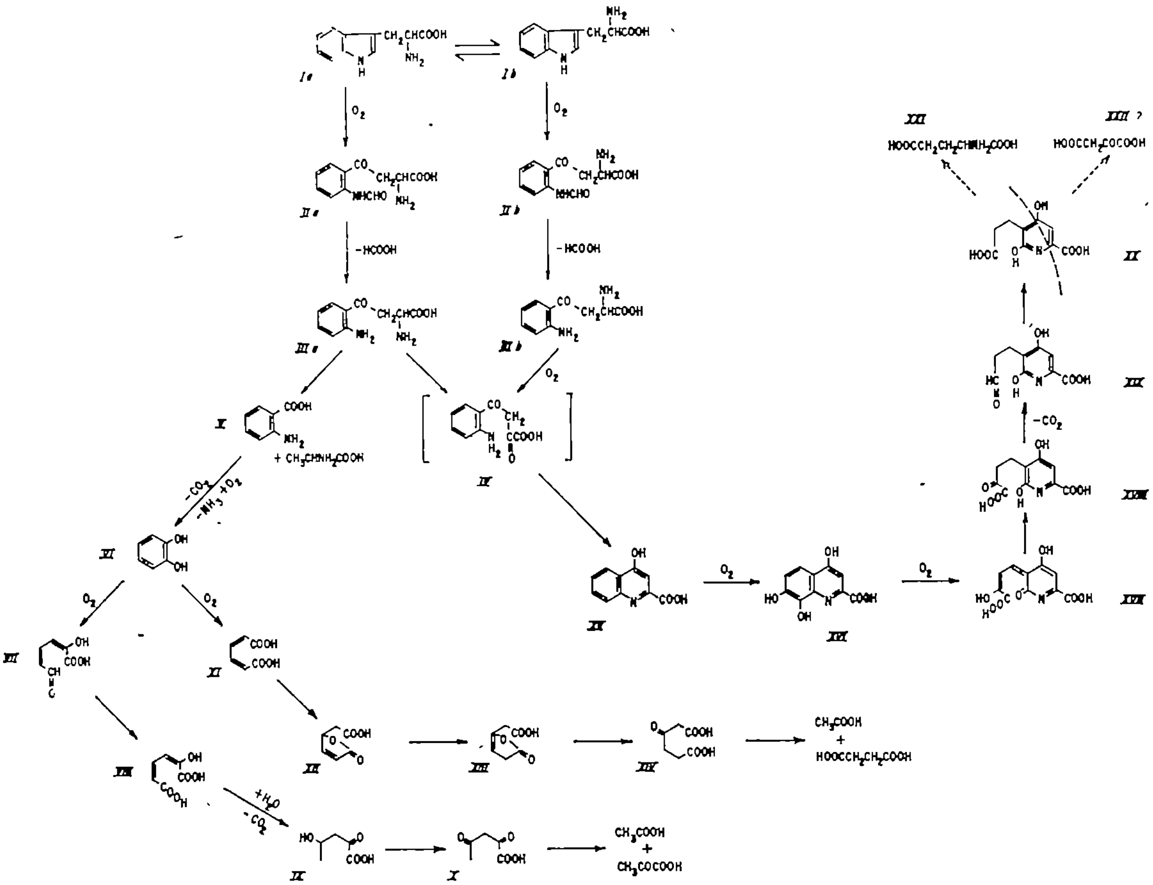

Fig. 1. Pathways of tryptophan metabolism in *Pseudomonas*

of tryptophan at a significant rate, Stanier *et al.*[13] made the suggestion that growth on D-tryptophan could be specifically used to enrich for quinoline-pathway organisms. We have isolated a strain of *Pseudomonas* (*DT*–2) (ATC,14676) from a D-tryptophan enrichment which exhibits inducible oxidative patterns typical of an aromatic-pathway organism, with this exception: the rates of oxidation of D- and L-trytophan as well as the extents of their oxidations are not dependent on the isomer of tryptophan which served as the carbon source of growth[14].

Crude cell-free extracts were prepared by alumina-grinding followed by high-speed centrifugation to remove the particulate components. Using such extracts, it was demonstrated that simple dilution or ageing allowed the stoichiometric accumulation of anthranilic acid (V) from D-tryptophan (I*b*), L-tryptophan (I*a*), or L-kynurenine (III*a*). The formation of kynurenine from either D- or L-tryptophan could be shown when extracts were treated with semicarbazide. These same extracts degrade pyrocatechol (VI) to a compound which appears to be β-ketoadipic acid (XIV), as demonstrated by its ready decarboxylation under 4-aminoantipyrine catalysis[15]. These experiments confirm the results obtained from whole-cell experiments, which suggest that the pathway for tryptophan metabolism in this organism is indeed the aromatic one.

Fig. 2 shows an experiment which demonstrates that D-kynurenine (III*b*) is not on the pathway of D-tryptophan metabolism in *DT*-2. The experiment utilized an aged, crude soluble preparation incapable of anthranilic acid oxidation. Accordingly, tryptophan and kynurenine decomposition could be observed at 310 mμ, the alkaline absorption maximum of anthranilic acid (V). Using much larger amounts of enzyme, it could be shown that D-kynurenine is slowly converted to anthranilic acid. Its rate of conversion is, however, far too slow to account for the conversion of D-tryptophan. The relative rates of conversion of L-kynurenine (III*a*), L-tryptophan (I*a*),

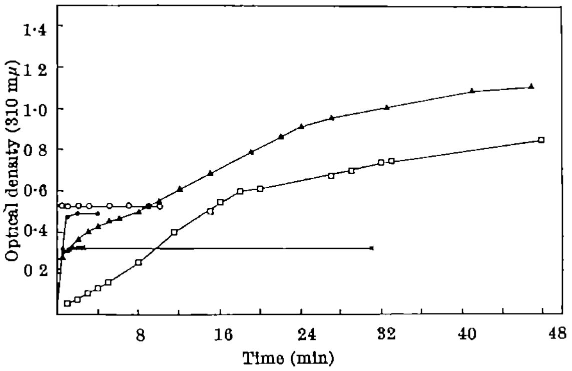

Fig. 2 Each cuvette contained· 0·1 ml. 0–40 per cent ammonium sulphate fraction (1 2 mg protein), 0 05 ml. rat liver formylase (Knox, W. E., in *Methods in Enzymology*, 2, 246, Academic Press, New York, 1955), 0·1 ml 0 02 *M* ascorbic acid (freshly neutralized), substrate additions as indicated in the figure, and 0·02 *M*, phosphate buffer *p*H 7·0 to a final volume of 3 0 ml. Temp., 26° C. ▲, 4 μmoles D-tryptophan + 0·5μ moles DL-kynurenine; □, 4μ moles D-tryptophan, ○, 0·5μ moles anthranilic acid, ●, 0·5μ moles L-kynurenine; ×, 0 5μ moles DL-kynurenine

D-trytophan (I*b*), and D-kynurenine (III*b*) to anthranilic acid (V) by the same enzyme preparation are, respectively, 100, 26, 9 and 0·1.

Anaerobic incubation of either D- or L- tryptophan with the crude soluble enzyme system resulted in the production of a racemic mixture. Ultra-violet spectrophotometry demonstrated that the tryptophan concentration at the end of the anaerobic incubation was unchanged. The extent of racemization was measured manometrically by following the oxidation of the L-isomer by moccasin venom L-amino-acid oxidase in the presence of catalase.

In later experiments, assays were performed spectrophotometrically at 328 $m\mu$, the absorption maximum of the enol-borate complex of indolyl-3-pyruvic acid produced, specifically from L-tryptophan, by L-amino-acid oxidase, the glutathione-activatable indole pyruvic acid tautomerase, and borate buffer[16,17]. No stimulation of the racemase activity could be demonstrated in crude preparations by the addition of pyridoxal phosphate, nor could any consistent stimulation be observed following ammonium sulphate precipitation and dialysis. However, the tryptophan racemase activity of eluates from DEAE-cellulose columns were consistently increased up to four-fold by the addition of pyridoxal phosphate with one-half maximal stimulation occurring at about 10^{-6} M. The purified enzyme has a *p*H optimum (*tris* buffer) at 8·3. The activity was inhibited by both 10^{-3} M hydroxylamine (90 per cent) and 10^{-3} M semicarbazide (70 per cent). No stimulation of the activity could be observed following the addition of 10^{-3} M pyridoxal, adenosine triphosphate, or glutathione.

Pyridoxal phosphate has been shown to be involved in a number of enzymatic amino-acid racemizations[18-21], while there is no evidence for its participation in other cases[22-24]; different mechanisms operate in other systems[25-27]. In the present instance, we could find no evidence for the α-keto acid, the α,β unsaturated acid, or the imino-acid as free intermediates. The most probable mechanism for tryptophan racemization is then through the azomethine formed by reaction with pyridoxal phosphate[28].

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CRIMP IN COTTON

Crimp as a New Characteristic of Cotton Fibres

CRIMP is an important property of wool fibres. Artificial crimping has been produced on synthetic fibres, culminating in 'stretch' or 'high bulk' textiles. The formation of artificial crimp has been reported for jute[1]. Since the geometry of the single fibre was thus shown to be an important fibre property, it was of interest to see whether a natural crimp occurs in cotton fibres. Preliminary observations have shown the appearance of certain waves on the cotton fibres. These observations were further investigated by applying the method of defining and measuring crimp of fibres[2-4], previously developed at this Institute, to cotton.

The crimp of cotton fibres was defined by two groups of parameters, each concerning a different aspect of the fibre properties: the static parameters describe the geometrical shape of the fibre under a given load, while the dynamic parameters characterize crimp by the behaviour of the fibre on extension. The measurement of the static parameters was carried out[3,4] on a crimp meter, and that of the dynamic parameters on the Instron tester.

Two independent static parameters were used[3,4]: the crimp diameter (*D*) and the crimp ratio (*C*). The values of the static crimp parameters were determined from two enlarged perpendicular projections of each fibre, obtained from the crimp meter[4]. The dynamic parameter of crimp is the specific uncrimping energy E_i/l_i, where E_i is the uncrimping energy and l_i the length of the fibre[5] in the uncrimped state at the *i*th extension cycle. Its value is determined from the load-elongation curve recorded on the Instron tester.

Two varieties of cotton were investigated: Deltapine 15 and Acala 1517. The mean values of the static and dynamic parameters obtained for 25 single fibres from each variety, having a test length of about 15 mm, were calculated according to the method of measurement previously defined[3,4]. The measurements were carried out in a controlled air-conditioned room at 20° C and 65 per cent relative humidity. The values of the crimp parameters for the Deltapine fibres were at all loads up to 25 mg found to be considerably higher than the corresponding values for the Acala fibres.

The mean values of D and C obtained when a load of 5 mg was applied to the fibres are given in Table 1.

Table 1

	Deltapine	Acala
D (mm)	0.31 ± 0.04	0.21 ± 0.05
$C \times 100$ (per cent)	6.8 ± 0.9	6.7 ± 0.7

The difference between the mean values of the crimp diameters of the Deltapine and Acala fibres was found to be significant at a 1 per cent level of confidence. On the other hand, no difference was obtained between the crimp ratio of these two varieties, thus indicating that D alone may characterize each of the tested varieties.

The values of the specific uncrimping energies (E_i/l_i; ref. 3) were calculated for fibres subjected to continuous extensions at a velocity of 0·1 cm/min up to a load of 1 g. Five subsequent extension cycles were carried out, one immediately after the other. It was found that a considerable decrease in the value E_i/l_i occurs in the second extension cycle for both cotton varieties, whereas no further significant decrease was noted in the subsequent cycles. The values obtained from the first cycle of each fibre tested deviated from the mean much more than in subsequent cycles. Therefore it seemed reasonable to choose the specific uncrimping energy of the second cycle as a representative dynamic parameter of the given variety.

The mean values for the first and second extension cycles are given in Table 2.

Table 2

Cycle (l)	Deltapine	Acala
$\frac{E_1}{l_1}$ $\frac{\text{g cm}}{\text{cm}} \times 10^{-3}$	3.60 ± 0.94	2.42 ± 0.48
$\frac{E_2}{l_2}$ $\frac{\text{g cm}}{\text{cm}} \times 10^{-3}$	1.85 ± 0.42	0.67 ± 0.18

The difference between the values of the specific uncrimping energies for the second cycle (E_2/l_2) was found to be significant at a 2 per cent level of confidence.

On close visual observation of the single fibres, the foregoing quantitative measurements are qualitatively confirmed, and the Deltapine fibres seem to be more crimped than the Acala fibres. It is to be expected that fibres with higher crimp should be more entangled in their lot. Hence the force (P) applied when separating a fibre from the lot was measured on the Instron tester, on 50 single fibres from each variety. The mean values obtained (Table 3) were found to be in accordance with the above visual impression and the crimp parameters measured:

Table 3

	Deltapine	Acala
P (mg)	295 ± 45	180 ± 35

The results show that the static, as well as the dynamic, parameters can be used as a sensitive means for differentiation between cotton varieties. It is interesting to note that no difference was found between the mean values of the cross-sectional area and of Young's modulus for the two varieties, indicating that the crimp of cotton fibres is a property of a given variety, not necessarily related to the fineness of the fibre or its general elastic behaviour.

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Some Aspects of the Behaviour of Crimp of Cotton Fibres

In handling the fibres during ginning, baling and withdrawal from the cotton lots, different forces are applied to each fibre. These forces cause deformations to the crimp of the fibre, and it seems that the recovery of crimp depends on the extent of the deformation as well as on time. The changes of crimp occurring when different loads are applied to a fibre were therefore examined and the recovery properties of the deformations, as a function of time, were investigated.

Two varieties of cotton were investigated: Deltapine 15 and Acala 1517. The dependence of crimp on load was studied by means of two independent static parameters: The crimp diameter (D) and the crimp ratio (C). The method for defining and measuring the values of these parameters were previously evolved at this Institute[2,4].

The mean values of D and C were calculated for 25 single fibres under five states of load (w), from 5 mg up to 25 mg. A decrease of the crimp diameter (D) with the load was observed for both cotton varieties, as shown in Fig. 1. This decrease may be represented (Fig. 2) by the simple functional relationship:

$$\frac{1}{D} = aw + b$$

The values of the constants a and b obtained for the two cotton varieties are given in Table 4.

Table 4

	Deltapine	Acala
a (mm^{-1} mg^{-1})	0·30	0·45
b (mm^{-1})	1·45	2·60

The values of the crimp diameter (D) of the Deltapine fibres were found to be significantly higher than the corresponding Acala values for loads up to 20 mg.

A decrease in the crimp ratio with load was also observed, but no difference between the values for the two varieties was found to exist for the loads applied. This decrease is represented in Fig. 3.

The recovery of crimp of fibres subjected to tensile deformations was measured by the dynamic parameters. The specific uncrimping energy ($E/1$), defined[3,5] as the amount of energy necessary to uncrimp a fibre of a unit length, was used. Its values were obtained from the load-elongation curve of each fibre, recorded on the Instron tester. The immediate recovery of crimp and the retarded recovery (after 24 h of relaxation) were measured.

The immediate recovery was examined by performing five subsequent cycles of extension (up to 1 g) at a velocity of 0·1 cm/min and unloading immediately one after the other. Twenty-five fibres from each variety, having a test length of about 15 mm, were investigated. A recovery of about 50 per cent from the initial specific uncrimping energy was obtained for the Deltapine fibres during the second extension cycle. The corresponding recovery for the Acala fibres was only about 25 per cent. These recovered uncrimping energies kept their values unchanged during the subsequent cycles of both varieties.

The retarded recovery was determined by performing two immediate extension cycles and then keeping the fibres in a relaxed condition for 24 h in a controlled air-conditioned room at 20° C and 65 per cent relative humidity. After rest, two successive additional extension cycles were carried out on each fibre. It was found that after the relaxation of the fibres the specific uncrimping energies of both cotton varieties increased to about 55 per cent from their original values, thus indicating a time-dependent recovery. At the fourth extension cycle, performed immediately afterwards, the specific uncrimping energies were again reduced to about the same values as at the second cycle.

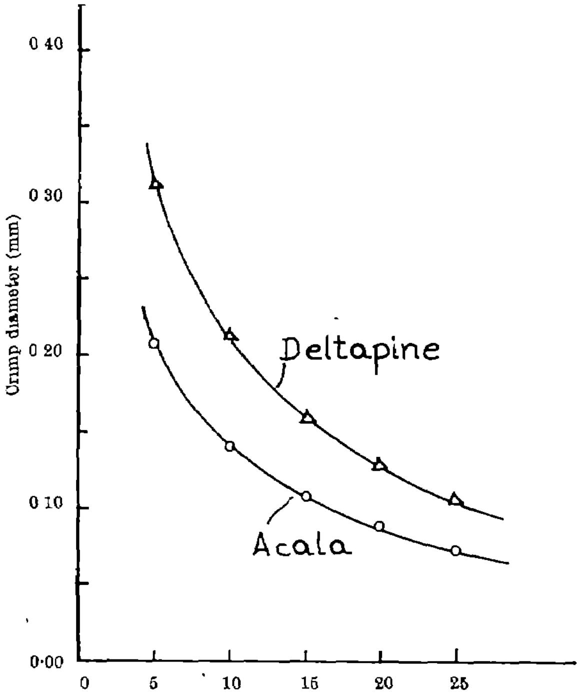

Fig. 1. Dependence of the crimp diameter (*D*) on the load (*w*)

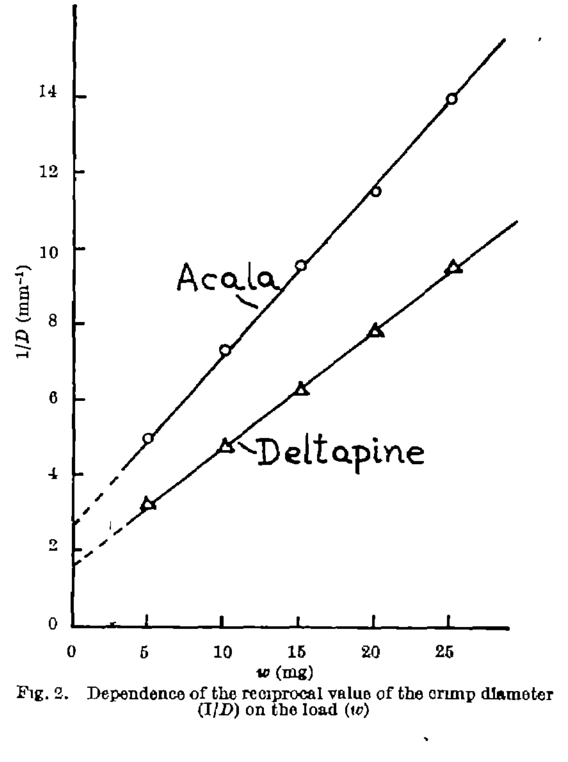

Fig. 2. Dependence of the reciprocal value of the crimp diameter (1/*D*) on the load (*w*)

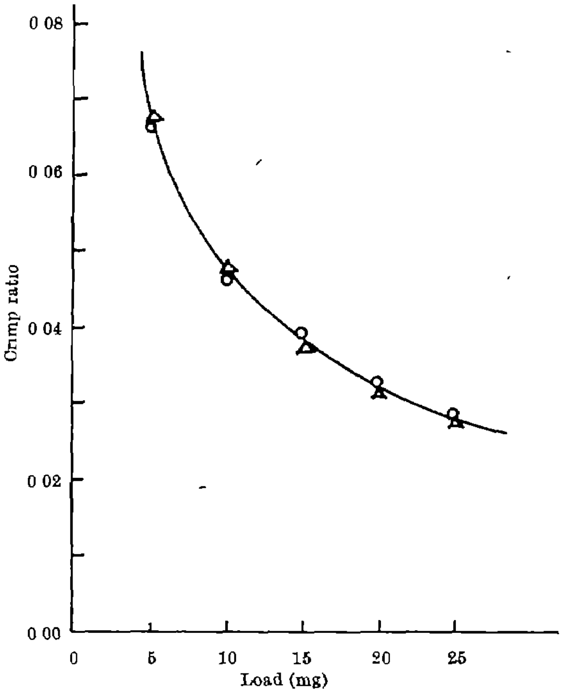

Fig. 3. Dependence of the crimp ratio (*C*) on the load (*w*) Δ, Deltapine; o, Acala

These results on the recovery of crimp show that the deformation of crimp which occurs during the extension of the fibre is composed of a reversible and an irreversible part. The reversible deformation includes an immediate elastic recovery and a retarded recovery. The immediate recovery of the specific uncrimping energies obtained at the second extension cycle keeps its value unchanged during the immediate subsequent cycles. It follows, therefore, that its value is independent of the forces applied to the fibre (up to 1 g). It appears that this stable part of the crimp of cotton is the important factor in the processing of fibres into yarns and fabrics, and affects the properties of the finished goods.

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LETTERS TO THE EDITOR

COSMOLOGY

Radio Background as a Criterion for Cosmological Models

As I have recently shown[1], the number counts of extragalactic radio sources according to the latest Cambridge survey[2] imply that in an evolutionary cosmology there is a minimum rate of evolution of the power of the radio sources, which depends on the time variation assumed for the spatial density of the sources. The particular cases when the spatial density of the sources is always proportional to that of ordinary galaxies and, alternatively, to the square of that of the galaxies, were found to require a diminution of mean source power exceeding 1·4 and 0·2 per cent per 10^8 years, respectively, at the present epoch. More generally, when no definite assumption was made regarding mean source density $\bar{n}(t)$ and mean source power $\bar{P}(t)$, it was found that agreement with the Cambridge results required a minimum present rate of diminution of the product $\bar{n}(t)\bar{P}^2(t)$ equal to 5 per cent per 10^8 years. In these calculations the Hubble time T was taken to be $1{\cdot}3 \times 10^{10}$ years.

The question now arises as to how this kind of evolution affects the radio background intensity at a given frequency due to the radio sources. Since it now appears possible to isolate the contribution of the radio sources to the background from that due to the normal galaxies[3], this observational approach to the cosmological problem may prove to be a very valuable one.

To deal with this matter consider any isotropic world model whose metric, in the usual notation, is:

$$ds^2 = c^2dt^2 - \frac{R^2(t)\{dr^2 + r^2d\theta^2 + r^2 \sin^2 \theta d\varphi^2\}}{(1 + kr^2/4)^2} \quad (1)$$

Let $E(\nu,t)$ (watts ster^{-1} (c/s)$^{-1}$ parsec^{-3}) be the emissivity of space at frequency ν and epoch t due to the radio sources of spectral index x. Then the sky brightness at the observer, at frequency ν_0 and epoch t_0, will be:

$$B(\nu_0,t_0) = c\int_{t^*}^{t_0} \left\{\frac{R(t)}{R(t_0)}\right\}^{3+x} E(\nu_0,t)dt \quad (2)$$

where t^* is the earliest epoch of emission of radiation contributing to the local radio background. In the case of the simple steady state model, in which $R(t) = e^{t/T}$ (T constant) and $E(\nu,t)$ is statistically independent of t, this yields:

$$B(\nu_0,t_0) = \frac{cT\,E(\nu_0,t_0)}{3 + x}$$
$$= 0{\cdot}26\, cT\, E(\nu_0, t_0), \text{ if } x = 0{\cdot}8 \quad (3)$$

In the evolutionary models the function $E(\nu,t)$ must be related to the power and spatial density of the sources since these depend on the epoch. The different power classes of the sources may be suitably labelled according to their emissive power P_0 (watts ster^{-1} (c/s)$^{-1}$) at the observed frequency ν_0 and at the epoch of observation t_0. Then, if $\bar{P}_0$ is a suitably defined mean power of the objects (observed in a given range of flux density) at the same frequency and epoch, the emissive power of the class P_0, at frequency ν_0 but at general epoch t, may be denoted by $P(l,t)$, where $l \equiv \log_{10}(P_0/\bar{P}_0)$ and $P(l,t_0) \equiv P_0$. Suppose now that the spatial density at epoch t of objects in the power class P_0, $P_0 + dP_0$, or equivalently l, $l + dl$ is $\eta(l,t)dl$. It follows that the emissivity due to all classes, at frequency of observation ν_0 but at the general epoch of emission t, is:

$$E(\nu_0,t) = \int_{-\infty}^{\infty} \eta(l,t)P(l,t)dl \quad (4)$$

It will be sufficiently general for the purpose to assume that for a limited range of past time:

$$\eta(l,t) \propto \{R(t)\}^{-p} \quad (5)$$

for all l, where p is a disposable constant. The cases already mentioned of proportionality of radio source density to that of the galaxies, and to the square of that of the galaxies, are given by setting $p = 3$ and $p = 6$ respectively. It is then appropriate to take:

$$P(l,t) \propto \{R(t)\}^{-q} \quad (6)$$

where q is a disposable constant. Hence, for the sky brightness at the observer due to objects of spectral index x one finds:

$$B(\nu_0,t_0) = c\, E(\nu_0,t_0)\int_{t^*}^{t_0} \left\{\frac{R(t)}{R(t_0)}\right\}^{3+x-p-q} dt \quad (7)$$

To agree with the number counts p and q cannot be arbitrary, as I have stated at the outset. In fact, taking $x = 0{\cdot}8$ the following inequality must be satisfied (equation (4·9) of my earlier paper[1]):

$$p + 2q > 6{\cdot}6 \quad (8)$$

I have verified that, for the special family of models in which $R(t) = t^n$ (n constant) and $k = 0$, this condition is necessary for a rising $\log (N/N_0) - \log S$ curve[2], at least as far as the peak of the Cambridge curve ($S \simeq 6 \times 10^{-27}$ watt m^{-2}(c/s)$^{-1}$ at 178 Mc/s). Consider as a representative example the Einstein–de Sitter model in the cases $p = 3$ and $p = 6$ and take t^* to correspond to the peak of the Cambridge curve.

(1) $p = 3$, $q > 1{\cdot}8$. For $q > 1{\cdot}8$ the contribution to the sky brightness from sources in any range of flux density S must exceed that for the case $q = 1{\cdot}8$ since $N(S)$ at each S will be greater. In the case of the Einstein–de Sitter model when $q = 1{\cdot}8$ I have shown[4] that at $S = 6 \times 10^{-27}$ watt m^{-2} (c/s)$^{-1}$ the corresponding mean epoch of emission is t^* where $t^*/t_0 = 0{\cdot}11$, taking $\bar{P}_0 = 10^{26}$ watts ster^{-1} (c/s)$^{-1}$ (at 178 Mc/s) as the Cambridge[2] evidence appears to suggest. Hence, substitution of these values gives as a lower limit for $B(\nu_0,t_0)$ in the Einstein–de Sitter model when $p = 3$:

$$B(\nu_0,t_0)_{\min} = 1{\cdot}04\, cT\, E(\nu_0,t_0) \quad (9)$$

(2) $p = 6$, $q > 0{\cdot}3$. A similar calculation yields a lower limit for $B(\nu_0,t_0)$, by setting $q = 0{\cdot}3$ and again integrating up to $S = 6 \times 10^{-27}$ watt m^{-2}(c/s)$^{-1}$ where now the mean epoch t^* of emission is found to satisfy $t^*/t_0 = 0{\cdot}34$. For this case

$$B(\nu_0,t_0)_{\min} = 1{\cdot}06\, cT\, E(\nu_0,t_0) \quad (10)$$

It appears, therefore, that for two widely differing but plausible values of the source density as a function of the epoch, assuming for each case the corresponding minimum possible rate of power evolution to give a rising $\log(N/N_0) - \log S$ curve, the Einstein–de Sitter cosmology predicts much the same sky brightness due to the radio sources of spectral index 0·8. Furthermore, for the assumed value of $\bar{P}_0$ this minimum sky brightness is 4 times that expected in the steady state cosmology without minimizing. It follows that if the Cambridge estimate of the luminosity function of not too distant sources were confirmed, assuming present possible ambiguities[5,6] had been removed from it, then $E(\nu_0,t_0)$ would be known and another criterion for cosmological models would be available.

In this connexion, investigators[7] at Jodrell Bank have claimed a considerably lower value for $\bar{P}_0$ (about $2{\cdot}5 \times 10^{24}$ watts ster^{-1} (c/s)$^{-1}$) than the value assumed here on the basis of the Cambridge evidence. In this case the peak of the Cambridge $\log(N/N_0) - \log S$ curve would correspond to a much nearer point in space than hitherto believed, and therefore the contribution to the radio background from more distant sources might be of the same order as that due to the sources within the survey. However, assuming the most reasonable variations of power and density beyond the point $S = 6 \times 10^{-27}$ watt m^{-2} (c/s)$^{-1}$, calculations show that in this case, both for $p = 3$ and $p = 6$, the background intensity for the Einstein–de Sitter model can be fairly definitely restricted to lying between $0{\cdot}2\ cT\ E(\nu_0,t_0)$ and $0{\cdot}5\ cT\ E(\nu_0,t_0)$. This differs from the result (3) for the steady state model by a factor of less than 2. Therefore, if the Jodrell Bank interpretation were proved correct measurements of the radio background would be less likely to provide a useful distinction between cosmological models.

Finally, a point worth observing is that since $\bar{n}_0\bar{P}_0^{3/2}$ is a known constant obtained from the number counts ($\bar{n}_0$ being the mean number of sources locally per unit proper volume of space), then $E(\nu_0,t_0) \propto \bar{n}_0\bar{P}_0 \propto \bar{P}_0^{-\frac{1}{2}}$. Hence on the basis of the Jodrell Bank luminosity function the expected background intensity in any cosmological model would be about six times that expected on the basis of the Cambridge luminosity function.

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RADIOPHYSICS

Decametric Wave-length Absorption resulting from the Nuclear Detonation of July 9, 1962

Following the high-altitude nuclear detonation on July 9 at Johnston Island there was observed a pronounced effect on the 10·006-Mc riometer data taken at the Air Force Cambridge Research Laboratories, Sagamore Hill Radio Observatory, in Hamilton, Massachusetts (lat. 42° 38′, long. 70° 49′). This riometer consisted of a Collins *R–390A–URR* receiver with a band-width of 1 kilocycle, and a dipole antenna oriented in an east–west direction.

Onset of the absorption occurred (Fig. 1) between 0403 and 0408 E.S.T. ($H + 3$ to $H + 8$ min), reached a maximum drop in signal-level of 6·9 db. at 0421 E.S.T. ($H + 21$ min), and was essentially back to normal by 0500 E.S.T.—slightly less than 1 h after the onset.

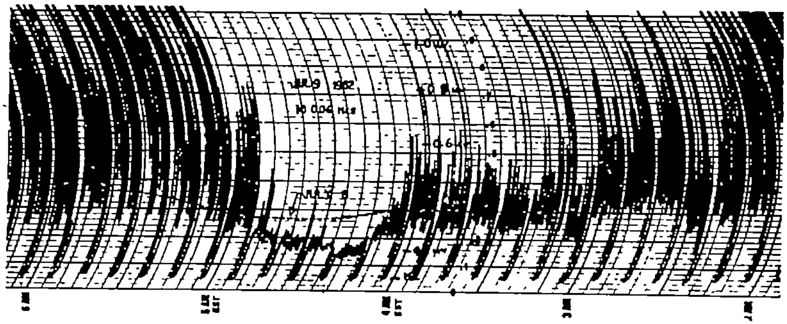

Fig. 1. 10 Megacycles absorption record of July 9, 1962, with previous day-level shown by dashed curve

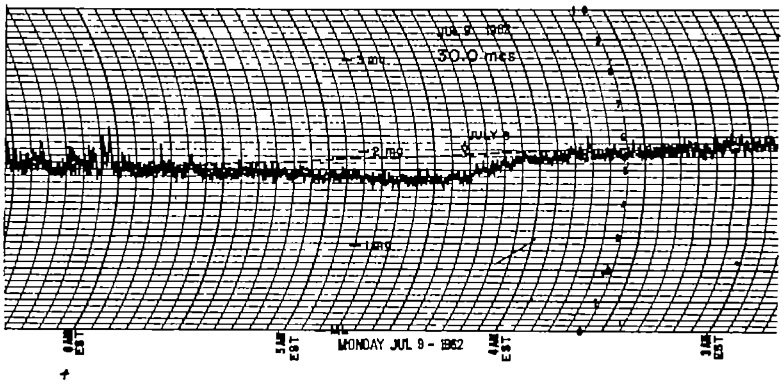

Fig. 2. 30 Megacycles absorption record of July 9, 1962, with previous day-level shown by dashed curve

Exact time of the start of the absorption was difficult to obtain as the normal sunrise effect decrease begins about this time of morning. Sunrise time for the *D*-layer altitude on July 9 occurred at about 0354 E.S.T. The plotted previous day-level (July 8) shows the normal decrease expected and, in fact, shows the largest decrease over the three days preceding the event day. On the actual day, no unusual solar activity was reported by other observatories. The decrease noted on July 9 was thus much greater than would normally have been expected.

Due to the large receptive angle of the dipole antenna, propagated signals, mainly of the teletype nature, are also recorded as part of the riometer signal. This gives uncertainty to the degree *D*-layer absorption contributes to the 6·9 db. drop in signal-level. Characteristics of the propagated signal is the increased excursion width of the recorded signal. With the increased excursion width some change in base-level occurs, but this should be small due to the fast time constants used in the receiver.

The drop-out of propagated signals during the event was pronounced. Levels shown for 0·5 h prior to 0400 E.S.T. represent the 'smallest' propagated excursions expected at this time of morning—in particular when compared with the larger excursion values obtained for the days of July 6, 7, and 8.

Sagamore Hill is situated almost 39° geomagnetically north of Johnston Island, thus any trapped particles must have passed well to the south of this Station as the sheath encircled the globe. This sheath appears to have been mainly responsible for the drop-out of the propagated signals, especially when they originated from the southern azimuths to this station.

That a true *D*-layer absorption occurred at this high a latitude is confirmed by results from a 30-Mc riometer also located at this Station. From Fig. 2 it can be seen that a similar decrease occurred at this shorter wave-length. Sunrise effect decreases are normally not observed with this equipment. The antenna used is a *yagi* pointed to the celestial-polar region of the sky—thus limiting observations to a more selective area and in the northern quadrant of azimuth. The change in db. was much less than that of the 10 Mc, as would be expected by the inverse-frequency-squared law of absorption; and may in part also be due to the beam orientation used. Maximum absorption of the 30-Mc signal was of the order of 0·5 db.

D-layer absorption occurring at this site may be the result of prompt neutrons from the burst decaying into β-particles, which were then guided into the *D*-layer via magnetic field lines[1]. These β-particles could have come in field lines some distance from the Sagamore Hill site and created a prompt absorption at that injection point. The travel time for the disturbance to reach this Station would then account for the delay time noted for the onset of absorption.

Detection of the effects of a high-altitude nuclear detonation with measurements made a large distance away from the detonation area, and well removed from the trapped radiation sheath, are well illustrated by these 10- and 30-Mc absorption observations.

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GEOPHYSICS

Observation of the (0,0) Band of the ($^1\Delta_g - {}^3\Sigma_g^-$) System of Oxygen in the Day and Twilight Airglow

THE (0,1) band of the ($^1\Delta_g - {}^3\Sigma_g^-$) system of oxygen was first observed in the twilight airglow in 1958 by Vallance Jones and Harrison[1]. The (0, 0) band, although theoretically at least 10 times stronger, was not detected from the ground because of re-absorption by oxygen in the lower atmosphere.

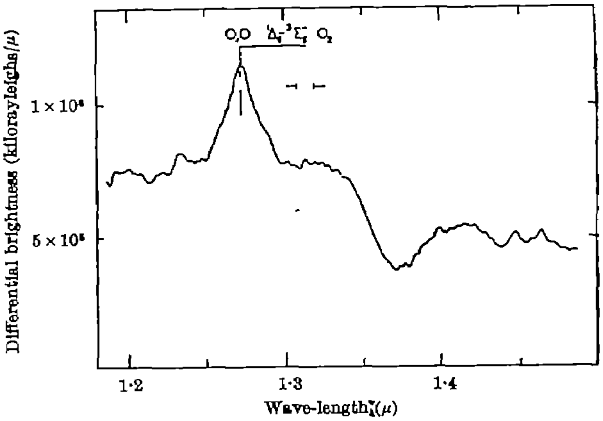

Fig. 1. Dayglow spectrum obtained from a height of 13 km at a zenith angle of 55°. The (0,0) oxygen (O_2) band is shown. The spectral slit width was 100 Å and the solar zenith distance was 40°

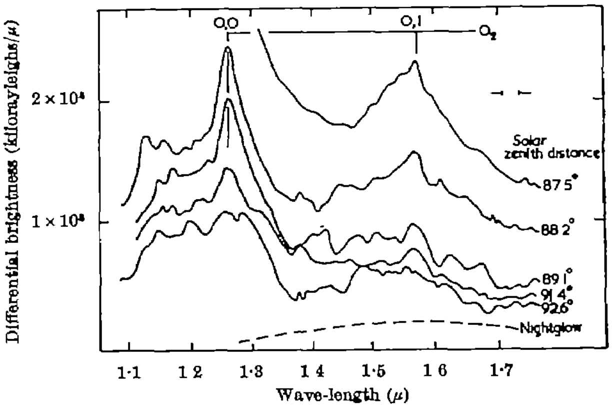

Fig. 2. Twilight airglow spectra obtained from a height of 13 km at a zenith angle of 75°. The spectral slit width was 250 Å and the solar zenith distance varied between 87·5° and 92·6°

Recently, it has become possible to make observations with a grating spectrometer flown in a *KC*–135 aircraft at a height of 13 km, and a successful observation of the (0,0) band in the day and twilight airglow has been accomplished.

Fig. 1 shows a dayglow spectrum obtained at a solar elevation angle of 50° and Fig. 2 shows a set of twilight spectra. The line of sight of the spectrometer was at 90° to the direction of the Sun in azimuth, and was at zenith angles of 55° and 75° for the dayglow and twilight spectra, respectively. The absolute brightnesses were obtained by comparison with the spectrum of a standardized low-brightness source. The (0, 0) band is present in the spectra at 1·27μ and is superimposed on a background due to scattered sunlight; in the present observations the weaker (0, 1) band was largely masked by noise.

The integrated areas of the oxygen band were measured after subtraction of the background and the total brightnesses of the oxygen band derived. These brightnesses were reduced to zenith by multiplying by cosine of the zenith angle and were corrected for molecular oxygen absorption by using the approximate value of the band extinction coefficient of 2·2 km^{-1} given by Vallance Jones and Harrison. This will give an approximate value only for the true brightness of the emission, but it is probably correct within a factor of two or three even at the large zenith angle.

Fig. 3 shows the results of the dayglow measurements obtained on April 1, 1962, at 45° N. and the twilight measurements obtained on February 4, 1962, at 20° N, plotted as a function of solar elevation angle. The dayglow intensity of the (0, 0) band is close to 10,000 kilorayleighs, which is in agreement with theoretical estimates made by Vallance Jones and Gattinger[2] in the course of their interpretation of the intensity and decay of the (0, 1) band in the twilight. Their interpretation ascribes the atmospheric emission of the $(^1\Delta_g - {}^3\Sigma_g^-)$ bands to the production of excited oxygen molecules by photodissociation of ozone. These preliminary observations also show that the twilight decay of the bands begins noticeably before sunset. This is to be expected, since the exciting solar radiation penetrates less deeply into the atmosphere as the Sun nears the horizon.

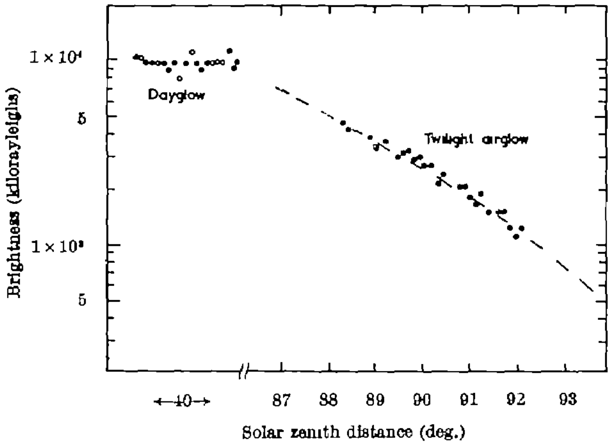

Fig. 3. The brightness of the (0,0) oxygen (O_2) airglow band as a function of solar zenith distance, as derived from Figs. 1 and 2. The brightnesses have been reduced to the zenith and corrected for absorption by atmospheric oxygen

The (0, 0) oxygen band is by far the most intense feature yet observed in the day airglow. The only other dayglow emissions detected so far are the sodium lines at 5893 Å (ref. 3) and the red lines of atomic oxygen at 6300 Å (ref. 4); both emissions have a zenith intensity of about 30 kilorayleighs.

This work was sponsored by the Geophysics Research Directorate, Air Force Division, Office of Aerospace, U.S. Air Force, under contract *AF* 19(604)-7265 with the University of Saskatchewan and also supported in part by a grant to Harvard University from the National Science Foundation. Installation of the scanning spectrometer aboard the *KC*-135 aircraft was made possible through the cooperation of Dr. G. Gassmann and Mr. T. P. Markham, of the Air Force Cambridge Research Laboratories.

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PHYSICS

Elementary Particles

THERE appears to be a relation between some basic laws of physics and the numerical value of certain natural constants when dimensional units are defined in a particular way.

The velocity of light is a universal constant and it can be assigned a value of one. From the uncertainty principle of Heisenberg, the product of the standard deviation of the position of a particle and the standard deviation of the momentum of a particle equals $\hbar/2$. If the dimensional units are assigned so that the standard deviations or variances equal one, then $\hbar = 2$. The square of the electron charge (e^2) then equals twice the fine structure constant or $2a$.

Suppose $y = f(x)$, where x is a random variable of mean zero and variance one. Then $y = \exp - x^2/2$ and the area squared equals 2π. When $x = \pm\pi$ then $y = \exp - \pi^2/2$. If $\pm\pi$ equals rest mass then y corresponds to charge. These may be the parameters of the components of all particles. If similar components combine to give a mass of 2π then the Compton wavelength equals 2 since $M\lambda$ equals 4π. The charge squared should be a function of $2 \exp - \pi^2/2$. The function seems to be $2 \exp(-\pi^2/2 + 2 \exp(-\pi^2/2 + 2 \exp - \pi^2/2))$ (ref. 1).

The difficulties in locating basic particle[2] suggest $Me/e^2 = Mp/g^2 = \pi/a$, where Mp equals the proton mass and g^2 is the nuclear force constant. In this case $g^2 = 2a$ when Mp is assigned the value 2π (ref. 3). Electrons and protons are then similar combinations of stationary states of the random variable.

If rest mass has a sign then combinations of opposite sign for charge and mass will have zero rest mass and zero charge. Energy is a random variable and can take any value. The neutrino is a particle with these properties.

The important role of chance and stationary states in basic processes is evident in the quantum theory. Perhaps these basic factors can also account for the various elementary particles and the numerical values of the constants which identify them.

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Dependence of the Anisotropy Axis Dispersion on Thickness of Thin Nickel-Iron-Cobalt Films

THE measurement of easy axis dispersion in thin ferromagnetic films is of interest in connexion with the explanation of their magnetic behaviour. Clow[1] has reported the variation of the dispersion of easy axis with film thickness for evaporated specimens of 82-18 per cent nickel-iron alloy in the thickness-range 50-2000 Å. In the work reported here, measurements of dispersion were made by a slightly different method on films of nickel-iron-cobalt alloy in the range 400-6000 Å in thickness.

The method of measurement used here has been described by Crowther[2] and is the low-frequency counterpart of the technique suggested by Peacock and Winsor[3]. A loop plotter, similar to the type described by Crittenden *et al.*[4], was used to observe the transverse flux pick-up when the film was switched with a 400 c.p.s. alternating field. The angle between the field and the hard axis of the film was varied until the net flux observed was equal to one-half of the maximum possible flux. The angle α_q between the field and the mean hard direction was measured. This angle was of the order of 1–4° and could be measured with an accuracy of ± 1/4°. Clearly this angle was such that when the applied field was zero, 75 per cent of the film had become magnetized in one sense along the easy axis. Measurements of α_q were made on films evaporated in a vacuum of approximately 10^{-5} mm mercury on to aluminium substrates held at an elevated temperature. Two batches of films were measured, each consisting of a series of successive evaporations from ingots of composition 80 per cent nickel–17 per cent iron–3 per cent cobalt. The difference between the two batches lay in their substrate temperature.

The substrates measured 11 cm × 8 cm and at their corners the films showed an easy axis dispersion characteristic of angle-of-incidence anisotropy[5]. Since, however, the pick-up coil of the loop plotter was of such a shape that it effectively detected flux changes only in a narrow strip across the centre of the specimen, contributions to the measurements from this source would not be expected.

Fig. 1 shows the measured variation of α_q with thickness for the two batches of specimens. It is seen to be roughly constant above about 1500 Å thickness, rising steeply as the thickness falls below that value. The difference between the curves for the two batches suggests that there may be a dependence of the dispersion on substrate temperature for thick films.

The dependence on thickness shown in Fig. 1 is so different from that reported by Clow for nickel–iron films that different mechanisms must be operating in the two cases.

To explain the dependence observed in nickel–iron films, Clow[1] has suggested that the dispersion increases with grain size, which might be expected to rise with increasing film thickness. Since the actual dependence of grain size on thickness has not been reported for either nickel–iron or nickel–iron–cobalt films, comparison on the basis of this model is impossible. However, there is some evidence[6] that the measured amplitude of dispersion is dependent on the self-demagnetizing field of the specimen, but only when this field becomes comparable to the anisotropy field. The nickel–iron specimens used by Clow were of a constant diameter of 1·5 mm (Clow, H, private communication), so that the demagnetizing field of a 2000 Å specimen was about 1 œ, compared with an anisotropy field H_k of 3 œ. The observed variation of dispersion may have been a result of the demagnetizing fields present. In the present specimens, however, the demagnetizing field of the thickest specimen was less than 0·05 œ, so that this explanation would not apply.

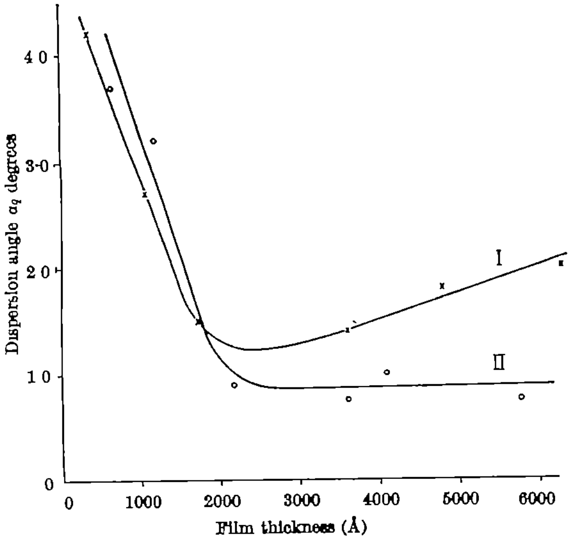

Fig. 1. Variation of dispersion angle α_q with film thickness for nickel–iron–cobalt films. I, Films with substrate temperature 350° C, H_k = 5–6 œ; II, films with substrate temperature 150° C, H_k = 10–11 œ

It has been reported that in the initial stages of growth the film consists of isolated islands of material, and that in this state the film displays super-paramagnetism[7]. The easy axes set up in this initial layer of material will probably be randomly oriented. As the film becomes thicker, the easy axes will become more closely aligned, owing to the high internal field of the ferromagnetic state. The influence of the initial layer with highly dispersed easy axis may be felt up to greater thicknesses. Such a model accounts qualitatively for the observations reported here.

I thank Mrs. J. Phillips for assistance in the preparation of the films.

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(Engineering) Ltd.,
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Correction to the High-Pressure Scale

CALIBRATION of the pressure scale may be accomplished by determining the pressure associated with, for example, volume discontinuities of known materials, such as bismuth[1]. The question arises as to whether the pressure on the calibration material is the same as the pressure generated by a ram or a surrounding liquid or solid medium.

The calibration material may be in a container that is closed, or open at top and bottom (a sleeve). When in a closed container, it can be shown that the pressure external to the container is different, in general, from the pressure within the container[2]. Whether in a closed or open container, the stresses on the contained material are not isotropic, with average stress in the specimen different from any applied stresses.

Many calibrations of specimen materials (bismuth and cæsium, for example) have been performed with specimens in containers of materials less compressible than the specimen materials. This practice has led to measured pressures (outside the containers) higher than within.

Transition points used for calibration of the absolute pressure scale in this manner so far reported are consequently in error by amounts dependent on relative physical properties and sizes of containers and specimens. Preliminary calculations indicate these errors can be several per cent of reported pressures.

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GEOCHEMISTRY

Further Isotopic Age Measurements on Rocks from North-eastern Greenland

SUBSEQUENT to the publication of our earlier communication[1] a few additional critical samples of basement rocks from north-eastern Greenland were obtained and analysed. These are given in Table 1. The rubidium–strontium determination on the potassium feldspar cogenetic with the biotite from the alkali granite (*GAA*–135) confirms the presence of an ancient basement in this region. Even this age of 2,290 ± 70 m.y. must be regarded as a minimum due to the possibility of earlier metamorphic events. Since the potassium–argon on the biotite from this rock gave an apparent isotopic age of 1,900 ± 50 m.y., it seems unlikely that the ^{87*}Sr/^{87}Rb ratio of the feldspar was affected by the Caledonian orogeny.

Table 1. ISOTOPIC AGES FROM ROCKS IN NORTH-EASTERN GREENLAND

I. Pre-Cambrian Basement

Sample No.	Rock type	Mineral	Isotopic age (m.y.)	Rb p.p.m.	Normal Sr p.p.m.	Radiogenic Sr p.p.m.	Location
GAA-135	Alkali Granite	Feldspar	Rb/Sr = 2290 ± 70	455	8·80	4·41	70° 20′ N./29° 47′ W.

II. Caledonian Infra-structural Rocks (Pre-Middle Devonian)

Sample No.	Rock type	Mineral	K/Ar Isotopic age (m.y.)	K (per cent)	^{40}Ar s.c.c./gm.	Location
GW-73*r*	Schist	Muscovite	K/Ar = 410 ± 10	6·74	$1·23 \times 10^{-4}$	73° 11′ N./27° 40′ W.
JH-146	Granodiorite	Biotite	K/Ar = 395 ± 10	7·29	$1·27 \times 10^{-4}$	73° 20′ N./26° 50′ W.

The potassium–argon ages on the muscovite from the schist (*GW*—73*R*) which was developed during the main phase of the orogeny and the biotite from the syn-orogenic granodiorite (*JH*–146) further confirm the observation that the Caledonian activity around the North Atlantic ended by 390 m.y. ago in Lower Devonian time.

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CHEMISTRY

Kinetic Law in the Adjustment Period of Natta's Polymerization

THE Ziegler–Natta polymerization of α-olefines has been widely investigated by many workers mainly from the practical point of view. On the other hand, the details of the reaction mechanism are not yet clear. There are, of course, some basic investigations of the reaction by Natta *et al.*[1], one of which is considered here; that is, the kinetic behaviour of the polymerization of propylene in the initial stages (Natta's 'the adjustment period'), where the polymerization-rate, r, changes with the polymerization time, t, from the initial rate, r_0, at $t = 0$, to the stationary rate, r_∞. The phenomenon can be divided into two types, that is, the build-up type and the decay type. The decay type was observed with the ground catalysts (sizes $\leqslant 2\mu$), while the build-up type was observed with the unground ones ($1 \sim 100\mu$). Analysing the latter types of rate change, Natta *et al.* found that:

$$t_N \propto 1/P \tag{1}$$

$$\ln t_N \propto 10 \text{ kcal}/RT \tag{2}$$

where t_N is the polymerization time at which the rate equals 3/4 of the value of the stationary rate, r_∞, and P the pressure of propylene. Natta suggested that the stationary reaction corresponds to a definite particle size of the catalyst, and any other catalyst with a different particle size would alter to this definite size.

In order to compare the kinetic behaviours of the two types, I have carried out the polymerization with the active catalyst (titanium trichloride-'ARA' of Stauffer Co., Ltd.) and obtained some decay curves as shown in Fig. 1. Analysing the results, we have:

$$(r - r_\infty)/(r_0 - r_\infty) = \exp(-k_K t) \tag{3}$$

$$k_K = AP\exp(-6·5 \text{ kcal}/RT) \tag{4}$$

$$r_\infty = BP\exp(-10 \text{ kcal}/RT) \tag{5}$$

$$r_0 = CP\exp(-9·8 \text{ kcal}/RT) \tag{6}$$

where A, B, and C are the frequency factors. Here, it should be noted that the rate equation of the stationary polymerization (5) is in agreement with Natta's result, except for the value of B. My value of B is larger than that reported by Natta, which may be attributed to the large surface area of 'ARA' (about 20 m^2/g by BET method with nitrogen adsorption).

From equation (3) we have, denoting by t_K, the time at which the rate equals 4/3 of r_∞:

$$t_K = \frac{1}{k_K}\ln\frac{3(r_0 - r_\infty)}{r_\infty} = \frac{\text{const.}}{k_K} \propto \frac{1}{P} \tag{7}$$

and from equations (7) and (4):

$$\ln t_K \propto \ln\frac{1}{k_K} \propto 6·5 \text{ kcal}/RT \tag{8}$$

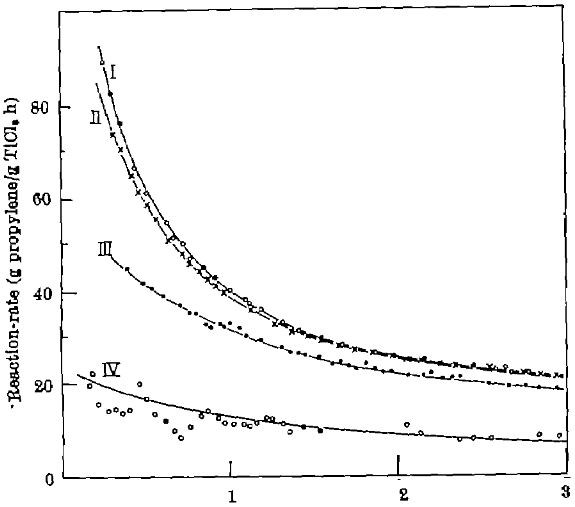

Fig. 1. Propylene polymerization-rate at different temperatures and pressures as function of polymerization time: I, 60° C, 550 mm mercury; II, 44° C, 650 mm mercury; III, 27° C, 710 mm mercury; IV, 10° C, 740 mm mercury. Other conditions are approximately the same (Ti ~ 2 g/L; Al/Ti ~ 1)

These relationships are formally the same as equations (1) and (2), respectively. In view of this, the following expression may be attributed for the curves of Natta's build-up type:

$$(r_\infty - r)/r_\infty = \exp(-k_N t) \tag{9}$$

with

$$k_N = A'P \exp(-10 \text{ kcal}/RT) \tag{10}$$

Furthermore, it should be pointed out that we have a new equation, differentiating equations (9) and (3) with respect to t, as:

$$\frac{dr}{dt} = -k(r - r_\infty) \tag{11}$$

where k is k_K or k_N.

The last equation, thus derived, is the kinetic law in the 'adjustment period' of Natta's propylene polymerization.

I thank Drs. R. J. Cvetanivić, S. Bywater, Y. Amenomiya and S. Affrossman for discussions.

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Catalytic Decomposition of Cobalt (III) Hexammine Cations on the Surface of Montmorillonite

THE stability of crystalline cobalt (III) hexammine chloride up to 100° C and in solution is well known[1]. Morel[2] has proposed the use of $Co(NH_3)_6^{3+}$ for the determination of the cationic exchange capacity of clay. The fast colorimetric determination of an excess of reagent would provide an accurate routine analysis for cationic exchange capacity of clay minerals such as kaolinite and montmorillonite; this method gives results in agreement with the classical ones, but with an alumino-silicate gel (cracking catalyst) steric hindrance precludes such an agreement.

Table 1

	(a)	(b)	(c)	(d)
Montmorillonite	100	118	100	112
Cracking catalyst	118	40	—	—

Cationic exchange capacity (m.equiv./100 g oven-dried, at 105°) data obtained from ammonium acetate method (*a*), from cobalt (III) hexammine colorimetric determination (*b*), from direct Kjeldahl determination (*c*) and indirect Kjeldahl determination (*d*).

Since the infra-red data for $Co(NH_3)_6Cl_3$ have been thoroughly examined by several authors[3-5], it may be of interest to compare them with the spectra obtained for the complex cation adsorbed on a clay surface. The film technique[6] is especially suitable for this purpose and montmorillonite constitutes, from this point of view, a very good material.

When films of lyophilized or air-dried montmorillonite saturated with $Co(NH_3)_6^{3+}$ at different pH are examined, besides the spectral features belonging to the clay lattice, bands are found which may be obviously attributed to the complex.

If the same films are either outgassed under vacuum at room temperature or dried at temperatures up to 100° C, the decomposition of the complex occurs within a few minutes, resulting in a considerable transformation of the spectra. The new bands indicate that ammonia evolved is strongly adsorbed as ammonium ions. When the sample is dried under vacuum at low temperature in a cell similar to that proposed by Wagner and Hornig[7] the complex is not destroyed. Fig. 1 shows the spectra obtained under these different conditions. In a recent paper, Mortland, Fripiat, Chaussidon and Uytterhoeven[8] demonstrated the very strong affinity of acid- or base-saturated montmorillonites for gaseous ammonia, the chemisorbed species existing as ammonium ions. For base-saturated montmorillonites they explain this transformation as follows: the chemisorbed water molecules are strongly polarized by the exchangeable cations and do exist in a dissociated form. NH_3 reacts with a proton giving NH_4^+. If montmorillonite saturated by $Co(NH_3)_6^{3+}$ is either dried or outgassed, the proton of residual water is available for the ammine ligands and the complex is destroyed. The charge balance is restored by hydroxyl anions produced by water dissociation.

When the complex has been destroyed exchange with sodium does not remove the cobalt ion but NH_4^+

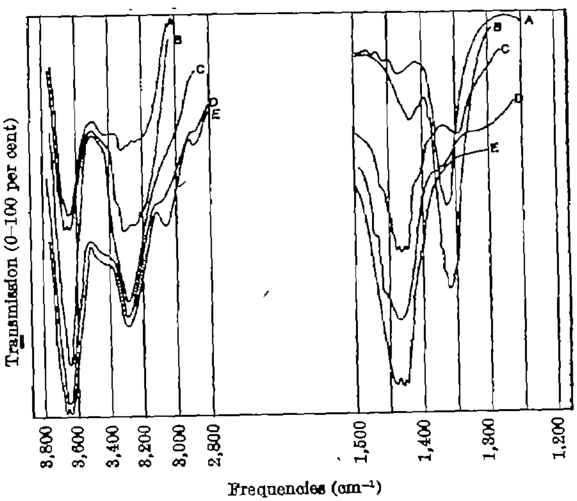

Fig. 1. *A*, dried at 20°; *B*, cooled and outgassed; *C*, outgassed at 20°; *D*, heated at 100°; *E*, NH_4-montmorillonite

is recovered in amounts equivalent to the cationic exchange capacity. Cobalt exists as probably cobalt hydroxide since it is in acid solution only (2 N) that it may be extracted. These facts are also supported by X-ray diffraction data: Co(NH$_3$)$_6^{3+}$ saturated montmorillonite expands as usual to 16 Å with glycerol while it does not expand when the complex has been destroyed.

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Detection of Nanogram Quantities of Sulphur Hexafluoride by Electron Capture Methods

As sulphur hexafluoride is now used as a gaseous insulating and arc-quenching medium in electrical equipment, there is some interest in methods for its estimation in mixtures with other gases. The accompanying gas chromatogram (Fig. 1) demonstrates that electron capture methods can be applied to this end.

The detector used was as previously described[1,2] and was operated with a constant applied potential of 24 V with the carrier gas, nitrogen, flowing from a 4 mm × 70 cm chromatograph column packed with 80–100 mesh silica gel with subsequent dilution making the total gas flow-rate 500 c.c./min.

The peak illustrated was obtained with a 1-μl. load of 1/2,500 by vol. of SF$_6$ in air, that is, with approximately 2×10^{-9} g of SF$_6$, giving a maximum concentration of SF$_6$ in the detector of the order of 5×10^{-8} (molar). The ionization efficiency in the detector under these deliberately non-optimum conditions was approximately 2 per cent.

A proportion, 1/60 by vol., of CCl$_2$F$_2$ was included in the sample for comparison purposes. The ionization efficiency for the three peaks shown are in the sequence SF$_6$ > CCl$_2$F$_2$ > O$_2$, in accord with the known arc-quenching properties of these gases.

The detector efficiency decreases as the applied voltage is increased, but does so less rapidly for SF$_6$ than for most other gases, implying that the negative ion formed is relatively heavy. Thus the only peak occurring in the chromatogram when the applied voltage is 42 V is that due to SF$_6$. It is suggested, therefore, that it may prove possible to apply electron capture detectors to the routine estimation of the SF$_6$ content of mixtures of known gases, without preliminary chromatographic separation.

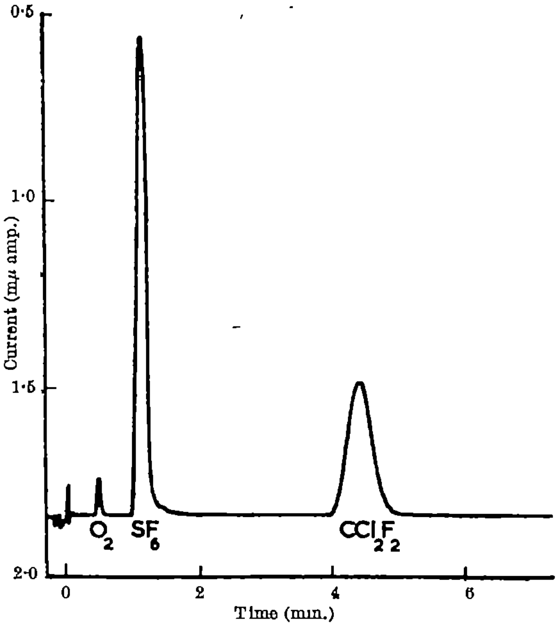

Fig. 1

I thank Mr. T. K. Morley and Imperial Chemical Industries, Ltd., for making available the sample of SF$_6$.

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CRYSTALLOGRAPHY

X-ray Line Broadening in the Alkali Halides

During the past few years many investigations have been made of the effects of cold work on the X-ray line profiles of polycrystalline metals. Analysis of the profile broadening has been used to determine particle size, stacking fault probability and strain relationships. Using these same analytical methods, work is now in progress here on the investigation of broadening produced by cold work on face-centred cubic compounds of the alkali halides. This communication covers three of the compounds which have been investigated, namely, sodium chloride, potassium bromide and sodium fluoride.

Initially, the method of cold work was that which had been used in the examination of metal—the filing of block specimens. However, since the broadening produced in the alkali halides is relatively small, and it was therefore desirable to produce the maximum effect, it was found that crushing the specimens in an agate ball mill produced the better results. Similarly, annealing conditions have been varied to achieve maximum resolution in each compound studied, optimum conditions being related to melting point on one hand and solubility in water at room temperature on the other. Thus the broadening of a potassium bromide specimen decreases rapidly at room temperature under normal atmospheric moisture conditions and maximum resolution was achieved under these conditions after seven days. Sodium fluoride and sodium chloride, on the other hand, showed very slight effects at room temperature, and heating to 500° C for 1 h in the case of sodium chloride and 47 h for sodium fluoride was used to produce the annealed specimen. Even under these conditions it was not possible to resolve the sodium chloride and sodium fluoride lines to the extent that was achieved with potassium bromide.

Rachinger's method was used to resolve the α_1 and α_2 doublet and the integral breadths were obtained graphically. Graphs showing the values of integrated breadths versus $N = (h^2 + k^2 + l^2)$ are shown in Figs. 1, 2 and 3. Also shown in the graphs is the integral breadth computed from the Scherrer formula assuming a particle size of 1,000 Å.

The correction for instrument broadening was carried out by subtracting the broadening of the annealed specimen from that of the worked specimen, assuming, in turn, Cauchy and Gaussian shapes for the line profiles. As a result of particle size changes during annealing, the correction of instrumental broadening introduced anomalous particle size effects which were eliminated before proceeding to the strain determinations. The annealed profiles of sodium fluoride and sodium chloride also appear to have some residual strain broadening so that the strain function which was determined later relates to the excess broadening produced by cold work and not to the total strain. It has been found by previous workers that the actual line profiles lie between the Cauchy and Gaussian approximations, so the final values of strain measurements were obtained by taking the average values from these two extremes.

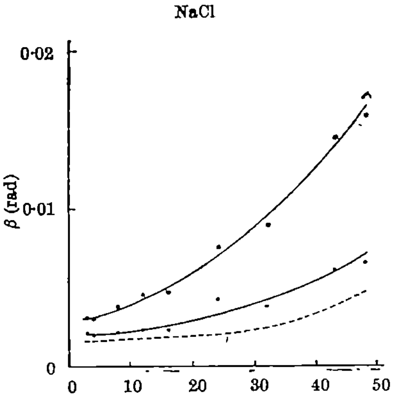

Fig. 1

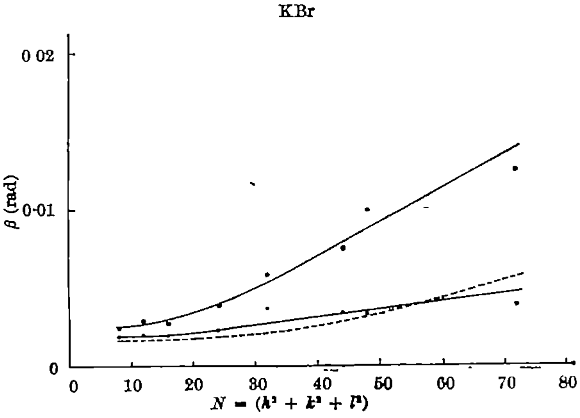

Fig. 2

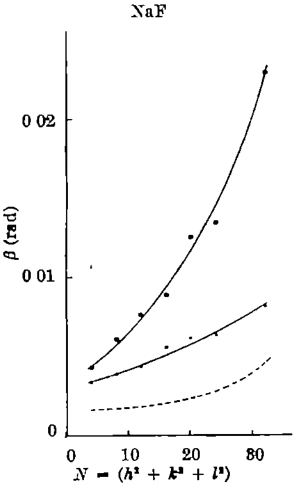

Fig. 3

Figs 1, 2 and 3. Graphs of measured integral breadth, β versus $N = (h^2 + k^2 + l^2)$, for sodium chloride, potassium bromide and sodium fluoride. Circles are for worked specimen and solid dots for annealed specimen. Broadening for particle size of 1,000 Å computed from $\beta = \frac{\lambda}{t \cos\theta}$ shown as broken line

The strain function $\xi \left(= \frac{\beta}{2} \cot\theta \right)$ was determined for the various orders of (100), (110), (111) reflexions and then averaged; this is compared with a mean value of the strain assuming isotropic strain distribution from the slope of the graph of

$$\beta^* \left(= \frac{\beta \cos\theta}{\lambda} \right) \text{ versus } d^* \left(= \frac{2 \sin\theta}{\lambda} \right)$$

where $\beta^* = \xi d^* + \frac{1}{t}$ and t is the average particle dimension, but in this case is not significant as explained here. The results of these strain determinations are shown in Table 1.

Table 1. STRAIN FUNCTION $\xi = \beta/2 \cot\theta$

Direction	[100]	[110]	[111]	Slope of $\beta^* - d^*$ graph
Sodium chloride	0·0022	0·0025	0·0025	0·0024
Potassium bromide	0·0024	0·0027	0·0030	0·0027
Sodium fluoride	0·0034	0·0037	0·0038	0·0037

Stress function = ξ × Young's modulus ($\times 10^{-8}$ dynes cm^{-2})

Direction	[100]	[110]	[111]
Sodium chloride	9·0–9·4	8·6	7·9–8·0
Potassium bromide	5·9–7·7	4·4–8·4	4·2–10·0

The particle-size dimensions could not be determined since the broadening due to particle size could not be separated from instrumental broadening, both of which influence the value of the intercept in the graph of β^* versus d^*.

In the case of sodium chloride an analysis of the profile shapes by a study of the Fourier coefficients of the broadening function, as outlined by Warren and Averbach, was carried out. The plots of A_n versus n confirm an appreciable strain broadening component in the worked specimen and a residual effect in the annealed specimen. Plots of the coefficients for multiple orders indicate a mean particle dimension of approximately 1000 Å, and absence of any peak shift precludes any apparent particle size effect resulting from stacking faults.

In order to obtain some idea of stress distribution in the crystals, the value of the strain function was multiplied by the respective values of Young's modulus for sodium chloride and potassium bromide. This could not be done in the case of sodium fluoride since the elastic constants for this compound were not available. It will be seen that the stress function for sodium chloride is reasonably constant for the three directions [100], [110] and [111]. In the case of potassium bromide the elastic constants quoted in the literature show a wide range of values; however, the spread of stress values obtained also suggests a constant stress in the three directions of about the same order of magnitude as for sodium chloride. Since three elastic constants are involved in the compilation of Young's modulus, the assumption of a constant value of stress in the three directions would enable one to compute the value of the elastic constants.

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METALLURGY

Oxidation of Cupro-Nickel Alloys in Steam-Oxygen Mixtures

In the course of experiments in which a cupro-nickel alloy containing nickel 31·3 per cent, iron 0·50 per cent and manganese 0·73 per cent, balance substantially copper, was subjected to corrosion by steam–oxygen mixtures relatively rapid oxidation has been observed leading to the formation of a thick scale of corrosion product within a period of a few days. The experiments were conducted in an autoclave containing water, steam and oxygen at a total pressure of approximately 900 lb./in.^{2} and a temperature of 250° C, the saturation vapour pressure of steam at this temperature being 577 lb./in.^{2}. The results obtained plotted as a weight gain-time curve are given in Fig. 1. The corrosion product had a laminated multi-layer structure containing alternate nickel-rich and copper-rich phases; within the latter aggregates of substantially pure metallic copper were a prominent feature.

Segregations of nickel-rich and copper-rich phases and the formation of metallic copper have previously been reported in the scale present on copper–nickel alloys after dry oxidation in the temperature-range 700–900° C[1,2]. At these higher temperatures the formation of the discrete oxide phases NiO, Cu_2O and CuO can be accounted for on the basis of ionic and electronic diffusion, and the diffusion-controlled displacement reaction $Ni + Cu_2O \rightarrow NiO + 2\ Cu$ has been postulated to explain the formation of metallic copper[3]. However, at the relatively low temperature of 250° C used in our experiments, it seems improbable at first sight that a mechanism dependent on cationic diffusion as the rate-determining process is consistent with the rapid oxidation observed (Fig. 1).

An optical micrograph of a typical field illustrating in section the striated nature of the corrosion product formed on a specimen after 30 days is presented in Fig. 2 together with associated compositional variations revealed by the electron probe X-ray micro-analyser along the line marked X–X. During scans of 9-min duration, the intensities of characteristic X-rays excited by 29-kV electrons were recorded on a chart, from which the concentrations of nickel and copper in the corrosion product layers were estimated, using the known concentrations of these elements in the unoxidized alloy as calibration standards. Errors arising from X-ray absorption of fluorescence in the specimen were thought to be less than those introduced by unevenness of the specimen surface and were therefore neglected. Additional slow scans with the micro-analyser revealed that the nickel-poor regions were also depleted in iron and manganese.

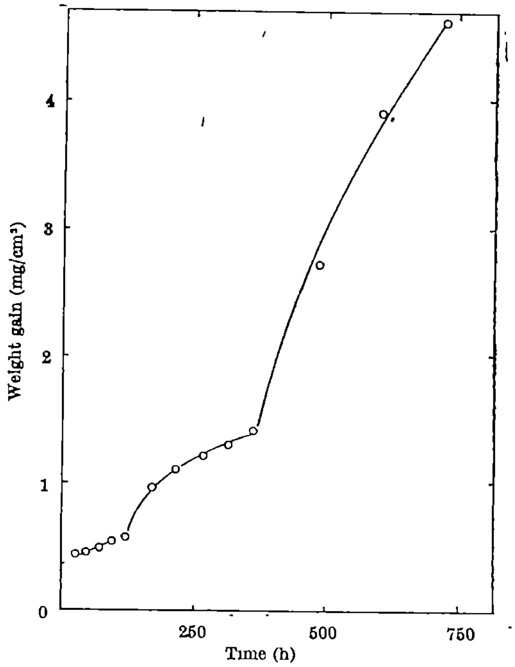

Fig. 1

The presence of metallic copper is immediately obvious when the scale is viewed under the optical microscope. The presence of the oxides NiO, Cu_2O and CuO was confirmed by X-ray diffraction analysis of a sample of corrosion product removed from the specimen surface.

On the basis of the foregoing evidence the main phases present in the respective layers labelled A–G in Fig. 2 have been identified as follows: A, unoxidized alloy + NiO; B, metallic copper + NiO; C, Cu_2O + NiO; D, predominantly NiO; E, predominantly metallic copper + a little Cu_2O; F, predominantly NiO; G, Cu_2O + a little metallic copper.

Some further metallographic evidence not reported herein confirms that the corrosion product layers are formed in the main as a consequence of inward diffusion of oxygen or some other oxygen-rich species. From Fig. 2 it is seen that at the boundary between corrosion product and uncorroded metal nickel is preferentially oxidized. It may be envisaged that the creation of vacancies in the solid solution lattice resulting from this process provides an enhanced opportunity for diffusion of the remaining cations, which will be predominantly copper, leading to the formation of agglomerates of substantially pure copper. Bands of metallic copper have been observed to appear at an early stage in the oxidation process when only a relatively small amount of corrosion product scale is present.

The degree of dispersion of the nickel-rich and copper-rich areas is presumably dependent on the respective rates of nucleation and growth of the

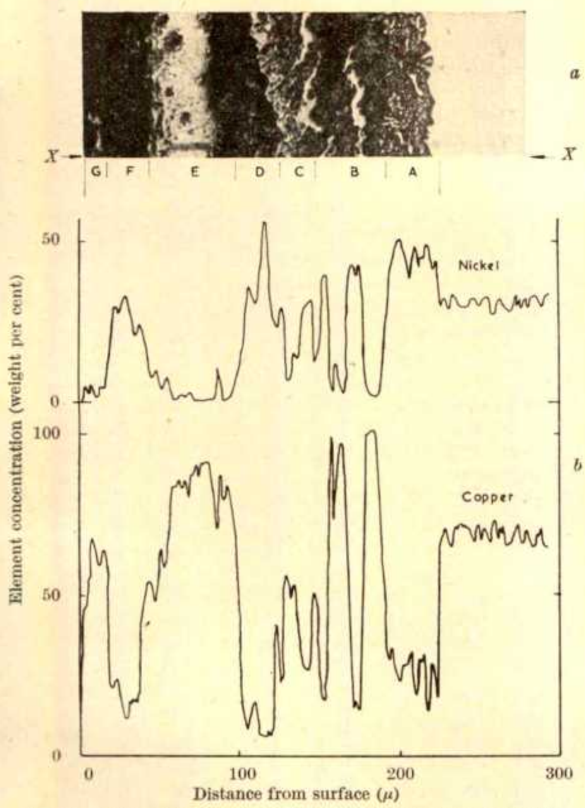

Fig. 2. *a*, Optical micrograph of a section through the corrosion layers; *b*, concentration curves of nickel and copper along the line *X–X*

phases, which will be influenced by the rate of inward diffusion of oxygen. Local variations in the latter dependent on space and time factors could give rise to the varying textures observed in different layers of the corrosion product scale.

We thank the International Nickel Co. (Mond), Ltd., for permission to submit this communication.

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CERAMICS

Corrosion of Corundum Crystals by Commercial Glasses

One of the most striking and least-understood aspects of refractory corrosion by molten glass is the relatively high rate of solution at the refractory-glass-gas interface, compared with that which occurs below the glass-level[1,2]. Any attempts to explain this phenomenon, or even measure it, are made exceedingly difficult by the complex nature of the materials involved[3,4]. This difficulty has been partially overcome by the use of single crystals of corundum as the refractory materials[5,6], thus eliminating factors such as porosity, pore size, pore shape, texture, etc.

Single crystals of corundum were immersed in four different commercial glasses (soda–lime–silica, barium crown, borosilicate and lead) for various times at temperatures ranging from 1,400°–1,500° C. Measurements were made both at the glass-level and below and rates of corrosion were calculated.

The measurements showed that not only did the rates of corrosion vary widely with different glasses, from 0·04 g cm^{-2} h^{-1} for barium at 1,500° C to 0·0005 g cm^{-2} h^{-1} for borosilicate at 1,400° C, but that there were also large differences between the rates of corrosion at the glass-level and below; for example, for soda–lime–silica glass, corrosion takes place approximately three times faster at the glass-level than below. It was also found that each glass impressed its own particular corrosion profile on the crystal (Fig. 1). The barium and the soda–lime glasses differed only in the acuteness of the indentation produced at the glass-level, but the lead glass caused a marked tapering effect on the crystal below the glass-level, as well as an indentation at the glass surface; the borosilicate showed practically no attack at the glass surface, but a gradual wear down the whole length of the crystal was observed. Photographs obtained of the cord formation showed that in some cases there was a tendency for the reaction product to fall, giving some protection to the lower part of the specimen (Fig. 2), and in others to rise to the surface, allowing fresh glass to reach the lower part of the specimen. This 'protection' effect however, did not appear to have any great influence on the glass-level phenomenon.

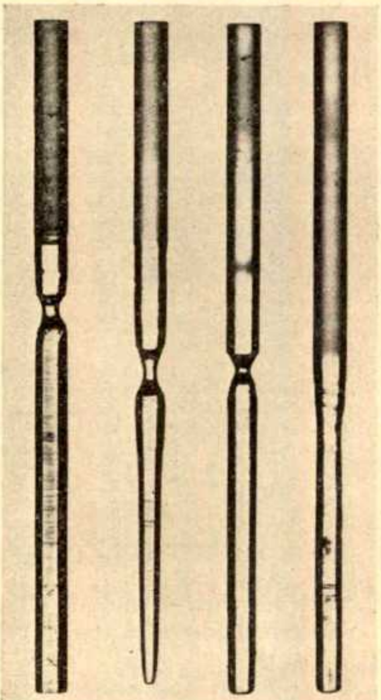

Fig. 1. Typical corrosion profiles obtained by immersing the corundum crystals in the four different glasses. The crystal on the extreme left has been immersed in soda–lime–silica glass for 5 h at 1,450° C and shows a sharp flux line indentation with practically no wear below this point; the specimen second from the left has been immersed in lead glass for 24 h at 1,500° C, it has a shallow and more elongated flux-line attack with considerable wear below the glass-level; the crystal second from the right has been immersed in barium crown glass at 1,500° C for $2\frac{1}{2}$ h and is similar to the specimen in soda–lime glass except for a sharper flux-line attack; the specimen on the extreme right has been immersed in borosilicate glass for 96 h at 1,400° C and shows practically no flux-line attack but a rather uneven wear down its whole length

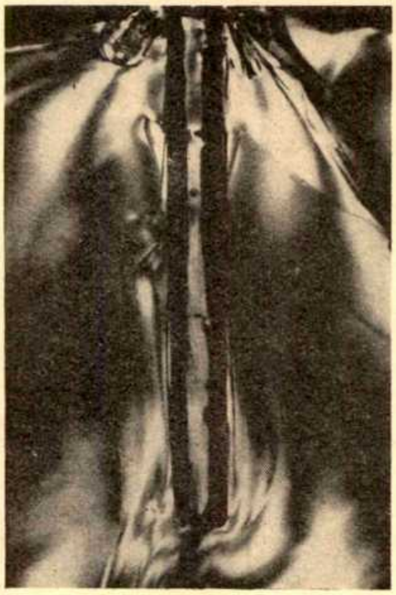

Fig. 2. A corundum crystal which has been frozen in borosilicate glass. The reaction product can be seen leaving the specimen and flowing downward; the photograph also shows some disturbance around the glass-level area

Even in cases where the reaction product rose to the surface, giving a relatively large amount of attack below the glass-level, specimens were still obtained which showed a marked indentation at the glass surface.

It was concluded that at least two corrosion mechanisms were involved; one at the glass-level, possibly closely associated with surface tension[7], and the other below the glass-level, where density currents and diffusion appeared to be the controlling factors.

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BIOCHEMISTRY

Fate of Carbohydrates during Thermal Degradation of Tobacco

THE least-stable and least-explored group of substances in tobacco are the 'dynamic constituents', composed mainly of carbohydrates, organic acids, and unidentified substances of a related nature which are characterized by a high degree of mutability. Because of the high content and the reactivity of glucose and fructose in 'bright' tobacco, carbohydrates are considered the major constituent which undergoes modifications in harvested tobacco. Various oxidative and condensation reactions have been postulated[1-3], but the detailed nature of carbohydrate conversions and related chemical changes in tobacco have not been investigated.

The objective of this work was to examine the transformations of carbohydrates during the thermal degradation of flue-cured, 0–78*X* bright tobacco, crop 1961. The stemmed leaf was packed in glass cylinders at a density of 335 g/1,000 cm^3. The cylinders were closed hermetically, sealed with wax, and exposed to constant external heat in the range 24°–60° C for 1–9 days. The tobacco and the gaseous atmosphere were examined for compositional changes resulting from the breakdown of carbohydrates. The nature of the thermal conversions of glucose in the tobacco system and the relative distribution of its breakdown products were also determined on the same tobacco, which was sprayed with a glucose uniformly labelled with carbon-14 (Merck and Co., Ltd.).

The total content of the reducing sugars in tobacco was analysed colorimetrically by the modified method of Hawkins[4]. Water-soluble acids were determined by the method of Darkis *et al.*[5]. The dibasic acids were analysed on the F.N.M. model 500 gas chromatograph with a 2-ft. 'Carbowax' column[6] and by paper chromatography, using a diethyl ether/formic acid/water (5 : 2 : 1) system. The moisture content was determined in a Freas oven at 100° C for 3 h. The total carbon dioxide and oxygen content in the ambient was analysed on a C.E.C. mass spectrometer, type 21–103*C*, at an ionizing voltage of 70 V, ionizing current of 50 μamp. and a magnet current of 251·0 m.amp. The evolution of radioactive carbon dioxide was measured in a liquid scintillator L.S.C. : 10*B* after precipitation from sodium hydroxide solution as a barium salt. The total radioactivity present in the tobacco residue and in its water and ether extracts was determined by combustion and by counting in the ionizing chamber (Dynacon *M* 6,000-recorder model *R*–1,000).

Thermal degradation decreased the amount of reducing sugars and increased the free acids and water content in tobacco. The *p*H of tobacco decreased from about 5·5 to 5·3–4·9 per cent, depending on the extent of changes in composition. No detectable decrease in the weight of the thermally degraded tobacco samples could be observed as would be the case if the 'analytically disappearing' carbohydrates were fully oxidized to carbon dioxide and water. However, some of the changes were connected with the evolution of carbon dioxide and with an almost equimolecular consumption of oxygen from the atmosphere. Correlation between the molar conversion of sugars and its various reaction products indicated that several mechanisms of carbohydrate transformation took place during the thermal degradation of tobacco. Small amounts of the total carbon dioxide which was liberated may arise from the decarboxylation of acids.

The rate and the extent of the reaction were very sensitive to temperature. Up to 50° C, the quantitative compositional changes connected with the transformation of carbohydrates were linearly related to an increase in temperature. At higher temperatures, these changes were exponential.

The results were confirmed by examining the thermal conversion of radioactive glucose which had been introduced into tobacco. Depending on the temperature conditions used, approximately 0·64–6·04 per cent of the total carbon from dynamic constituents and/or 0·47–1·92 per cent of the total carbon from the radioactive glucose present in tobacco was oxidized to carbon dioxide. The material balance and the examination of the glucose conversion products indicated that a part of the 'analytically disappearing' carbohydrates lost its reducing power due to condensation with other naturally occurring constituents in the leaf. Depending on the temperature conditions, 5·8–46·0 per cent of the glucose was converted to high-molecular-weight complexes, remaining on the leaf during conventional sugar extraction. In addition, 7·7–29·5 per cent glucose was converted to a mixture of non-reducing, water-soluble, partial oxidation products and complexes. Approximately 1·7–3·6 per cent glucose was converted to the ether soluble compounds. Furfural and hydroxymethylfurfural were identified as some of the reaction products[7] by the ultra-violet and infrared analyses after elution from paper chromatograms at the R_F values of 0·78–0·84. Paper chromatograms of the ether extracts from tobacco, scanned on the Scanogram II (model R.S.C.–160), showed also the presence of wide area of radioactivity at the R_F range of 0·07–0·38. These glucose degradation products have not yet been identified. The gas chromatographic examination gave no evidence that dibasic acids were formed from glucose during thermal degradation of tobacco.

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Autonomy of Protein Synthesis in Mutant Staphylococci with Impaired Respiration

ACCORDING to present concepts about the mechanism of protein synthesis, the ribosomes act as nonspecialized structures which synthesize the protein dictated by the transient attachment to the ribosome of a short-lived 'messenger RNA'[1]. It has been observed recently that the synthesis of messenger RNA in the bacterial cell is selectively inhibited by the actinomycins[2]. This specific mechanism of action of actinomycins gives a unique opportunity for analysis of the effects of blocking the synthesis of messenger RNA in the cell on the synthesis of proteins in the ribosomes. These effects can be comparatively investigated in normal staphylococci, as well as in mutant staphylococci with impaired respiration, which have been examined in some detail as a possible microbial model for cancer cells[3].

For an analysis of this problem we used the parent culture of staphylococci (*Staphylococcus aureus* 209), as well as mutants with impaired respiration induced by ultra-violet radiation, ultra-violet-2[4]. Parent staphylococci were grown in meat-peptone broth in Erlenmeyer's flasks containing 200 ml. of the nutritive medium, at 37°. Before the beginning of an experiment each flask was inoculated with 2 ml. of 24 h culture of staphylocci grown in the broth in shaking flasks and containing 6 × 10^9 of microbial cells per ml. Observations showed that in the prescribed conditions the logarithmic phase of growth of the culture begins 1 h after inoculation and continues for at least 3 h. Pure crystalline actinomycin *C* was used as an inhibitor of 'messenger RNA'. The inhibitor was added 1 h after inoculation, at the beginning of the logarithmic phase of growth of the culture. For an evaluation of the action of the inhibitor on the synthesis of proteins and of nucleic acids in the cells of staphylococci, two samples of 27 ml. each were taken from each flask: the first sample was taken immediately after addition of the inhibitor, and the second sample 3 h later. Of each sample 2 ml. were used for assays of protein content, and 25 ml. for assays of nucleic acids.

In the work with the slower growing mutants we used nutritive broth containing peptone (0·5 per cent), glucose (1 per cent), sodium chloride (0·5 per cent), and Hottinguer digest (0·5 per cent). Inoculations were made with 2 ml. of 48-h culture of mutants grown in shaking flasks and containing 6 × 10^9 of microbial cells per ml. In the parent culture of staphylococci growing logarithmically three-fold increase of the density of the culture is attained approximately in 3 h, but the same increase of density in the culture of mutants is reached only after 17 h. Therefore, in the work with mutants, the first sample for assays was taken immediately after addition of the inhibitor, and the second sample 17 h later.

Bacterial cells contained in each sample were separated from the nutritive medium by centrifugation, washed with distilled water, and used for assays. The protein content was measured by Folin's method[5]. Ribonucleic and deoxyribonucleic acids were extracted separately. RNA was extracted from microbial cells by 10 per cent perchloric acid at −4° for 17 h and assayed by the orcinol reaction. After removal of RNA from cells the latter were extracted with 10 per cent perchloric acid in the water bath at 90° for 15 min. and DNA contents of the extracts assayed by the diphenylamine reaction. Measurements are expressed in units of optical density. They were always made in duplicate, and frequently repeated.

Table 1 shows the results of our experiments. In the work with the parent culture of staphylococci the concentration of actinomycin used by us was 0·3 μg/ml., and in the work with mutants 0·006 μg/ml., since the latter were 50 times more sensitive to the action of actinomycin[6]. It is clear that actinomycin *C* in the concentration of 0·3 μg/ml. completely blocks (96 per cent of inhibition) synthesis of RNA in the cells of parent staphylococci. Under these conditions protein synthesis in the same cells is decreased by 60 per cent. An entirely different picture is observed in mutant staphylococci with impaired respiration. Actinomycin *C* in the concentration of 0·006 μg/ml. completely (by 99 per cent) inhibits synthesis of RNA in the cells of mutants, but this block produces no

Table 1. EFFECT OF ACTINOMYCIN *C* ON THE SYNTHESIS OF PROTEIN AND NUCLEIC ACIDS IN THE PARENT STAPHYLOCOCCI AND IN THE MUTANTS WITH IMPAIRED RESPIRATION ULTRA-VIOLET-2

Culture	Time incubated (h)	Actinomycin (μg/ml.)	Turbidity	Protein	RNA	DNA
1. Parent	0	0	0·168	0·058	0·272	0·081
2. The same	3	0	0·548	0·209	0·781	0·152
3. The same	3	0·3	0·336	0·138	0·291	0·122
Percentage of inhibition			56	60	96	42
4. Mutants ultra-violet-2	0	0	0·117	0·052	0·268	0·067
5. The same	17	0	0·439	0·183	0·496	0·113
6. The same	17	0·006	0·417	0·190	0·270	0·110
Percentage of inhibition			7	0	99	6

effect on protein synthesis, which continues at the same rate as in the control culture to which no actinomycin has been added.

The significance of these observations is self-evident. In the cells of parent staphylococci protein synthesis is controlled by 'messenger RNA', and as soon as the synthesis of messenger is blocked by the actinomycin the rate of protein synthesis decreases immediately. In the cells of mutant staphylococci this control mechanism is evidently lost, and protein synthesis goes on for some time autonomically, in spite of complete suppression of the synthesis of 'messenger RNA'. In other words, protein synthesis in the cells of mutant staphylococci is not controlled to the same degree as in the cells of parent staphylococci.

The validity of this conclusion is supported by some additional evidence. In accordance with some recent observations on the mechanism of streptomycin action in bacteria, streptomycin impedes the attachment of messenger RNA's to bacterial ribosomes, and in this way blocks the synthesis of proteins in the cell[7,8]. Since, in the mutant staphylococci examined by us, protein synthesis in the ribosomes is more autonomic and does not depend on the messenger RNA to the same degree as protein synthesis in the ribosomes of parent cells, one could theoretically expect that these mutants should be less vulnerable to the action of streptomycin than the parent cells. In fact, Gause and Kochetkova[9] in 1959 observed that mutant staphylococci with impaired respiration, ultra-violet-2 and ultra-violet-3, are five-times less vulnerable to the bacteriostatic action of streptomycin than the parent cells. At that time this observation was unexplained, but now one can see that it well agrees with our observation on the autonomy of protein synthesis in the cells of mutant staphylococci with impaired respiration.

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Substrate Specificity of Maltose Phosphorylase

MALTOSE phosphorylase from *Neisseria meningiditis*, discovered by Fitting, Doudoroff[1], and Scherp[2], catalyses the following reaction:

$$\beta\text{-D-glucose 1-phosphate} + \text{glucose} \rightleftharpoons \text{maltose} + \text{phosphate} \quad (1)$$

Studies of specificity[1,2] showed that of many sugars tested only D-xylose could replace glucose as the acceptor for glucose transferred from the phosphate donor. The product was 4-*O*-α-glucosylxylose[3]. The sugar phosphate could not be replaced by the α-anomer[1,2].

Maltose phosphorylase has since been found in beer *Lactobacilli*[4] and *N. perflava*[5] and enzyme from the latter source can use 2-deoxy-D-glucose, D-glucosamine and *N*-acetyl-D-glucosamine instead of the glucose acceptor, giving rise to analogues of maltose.

We are interested in maltose phosphorylase because it is one of the few enzymes capable of synthesizing the α-1,4-glucosidic linkage *ab initio*, and may perhaps be the source of the primer required by other enzymes that synthesize the same linkage, for example, starch phosphorylase. Furthermore, the capacity of the enzyme to synthesize analogues of maltose leads to new substrates for other carbohydrases.

This latter aspect of maltose phosphorylase action has been examined with maltose phosphorylase from *N. meningiditis*. A range of sugars, many not previously tested as acceptors, were incubated with β-D-glucose 1-phosphate and enzyme. The following sugars could replace D-glucose and their rates of reaction relative to glucose (100) are also given: 2-*O*-methyl- (90), 6-*O*-methyl-D-glucose (20), 2-deoxy- (60), 3-deoxy- (55), 6-deoxy-D-glucose (95), 6-deoxy-6-fluoro-D-glucose (80), D-xylose (50). The following sugars were inert: 3-*O*-methyl- and 2,3-di-*O*-methyl-D-glucose, 1,5-anhydro-D-sorbitol (1-deoxy-D-glucopyranose), D-galactose, nigerose, isomaltose, sophorose, laminaribiose and gentiobiose. Where the sugar reacted as an acceptor, as shown by release of inorganic phosphate (equation 1), the synthesis of a new disaccharide was confirmed by its isolation from a paper chromatogram followed by acid hydrolysis to yield glucose and the test sugar.

Only D-xylose was previously known to replace D-glucose with the *N. meningiditis* enzyme. Five of the six new acceptor substrates are seen to be more reactive than D-xylose and therefore presumably conform more closely to the specificity requirements of the enzyme. It is of interest that any modification of glucose at C-1, as in 1-deoxyglucose, the glucose disaccharides and also methyl α-glucoside[1], entirely eliminates acceptor activity.

Our main interest in these investigations of specificity lies in the question whether the sugar phosphate can also be replaced by a 'foreign' sugar phosphate. In this case a maltose modified in the non-reducing glucose moiety would be formed. Such a disaccharide can be expected to provide other maltose-splitting enzymes with a more searching test of specificity than when the modification is carried in the reducing glucose moiety. To this end the 1-phosphates of β-D-galactose, β-D-xylose, 2-*O*-methyl- and 3-*O*-methyl-β-D-glucose have been synthesized and tested with maltose phosphorylase and glucose.

β-D-Galactose 1-phosphate was inert as a sugar donor; neither is free D-galactose an acceptor[1]. The phosphates of xylose and 2-*O*-methylglucose also failed to react, although the free sugars can substitute for glucose as acceptors. 3-*O*-Methyl-β-D-glucose 1-phosphate, however, reacted with glucose at a rate equal to that of β-glucose 1-phosphate itself, yet free 3-*O*-methylglucose is not an acceptor. Clearly maltose phosphorylase has different specificity requirements as between donor and acceptor molecules.

The synthesis of a new disaccharide, presumably 4-*O*-[3-*O*-methyl-α-D-glucopyranosyl]-D-glucose, has been confirmed by its isolation and hydrolysis to glucose and 3-*O*-methylglucose. Furthermore, the enzyme was apparently able also to transfer 3-*O*-methylglucose from the β-1-phosphate to 2-*O*-methyl-

D-glucose, 2,- 3- and 6-deoxy-D-glucoses, 6-fluoro-6-deoxy-D-glucose, and D-xylose, as shown by release of inorganic phosphate.

To our knowledge, this is the first time that any phosphorylase has been found to react with a 'foreign' sugar phosphate. This finding prompts the further pursuance of such investigations with this and other phosphorylases, with the prospect of the synthesis of modified sugar polymers having interesting chemical and biological properties.

We thank Dr. H. Scherp for the gift of *N. meningiditis*, Miss Anastasia Theofanides for culturing the organism, and the Department of Scientific and Industrial Research for a Special Research Grant.

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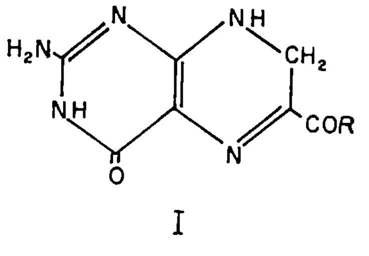
Synthesis of the Yellow Pteridine, Isosepiapterin

ISOSEPIAPTERIN is the name given to a yellow pteridine which occurs in *Drosophila melanogaster*, accumulates in the *sepia* mutant, and has been isolated in relatively large amounts from the blue-green alga, *Anacystis nidulans*[1]. Its structure (I, R = CH_2CH_3) and that of a closely related compound, sepiapterin (I, R = $CHOH{\cdot}CH_3$), have been elucidated chiefly by the work of Nawa[2] and Forrest *et al.*[3].

Present concepts[4] of the mechanism of action of thiamine suggest the intermediate formation in the decarboxylation of pyruvic acid of a relatively stable carbanion which can react non-enzymatically, for example, with acetaldehyde to give acetoin. Since a reduced form of 2-amino-4-hydroxypteridine (probably the 7,8- or 5,8-dihydro derivative) adds anionic reagents exclusively at the 6-position[5], it seemed feasible to use the catalytic action of thiamine to produce an anion from a keto-acid which, on reaction with the reduced pteridine, would give isosepiapterin or an analogue of it. Indeed, incubation of reduced 2-amino-4-hydroxypteridine, pyruvic acid, and thiamine at *p*H 9·0 yielded a small amount of a yellow compound (about 4 per cent of theoretical based on 2-amino-4-hydroxypteridine) which was purified as described here. This compound had an ultra-violet absorption spectrum identical with that of isosepiapterin and was oxidized by alkaline permanganate to 2-amino-4-hydroxy-6-carboxypteridine. Its structure is therefore believed to be I (R = CH_3).



I

Using α-ketobutyric acid instead of pyruvic acid, the following experiment was then carried out. 2-Amino-4-hydroxypteridine (Table 1) in 0·1 N sodium hydroxide (2·0 ml.) was hydrogenated in the presence of Adams' catalyst. After completion of the reduction (as evidenced by the complete disappearance of fluorescence), the reaction mixture was filtered and neutralized (2 N hydrochloric acid) as rapidly as possible. To this filtrate, *tris* buffer, α-ketobutyric acid, and thiamine were added (Table 1) and the whole was allowed to stand at 37° in the dark, when the solution gradually became yellow in colour. After 48 h, the solution was adjusted to *p*H 5 and applied to a column of 'Florisil'. After washing the column with water, the yellow band adsorbed at the top of the column was eluted with 20 per cent aqueous acetone and the eluate was concentrated. The yellow product was further purified by successive paper chromatography using the solvents 4 per cent sodium citrate and 1-propanol : 1 per cent aqueous ammonia (2 : 1). After elution from the final chromatogram and concentration *in vacuo*, the compound was obtained as yellow needles. It was found to be identical spectrophotometrically and chromatographically (Table 2) with natural isosepiapterin (I, R = CH_2CH_3) and was oxidized by alkaline permanganate to 2-amino-4-hydroxy-6-carboxypteridine. The yield in the first experiments was again low (3 per cent), but by varying the conditions (Table 2) a maximum yield of 22 per cent was obtained. This yield appears reasonable, since the overall reaction must involve a re-oxidation of the initial tetrahydro-2-amino-4-hydroxypteridine to a dihydro derivative (and presumably all three dihydro compounds—5,8; 7,8; and 5,6—may be obtained) followed by attack of the carbanion intermediate and then a further oxidation to the 7,8-dihydro compound.

Table 1

2-Amino-4-hydroxy-pteridine reduced (mg)	Isosepiapterin produced (mg)	Percentage of conversion of 2-amino-4-hydroxypteridine
5	0·2	3
1	0 14	10
0·5	0·12	17
0·2	0·06	22

Reaction mixture consisted of the reduced pteridine, 0 5 M; α-ketobutyric acid, 0 2 ml.; 0·5 M thiamine, 0·1 ml.; 0·1 M *tris* buffer, *p*H 9·0, 2·0 ml. The percentage conversion was calculated using the extinction coefficient of isosepiapterin at 440 mμ.

Table 2

Compound	Solvent		
	4 per cent sodium citrate	1-Butanol : acetic acid : water (4 : 1 : 1)	1-Propanol· 1 per cent ammonia (2 : 1)
6-Acetyl compound	0·27	0·34	0·41
6-Propionyl compound	0 27	0·49	0·53
Isosepiapterin	0·27	0·49	0·53
Sepiapterin	0·35	0 29	0·44

Synthesis of sepiapterin (I, R = $CHOH{\cdot}CH_3$) should be possible using this same procedure with α-keto-β-hydroxybutyric acid as the source of the side-chain. However, under conditions developed for maximal isosepiapterin synthesis, no sepiapterin was formed. When the OH group of this keto acid was protected by methylation (that is, using α-keto-β-methoxybutyric acid), a yellow compound was obtained in very low yield after reaction with reduced 2-amino-4-hydroxypteridine and thiamine, and this is presumably I (R = $CH(OCH_3){\cdot}CH_3$). However, we have not been able to hydrolyse this compound without destroying the yellow colour and fluorescence.

Although this synthesis of isosepiapterin is not unambiguous, it provides good confirmatory evidence for the structure of the compound and it might be developed for the synthesis of new compounds of the same type and/or derivatives of them. In addition, it opens up a possible new approach to the biosynthesis of isosepiapterin and sepiapterin and the related naturally occurring compounds such as biopterin (to which sepiapterin can be converted by chemical means). We are at present investigating the possible biochemical significance of this synthetic pathway.

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Removal and Regeneration of the Cell Coating in Tumour Cells

Some ascitic tumour cells are coated with a mucopolysaccharide stainable by the Hale reaction[1]. Experiments were carried out to characterize more specifically the chemical nature of this cell coating and to study the dynamics of cell coat formation. Cells of ascitic mammary carcinoma *TA*3 were used *in vitro*. Experiments were carried out to determine whether some proteases and mucinases remove the cell coating, and, after removal, regeneration of the cell coating occurs.

Ascites cells were washed with and resuspended in Eagle's medium spiner, unless calcium was needed for enzyme activity, and then incubated at 37° C, with slow stirring, in the presence of one of the following enzymes or mixture of enzymes : pancreatin, crystalline trypsin, crude papain, crystalline papain, crystalline pepsin, testis hyaluronidase, and receptor-destroying enzyme (Behringwerke, Ag., Marburg-Lahn). The enzyme concentration was 1 or 2 mg/ml. of culture medium, except in the experiment with the receptor-destroying enzyme, in which case 4 drops of the buffer-enzyme solution per ml. were used. After 30–60 min of incubation* cells were washed in fresh Eagle's medium. Cells exposed to trypsin were incubated for 5 min at 37° C or overnight at 4° C. Treated cells were fixed in Love solution[2], and sections or smears were stained with Hale[3] or periodic acid–Schiff methods.

The cell coating, as stained with the Hale reagents, was removed partially or totally by pancreatin, trypsin, pepsin, and receptor-destroying enzyme. In contrast, crude papain, crystalline papain, and testis hyaluronidase were completely ineffective. Receptor-destroying enzyme had no action on the periodic acid–Schiff-positive component of the cell coat, and indeed this component may show even better after the enzymatic treatment. Trypsin, however, eliminated both the Hale and the periodic acid–Schiff positive material of the coat.

(Other experiments were performed to see if enzymes would remove the Hale-positive coating from fixed dead cells attached to a glass surface. Receptor-destroying enzyme was very effective in this respect after incubation of the cells with the enzyme for 5 h at 37° C. Pure neuramidase also very actively removed the cell coating. Pepsin and trypsin, in shorter incubation, were ineffective, and, in some instances, the coating stained more intensively with pepsin.)

To study the regeneration of the cell coating, washed *TA*3 cells were treated with receptor-destroying enzyme. After being uncoated by this procedure, they were cultured in Leighton tubes (Eagle's medium plus 10 per cent bovine fœtal serum). The cells were withdrawn 1, 2, 3, 4, 5, and 22 h after incubation (37° C), fixed in Love, and stained with Hale. It was observed, in these circumstances, that the cell coating reappeared in cells after a minimum of 1 h of incubation. However, the coats were less spinny than in the normal control cells.

The action of receptor-destroying enzyme and neuramidase on the cell coat indicates that sialic acid (neuraminic acid) is one of the components of the coating. This is in agreement with the indirect proofs provided by Weiss[4]. Since testis hyaluronidase is inactive, mucopolysaccharides attacked by this enzyme did not co-exist with sialic acid at the cell surface.

These experiments suggest that the Hale stain depends on the contiguity of the galactosamine–neuraminic acid bond at the cell surface. Neuramidase breaks this bond, causing a release of neuraminic acid, and then the Hale staining becomes negative. In contrast, the periodic acid–Schiff stain acts on galactosamine, as this stain is unaffected or even increased by previous neuramidase treatment.

The sialic acid must be bound also, directly or indirectly, to proteins because some proteases remove the Hale-positive coating from live cells *in vitro*. However, destruction of the coat does not occur when dead, fixed cells are exposed to proteases. Under these conditions, the Hale-positive coat may stain intensely, probably because masking proteins are removed[1].

The experiment on cell-coating regeneration shows that *TA*3 cells start to restore their coats of sialomucin within 1–2 h. It seems likely that cells acquire their sialomucin coat from products made in the Golgi zone[5]. The Golgi zone also has Hale-positive material, and this substance may be removed from sections by the receptor-destroying enzyme or sialidase.

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Effects of Morphine on Uptake of Glucose and Synthesis of Glycogen in Muscle of Normal and Chronically Morphinized Rats

THERE is as yet no satisfactory explanation for the phenomenon of drug addiction. Morphine-induced biological dependence of a type which might be the basis of addiction in higher animals has not been demonstrated in unicellular organisms which have metabolisms independent of nervous or hormonal control, and no convincing explanation for such induced dependence arises from knowledge of the effects of morphine on the nervous system alone. There is little doubt that the chemical basis of addiction involves hormonal systems, and we further suspect that the site of addicting action may lie in mechanisms whereby hormonal effects are superimposed on the more primitive, intrinsic controlling mechanisms of cellular metabolism.

This view is encouraged by the results of experiments in which, preliminary to a more extensive study of the effects of morphine on the hormonal control of metabolism, we have compared the effects of morphine *in vitro* on the rates of glucose-uptake and glycogen synthesis in excised diaphragms of normal and of chronically morphinized rats.

Experimental procedures were based on those of Gemmill[1,2], Verzár and others[3,4]. Diaphragms from decapitated rats were trisected, the middle, vertebral portion used for the estimation of initial glycogen (we have confirmed the reliability of this procedure) and the two similar lateral portions were incubated in separate vessels for 2 h at 37° and *p*H 7·4 in aerated, phosphate-buffered saline (2 ml.) containing glucose (0·15 per cent), one containing morphine. Chronically morphinized rats were rats which had received daily injections of morphine (0·3 mg/100 g body-wt.) for 6 weeks. The control rats had received daily injections of saline for the same period. Glycogen was determined by the method of Good *et al.*[5] and glucose by Nelson's method[6].

The rate of uptake of glucose by diaphragms of normal rats was increased by the presence of morphine (1·9 × 10^{-4} – 1·6 × 10^{-3} M) in the buffered glucose–saline medium. In some experiments a fourfold increase was observed and in no case was the rate of glucose-uptake decreased by the addition of morphine. On the other hand, addition of morphine to diaphragms of chronically morphinized rats decreased the rate of glucose-uptake. The effects of morphine in a bicarbonate-buffered medium were similar to those in the phosphate-buffered medium. The results of experiments with 7·7 × 10^{-4} M final concentration of morphine are expressed in Table 1.

In the absence of added morphine there is no significant difference between the rates of glucose-uptake by diaphragms of normal and of chronically morphinized rats. This is confirmed by comparing chronically morphinized rats with control rats which had received saline injections (Table 2). On the other hand, the mean glycogen content of the diaphragms of the morphinized rats is somewhat higher than is that of the saline-injected controls ($P < 0{\cdot}1 > 0{\cdot}05$).

Table 1 EFFECTS OF MORPHINE (7·7 × 10^{-4} M) ON GLUCOSE-UPTAKE BY DIAPHRAGMS OF NORMAL AND CHRONICALLY MORPHINIZED RATS

	Glucose-uptake, mg/g wet tissue/h Mean ± *S.E.* (range) Control	+Morphine	Difference	*t*-test
Normal rats (21)	1·58 ± 0·141 (0·34–3·00)	2·58 ± 0·204 (1·45–4·00)	+1·0 ± 0·192	($P < 0{\cdot}001$)
Chronically morphinized rats (6)	2·46 ± 0·452 (0·98–4·08)	1·73 ± 0·355 (0·66–3·14)	−0·73 ± 0·269	($P < 0{\cdot}05$)

Table 2. INITIAL GLYCOGEN CONTENT AND RATE OF GLUCOSE-UPTAKE OF DIAPHRAGMS OF NORMAL AND CHRONICALLY MORPHINIZED RATS

	Glycogen, mg/g wet tissue Mean ± *S.E.* (range)	Glucose-uptake mg/g wet tissue/h Mean ± *S.E.* (range)
Control rats, saline-injected (12)	1·03 ± 0·145 (0·71–2·16)	1·83 ± 0·239 (0·96–3·54)
Chronically morphinized rats (16)	1·43 ± 0·157 (0·70–3·14)	1·87 ± 0·249 (0·38–4·08)

The rates of change of glycogen content during incubation of diaphragms of both normal and chronically morphinized rats are less affected by added morphine than are the rates of glucose-uptake. The mean rate of glycogen 'synthesis' in 9 normal rats was increased by addition of morphine (7·7 × 10^{-4} M) from 0·275 ± 0·132 to 0·347 ± 0·127 mg/g tissue/h, but we cannot claim that the difference, +0·072 ± 0·162, is significant. In 6 chronically morphinized rats the mean rate of glycogen synthesis was decreased by addition of morphine from 0·798 ± 0·206 to 0·398 ± 0·246, the mean difference being −0·40 ± 0·189 ($P < 0{\cdot}1 > 0{\cdot}05$). It would appear that the effects of morphine on glycogenesis or on glycogenolysis are secondary to direct effects on glucose-uptake. This view is further supported by our observations that morphine does not influence the rates of oxygen consumption and that it has relatively negligible effect on lactate production *in vitro* by diaphragms of both normal and chronically morphinized rats.

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Molecular Weights of Enzymes

IN a recent article Wright[1] put forward the hypothesis that the molecular weights of the enzymes tabulated by Dixon and Webb[2] fall near three series of numbers expressed by $2^n \times 12{,}000$, $2^n \times 16{,}000$ and $2^n \times 19{,}000$, n taking integral values 0–4. This hypothesis was based entirely on the experimental values themselves, mainly by means of graphical representation. A numerical test of the degree of fit of the data to the hypothesis was applied and indicated a good agreement; however, since this involved the arrangement of experimental values into groups, it contained the possibility of prejudice. It therefore seemed worth while to apply the more exacting method of the 'correlation function' used by Johnston, Longuet-Higgins and Ogston[3] to test the Svedberg hypothesis[4].

For this purpose, the null hypothesis is that values of log M are rectangularly distributed within the interval between the two successive predicted log values in which each falls. The predicted log values were those of Wright's hypothesis; the 59 experimental values used were taken from Dixon and Webb's table, omitting those for which a wide range is given. The estimated value of $F(N)$, the 'correlation function', was $+\ 0{\cdot}203$ with a standard error of 0·075. Since $F(N)$ is normally distributed, the significance of its deviation from zero is obtained from tabulation of the normal distribution, giving $0{\cdot}01 > P > 0{\cdot}001$; its positive value indicates grouping of the experimental around the predicted values.

Since this test does not involve a preliminary grouping of the experimental values it might appear to support the subjective judgments on which the hypothesis was based. However, since the relation of the experimental results to the predicted numbers is entirely defined by those numbers which were themselves based on a subjective judgment, the support is more apparent than real. All that it should be regarded as doing is expressing the agreement with hypothesis in a numerical form. Since the hypothesis itself contains four parameters (12,000, 16,000, 19,000 and 2) derived from the data, a good degree of fit is not surprising, and it is conceivable that other four-parameter hypotheses could be found giving equally good fit. Its author wisely councils caution.

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PHYSIOLOGY

Intimate Nature of Muscular Contraction

IT was originally proposed by Astbury[1] that muscular contraction was due to coiling or folding of the contractile proteins. A model of the structure of muscle sarcomere was recently proposed by A. F. Huxley and Neidergerke[2] and by H. E. Huxley and Hanson[3]; their model shows that filaments of actin and myosin slide past each other during contraction. A. F. Huxley[4] proposed a hypothesis of contraction in which it is assumed that active shortening or the development of tension is brought about by generation of relative translational forces between actin and myosin filaments at a series of points in the region of overlap.

In unstriated muscle there is evidence that contraction is due to coiling or folding of the contractile material. The effect of various substances on the contractile mechanism can be tested by using the usual glycerol-extracted muscle[5]. In a second method the muscle is allowed to die in solutions of various substances[6,7]. As the muscle dies its permeability and thus the excitability are destroyed, and the substances can come into direct contact with the contractile mechanism. Using both these methods it has been found that agencies, both physical and chemical, which denature proteins cause unloaded pieces of the transverse muscle of the frog, *Rana tigrina*, to lengthen without the application of any external force. Denaturation of proteins is caused by acids, bases, amides, detergents, guanidine and urea. Some of these substances cause relaxation of unloaded muscle when treated as here; these are acids, bases, urea, formamide and acetamide. It is therefore presumed that the contractile mechanism relaxes actively in these experiments.

Heat is a physical agent which denatures proteins. Its effect on unstriated muscle is most interesting. When unloaded circular strips of frog's stomach muscle are heated to 50° C for 10 min, some of them contract by 2–5 per cent and others lengthen by 10–30 per cent; further heating up to 60° C causes all of them to lengthen by 50–100 per cent. Muscles which have been heated to 50° C and are thus killed lengthen further if treated by the foregoing substances, which denature proteins. There is no doubt, therefore, that these substances act on the contractile mechanism. The action of heat and chemicals is thus complementary, and therefore they most likely exert an identical action on the contractile mechanism in causing relaxation.

The lengthening of unstriated muscle produced by denaturing agents may be due to proteins other than the contractile ones. That the contractile proteins take part in the lengthening is shown by the observation that if living unloaded frog's stomach muscle is immersed in urea, acetamide or formamide it becomes inexcitable but lengthens by more than 100 per cent in 1 hr; if the muscle is then transferred to saline it contracts and recovers its excitability[7]. The proteins in the living muscle had thus not yet been denatured in the usual sense, so that the lengthening could not have been due to the non-contractile proteins; the lengthening of the living muscle is presumably due to the close relationship between denaturation and relaxation. The effects of heat are similar[8]. If unloaded frog's stomach muscle is heated beyond 45° C, it becomes inexcitable but relaxes; if it is not heated beyond about 48° C, then it recovers. Relaxation continues if the muscle is heated to 60° C. The relaxation of living muscle passes imperceptibly into that of dead muscle. It is interesting to note that proteolytic enzymes, trypsin, papain and pepsin with hydrochloric acid cause lengthening of unloaded frog's stomach muscle whether living or killed by heating to 50° C (ref. 9); these enzymes are presumed to denature proteins before hydrolysing them. The lengthening of unstriated muscle by heat and other denaturing agents may be considered as the unfolding of peptide chains during denaturation. These experiments, therefore, elucidate the nature of both denaturation of proteins and relaxation of muscle.

Though unstriated muscle shows no special separation of two types of filaments as found in striated muscle, it is unlikely that there is any fundamental dissimilarity in contraction of the two kinds of muscle at the molecular level. Urea and other denaturing agents do not cause lengthening of isolated, unloaded, striated muscle as it is fully relaxed initially owing to the absence of tonus as found in unstriated muscle. But there appears to be some similarity in the contractile mechanisms of the two muscles as urea causes relaxation of loaded, glycerol-extracted, striated muscle fibres[10]. It is possible to apply the sliding filament theory to unstriated muscle. According to this theory the basic process in contraction of striated muscle is one of sliding; but some folding or crumpling of filaments at their ends may take place at higher degrees of shortening[11]. It may be assumed that in unstriated muscle the first stage is over, that is, the filaments have already slid, thus producing tonus of the isolated muscle and also accounting for the absence of cross-striations; its further shortening can only occur by folding of the contractile proteins.

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Permeability Response of Cerebral and Cutaneous Blood Vessels to Vasoactive Agents

IN 1922, Forssman showed that solutions injected centripetally into the ligated right common carotid artery of the guinea pig were swept by the flowing blood first into the right subclavian artery and thence into the right vertebral artery[1]. His findings on the distribution of such injected materials have since been repeatedly confirmed, and the technique offers a ready means of subjecting the right half of the brain-stem and the right fore-limb in this species simultaneously to the action of possible vasoactive substances.

This route was later used by Broman in his investigation of the effects of various agents on the permeability of cerebral capillaries to trypan blue[2]. In this communication, we record some observations on the effect of certain materials on capillary permeability in the brain, among them some of the recently available pharmacological agents known to affect the permeability of capillaries in the skin of guinea pigs at a site of intradermal injection.

Guinea pigs were anæsthetized with 'Nembutal' and light ether, and the right common carotid artery exposed. One millilitre of a two per cent solution of Evans blue was injected into a saphenous vein. The carotid artery was then ligated, and the material under examination was injected in a volume of 0·3 ml. during a period of about 15 sec. When blueing of the fore-paw occurred, the colour generally developed after about five minutes. The animals were killed after 2 h, the brain was removed and fixed in formol-saline, and 24 h later it was examined for any blue coloration of the brain-stem on the right side. The materials injected and the results obtained are shown in Table 1. Broman made no mention of any colour-change in the right fore-paw, but as regards the staining of the brain-stem, our findings with histamine, sodium deoxycholate and Forssman antiserum agree with his.

It is apparent that in quantities verging on, and sometimes exceeding, the lethal dose, the small blood vessels of the skin and brain differ greatly in their permeability response for this dye to the materials used. Histamine, substance 48/80 and bradykinin all caused leakage of the dye into the tissues of the fore-paw, but no raised permeability was apparent in the vessels of the brain-stem. A blue coloration of the cerebral tissues was seen only after the injection of sodium deoxycholate and of a high-titre Forssman antiserum. These observations show that it is unsafe to extrapolate from experiments on the skin to the permeability effects of these substances on the small blood vessels of the brain.

Table 1

Substance	Quantity	Blue coloration	
		Fore-paw	Brain-stem
Histamine acid phosphate	0·3 mg/kg	+	—
Substance 48/80	5 mg/kg	+	—
Bradykinin	80–500 µg/kg	+	—
5-HT creatinine sulphate	500–2,000 µg/kg	—	—
Sodium deoxycholate	12·5–25 mg/kg	—	+
Forssman antiserum	12·5–50 µl./kg	—	+

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Heparin and Blood Lipids in Fasted Individuals

IT is well known that intravenous administration of heparin induces in hyperlipæmic individuals a clearing of the plasma turbidity[1]. This effect has been explained by changes in physicochemical properties of the circulating lipids, consisting mainly of hydrolysis and dissolution of the fat in the plasma, due to the activation of a lipæmia-clearing mechanism[2]. There is also a suggestion that heparin could accelerate the removal of hydrolysed lipids from the blood stream since it was found that it induces a decrease in total fatty acids of plasma in hyperlipæmic rats[3].

The clearing effect of heparin has mainly been studied in hyperlipæmic individuals. There is a doubt whether heparin has any effect in non-hyperlipæmic states, since it has been suggested that it induces changes in serum lipoprotein spectrum only in hyperlipæmic subjects[4–7].

Therefore an investigation was undertaken to investigate the effect of heparin on plasma lipids and esterase activity in fasted subjects.

This effect was examined in 26 normal individuals after a 12 h fasting period. Blood was examined before the experiment and 5, 60 and 180 minutes after the intravenous administration of 2,000 units of heparin (Boots Pure Drug Co.).

An equal number of normal non-treated individuals were also examined in order to observe any possible spontaneous changes in blood lipids and esterase activity values during the experiment.

5 ml. of blood were drawn and mixed with 10 mg of potassium oxalate to prevent clotting and the plasma was separated by centrifugation.

The total lipids were measured by the sudan black method[8,9], the lipid phosphate by the Zilversmit and Davis method[10] and the plasma esterase activity by the method of Tuba and Hoare[11], using tributyrine as substrate.

The results are presented in Table 1. Total lipids and lipid phosphate are expressed in mg/100 ml. of plasma, and esterase activity as Tuba–Hoare units. The values are presented as mean values ± *S.E.* Normal non-treated individuals show practically no changes in total lipids or esterase activity of plasma

Table 1. Effect of Heparin on Lipids and Esterase Activity of Plasma

	Heparin			Control		
Time	Total lipids	Lipid phosphate	TBEsterase	Total lipids	Lipid phosphate	TBEsterase
Before injection (min)	324 ± 7·34	9·97 ± 0·26	351 ± 14·2	329 ± 7·24	10·28 ± 0·32	363 ± 15·0
5	257 ± 10·30	9·90 ± 0·29	419 ± 15·7	331 ± 6·40	10·26 ± 0·26	361 ± 14·2
60	279 ± 10·80	9·80 ± 0·27	384 ± 15·0	345 ± 7·72	10·14 ± 0·30	359 ± 14·3
180	317 ± 7·74	9·62 ± 0·25	356 ± 14·7	351 ± 7·18	10·19 ± 0·29	358 ± 15·1

during the experiment. Heparin-treated individuals show a significant decrease in total lipids ($P < 0·001$) accompanied by simultaneous increase in esterase activity ($P < 0·02$). These changes are observed 5 min after heparin administration. A return to the initial values was observed three hours after the beginning of the experiment. There was no change in plasma lipid phosphate in both groups. The decrease of plasma lipids and the increase of esterase activity observed indicate that heparin activates the plasma-clearing mechanism not only in states of hyperlipæmia but also in non-hyperlipæmic subjects.

Furthermore, the decrease of total plasma lipids could suggest that clearing is not only due to a change of the physicochemical properties of lipids but also to an accelerated removal of them from the blood stream. This conclusion is in agreement with the observations that heparin produces a distinct fall in the plasma lipids when given intravenously with different fat preparations[12–14] and in plasma total fatty acids when given in hyperlipæmic rats[9].

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Effect of Bradykinin on Isolated Coronary Arteries

During an investigation of the comparative pharmacology of isolated vascular smooth muscle, it was noticed that the coronary arteries of the sheep were caused to contract by very small doses of bradykinin (Sandoz). Other substances were less active.

Spiral strips 2·5–4·0 cm long were cut from the left circumflex coronary artery of recently killed sheep or dogs, and were suspended in an isolated organ bath containing 10 ml. Tyrode solution, oxygenated with 5 per cent carbon dioxide in oxygen at 38° C. An isotonic frontal writing lever was used, counter-balanced to exert 2*g* tension. All preparations were left for 2 h before testing.

In 20 sheep coronary strips bradykinin caused a contraction within 15–30 sec. Repeated administration at 20-min intervals did not lead to a decrease in the response, which was usually constant, but sometimes increased in size over several hours. Fig. 1 shows a typical dose-response relationship. Many preparations were very sensitive to bradykinin, responding with a good contraction to as little as 0·001 μg/ml.; a few preparations required up to 0·1 μg/ml. Acetylcholine caused a contraction in doses of 0·025–5 μg/ml., and 5-hydroxytryptamine in doses of 0·3–1·0 μg/ml. Histamine caused contraction after administration of 100 μg/ml. The contraction caused by acetylcholine, but not that of bradykinin, was abolished by atropine. Adrenalin and noradrenalin caused relaxation in large doses (100 μg/ml.). In 2 of 8 isolated strips from dog's coronary arteries bradykinin and acetylcholine caused relaxation; they never elicited a contraction in the doses which were effective in sheep.

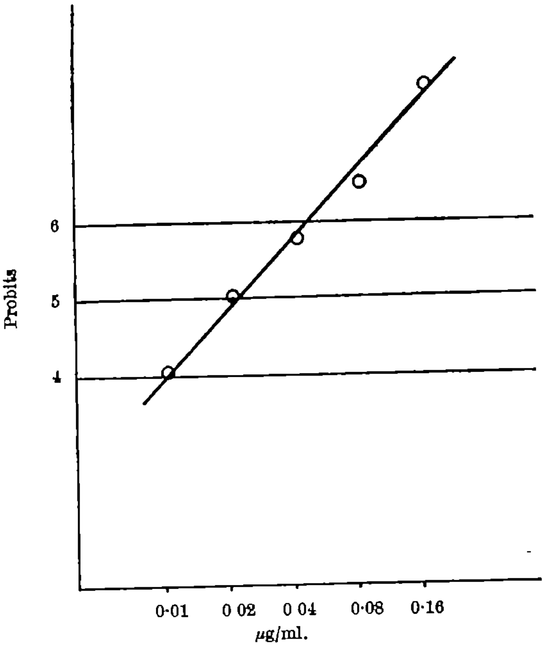

Fig. 1

It is interesting that this effect of bradykinin on the coronary vessels appears to vary with the species, and the great sensitivity of the sheep coronary vessels may make this preparation suitable for use in bio-assay.

I thank Sandoz Products, Ltd., for supplies of synthetic bradykinin.

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Iron Content of Mammalian Breast Milk: Measurements in the Rat and in a Marsupial

LITTLE attention has been paid to the physiology of iron transfer through the mammary gland into milk, although a considerable amount of work has been done on absorption through the intestinal epithelium and on transfer from mother to foetus through the placenta. Direct measurements of the iron content of milk have mostly been confined to man and dairy animals. The average concentration of iron is approximately 0·5 μg/ml. in both human and bovine milk[1]. This value is lower than the levels of plasma iron in the respective species. In rat milk, on the other hand, a mean iron concentration of 7 μg/ml., a figure which is higher than plasma iron values, was reported by Cox and Mueller[2].

During the course of work by us and others in this Department, on various aspects of iron metabolism in lactating animals and in the developing young, we have accumulated a large series of measurements on milk iron in the laboratory rat and in the quokka, a macropod marsupial indigenous to Western Australia[3]. The results of these studies will be reported in detail elsewhere. It seems, however, appropriate at this stage to direct attention to certain similarities between the two animal species in respect of the iron content of milk.

Briefly, in both rat and quokka the iron content of milk assumes values considerably higher than the plasma iron and there is in both species a clear pattern of change in milk iron content with different stages of lactation. The iron in rat milk is highest at the beginning of lactation and then diminishes gradually (Fig. 1). It is of some interest to note that in the series represented in the graph the mean iron concentration taken over the whole period of lactation agrees well with the figure of 7 μg/ml. given by Cox and Mueller. In the quokka, where the young are confined to the marsupial pouch and to an exclusive milk diet for nearly six months of their extra-uterine life, milk iron remains consistently high throughout this period but a drop in the concentration occurs with the onset of weaning (Fig. 2).

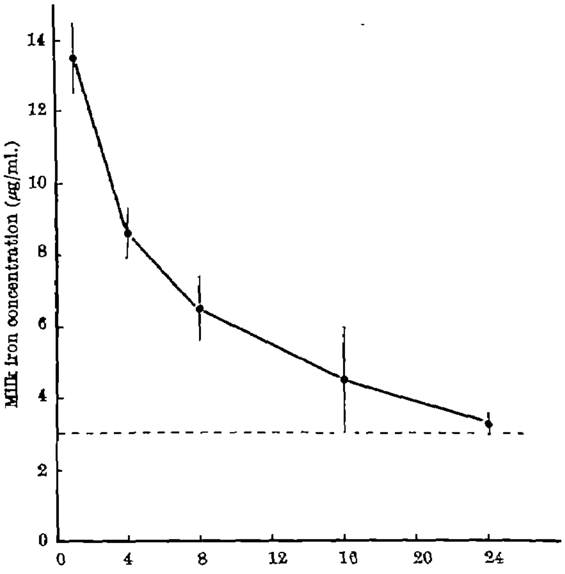

Fig. 1. Iron in rat milk. Each plot with a vertical bar represents the mean and its standard error for a group of 16 rats, except on day 1, where the group consisted of 9 rats. The horizontal broken line represents the mean plasma iron concentration in lactating rats

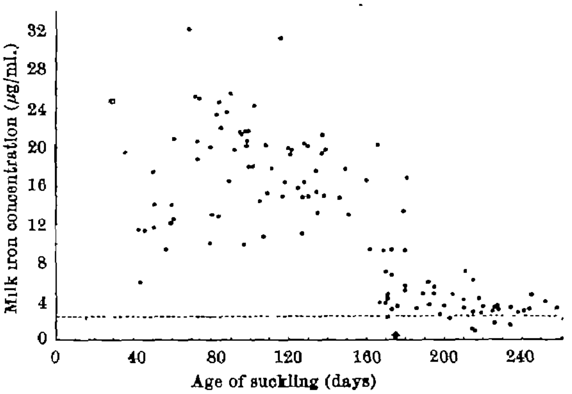

Fig. 2. Iron in quokka milk. Each plot represents a single measurement. The horizontal broken line represents the mean plasma iron concentration in lactating quokkas. The arrow on the abscissa indicates the average age at which weaning begins. The age of the young was estimated from measurements of physical characters as described by Shield and Woolley (ref. 7)

A physiological mechanism capable of concentrating iron into the milk is of obvious biological significance in those mammals the young of which are born relatively 'immature'. In the young quokka, the hæmoglobin concentration of blood rises from approximately 9 g/100 ml. at one month of age to 13 g/100 ml. at six months. The amount of non-hæmoglobin storage iron accumulated in the liver of six-month-old animals is greater than the corresponding fraction in maternal livers, though the weight of these young is only one-sixth to one-seventh that of the adult females (Kaldor, to be published).

In the case of the new-born rat, which grows faster and suckles for a much shorter period than the young quokka, it is more difficult to give a clear interpretation of the physiological significance of high maternal milk iron values. The suckling rat becomes anæmic before weaning[4] and this anæmia has been attributed to the inability of erythropoietic mechanisms to match the rate of body growth[5]. It has been shown that the iron content per unit body weight is also reduced at the time of onset of weaning[6], but in our own series young rats accumulated iron in proportion to their body weight throughout the suckling period (Ezekiel and Morgan, *J. Physiol.*, in the press). We are, as yet, unable to explain this difference in results.

Work is now in progress in this Department on the mechanism of iron transfer from plasma to milk. It is hoped that such studies may contribute to the general problem of iron transfer across cellular barriers.

This work was supported by the National Health and Medical Research Council of Australia and the Medical Research Grants Fund, University of Western Australia.

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HÆMATOLOGY

Johnson Type Haptoglobin

DURING our screening for haptoglobin patterns in different Jewish ethnic groups[1,2], we found a sample from a Kurdish boy (from Urmia in the Kurdish part of Persia) which appeared to contain a 'Johnson type of haptoglobin pattern'[3]. (We thank Prof. H. Harris (London) for comparing it with a known sample and identifying it.) Further examination of the family revealed the following results (Figs. 1 and 2). The mother of the propositus had a Johnson pattern of Hp and the father a Hp^{2-1} pattern; of the 7 children two had Johnson type Hp, one Hp^{1-1}, one Hp^{2-1}, two 'ahaptoglobinæmia' and one a hypohaptoglobinæmia. Two-and-a-half-fold concentration of the two ahaptoglobinæmic and the hypohaptoglobinæmic sera revealed the presence of haptoglobin in all of them. However, the exact pattern in them could not be determined.

In view of the presence of normal Hp^{1-1} in one of the children, it has to be assumed that the Johnson type haptoglobin is a combination of normal Hp^1 and Hp^J. The Hp^J is probably a mutation at the Hp^2 locus, or an additional allele. Lately Smithies has demonstrated biochemical evidence for a Hp^{2J} gene which is apparently a Hp^2 mutation[4]. Furthermore, he has demonstrated two Hp^1 polypeptides, a fast (Hp^{1F}) and a slow (Hp^{1S}). In the haptoglobin of the Johnson type Smithies demonstrated a Hp^{1S} and a Hp^{2J} polypeptide[4].

The hypohaptoglobinæmia which was found in three children is particularly interesting. The Hp^{2-1} pattern in the third generation from a father with a hypohaptoglobinæmia indicates that the hypohaptoglobinæmia is a combination of normal Hp^1 and Hp^{2J}. In family investigations Giblett and Steinberg found that a child of Hp^{2-1} (mod.) × Hp^{2-1} (mod.) mating was hypohaptoglobinæmic. Although they called it tentatively Hp^{2-2} they were not certain of the phenotype[5]. A phenotype Hp^2/Hp^{2M} looks just like Hp^{2-2}, but it can also be ahaptoglobinæmia. It was lately demonstrated that Hp^{2M}, is another Hp^2 mutation[4].

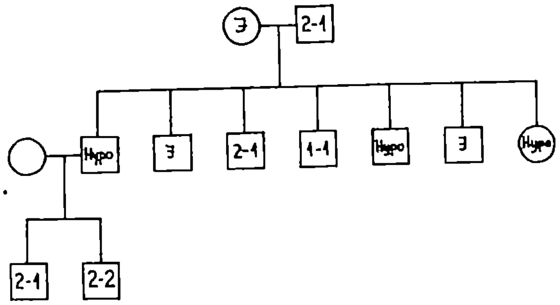

Fig. 1. The family tree

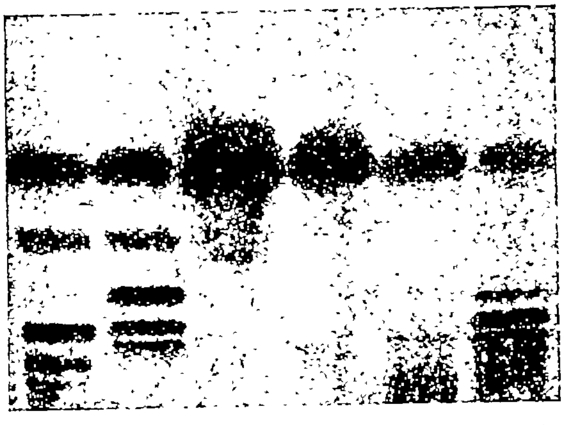

Fig. 2. Haptoglobin patterns (benzidine stain). Left to right: Hp^J, Hp^{2-1}, haptoglobinæmias (2·5-fold concentrated serum), Hp^{2-1}

It would appear, therefore, that the presence of Hp^2 in a genotype is associated with a decrease in haptoglobin production so that (in general) Hp^{1-1} > Hp^{2-1} > Hp^{2-2}.

The mutant forms of Hp^2 (that is, Hp^{2M} and Hp^{2J}), especially when associated with Hp^2, are even more likely to be associated with a decreased (or absent) haptoglobin. Only further family studies will prove if this hypothesis is correct.

Lately, Giblett found two more examples of the Johnson phenotype in samples from Hawaii. This makes a total of eight different families in the world known to contain the Hp^{2J} gene (or a slightly modified form) (Giblett, E., personal communication).

We thank Dr. E. Giblett for advice.

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Waldenström's Macroglobulinæmia observed in Two Brothers

PRIMARY macroglobulinæmia (Waldenström's disease) has been observed in two brothers aged respectively 65 and 63 years. The clinical and hæmatological findings are summarized in Table 1. These two patients showed almost the same clinical picture: hæmorrhagic disorders with frequent epistaxis, hæmatoma, melœna; anæmia, increase of the lymphoid cells in the circulating blood, and in bone marrow. The eye grounds show a dysproteinæmic aspect and bilateral bleedings. Patient 1 (Louis D.) shows enlarged lymph nodes and splenomegaly.

Both patients have a high sedimentation-rate (140 mm/h), hyperproteinæmia (110 g/l.), and an *M* component (a narrow band in γ_1 zone) is observed in paper electrophoresis. This *M* component belongs to the biochemical type $\gamma_1 M$ ($\beta_2 M$) and does not penetrate into starch gel during electrophoresis.

The sedimentation patterns obtained in a Spinco analytical ultracentrifuge equipped with a Philpot–Svensson optical system shows three heavy components in both sera. These macroglobulins correspond to about 50 per cent of the whole serum proteins.

Table 2 shows the sedimentation-rate and the percentage of heavy components in the serum of the two brothers. Figs. 2 and 3 show the almost identical patterns obtained during ultracentrifugation of serum samples with increasing dilutions.

Table 1. CLINICAL AND HÆMATOLOGICAL FINDINGS

	Louis D.	Paul D.
	65 years old	63 years old
Hæmorrhagic disorders	Epistaxis once weekly, multiple hæmatoma, melœna	Epistaxis twice weekly, multiple hæmatoma
Blood	Anæmia R.B.C 2·5 m W.B.C. 9 000 Lymphoid cells 38–62 per cent	Anæmia R.B C. 2·8 m. W.B.C. 6·000 Lymphoid cells 23–54 per cent
Sternal puncture	Lymphoid elements 80 per cent Plasma cells 1 per cent	Lymphoid elements 50 per cent Plasma cells 10 per cent
Lymph glands	Enlarged Proliferation of cells of the lymphoid system overflowing the capsule	No palpable
Spleen	Enlarged	No palpable
Roentgen of the skull	No skeletal changes	No skeletal changes
Eye grounds	Enlargement of retina veins	Bilateral bleedings
Clotting tests	Normal	Normal
Sedimentation-rate	140 mm	140 mm
Total serum proteins	110 g/l.	110 g/l.

Table 2 ULTRACENTRIFUGATION COMPONENTS

	A		M_1		M_2		M_3	
	S_{20}	*p*. 100	S_{20}	*p*. 100	S_{20}	*p*. 100	S_{20}	*p* 100
Louis D.	4·5	51	19 2	43	29·5	6	35·7	
Paul D.	4·6	48	18·9	42	28·3	7	33·7	3

The mother of our two patients, 80 years old, showed no clinical manifestations and the ultracentrifugal analysis did not show any hyper macroglobulinæmia. However, we observed a minor narrow γ_1 band in paper electrophoresis and considerable increase of $\gamma_1 M$ globulin in immunoelectrophoresis.

It is interesting that the two brothers started Waldenström's disease at the same age (61 years), showing the same clinical manifestations and hæmatological disorders. Even more, the abnormal macroglobulinic components observed in the ultracentrifuge showed identical characteristics in both brothers, indicating a very similar or even identical physical structure.

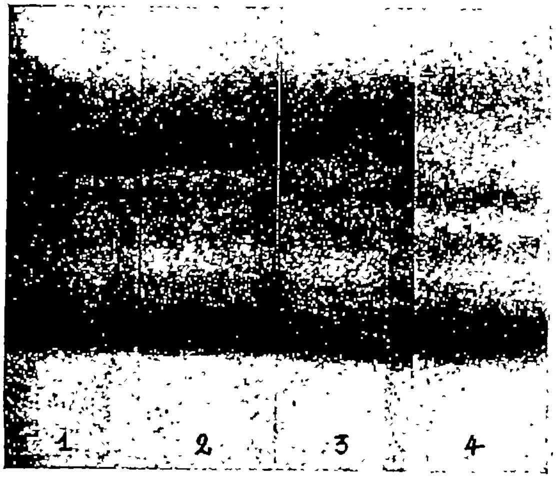

Fig. 1. Paper electrophoresis (veronal buffer *p*H, 8·6, staining with amidoblack 10 *B*)
Electrophoretical pattern of the serum in family D. 1, Louis D.; 2, Paul D., his brother; 3, Madame D., their mother; 4, Serum of normal human adult

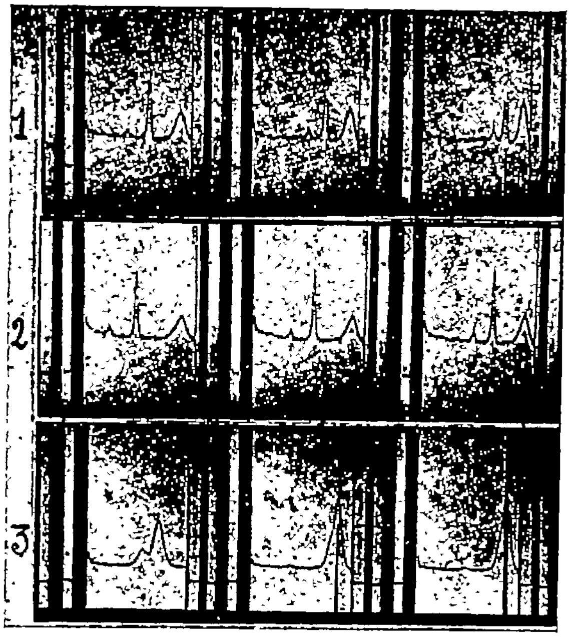

Fig. 2. Ultracentrifugation patterns of serum. 1, Louis D., 2, Paul D, 3, Madame D

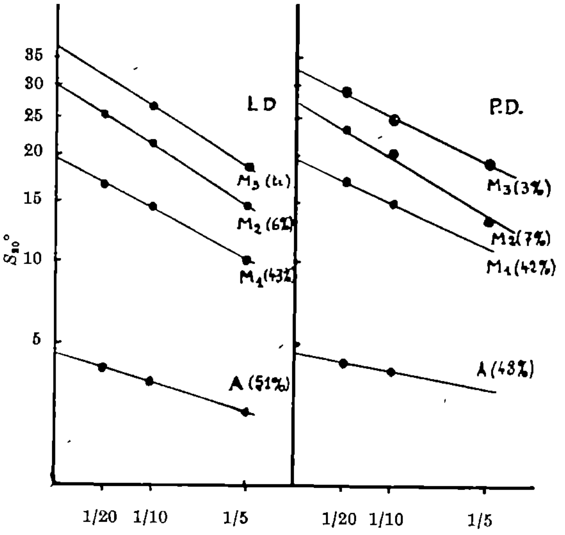

Fig. 3. Diagrams of the sedimentation-rate of the macroglobulinic components and their relationships with the dilution of serum

These findings, together with the observation of a minor *M* component of the $\gamma_1 M$ type in the serum of the mother, suggests the idea that Waldenstrom's disease might be an inherited syndrome due to some genetical disorder; the illness being only observed in the homozygotes of this abnormal gene. Unfortunately the familial studies are very often impossible as this disease is almost usually observed in people aged 50 years and more. So, one or both parents might be dead at that time and the observation of the grandparents is therefore absolutely impossible.

We hope that the publication of these exceptional findings will be followed by the study of the ascendants in other cases of 'primary macroglobulinæmia'.

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Hæmogram of Normal and Starved Rats

THE bioassay of plasma erythropoietin by the starved rat method was introduced by Fried *et al.* in 1957[1] and has since been utilized by other investigators for evaluating erythropoietic stimuli. Gross changes in peripheral blood are apparent in all rats prepared for assay by withdrawal of food for varying periods of time.

The hæmograms of Sprague–Dawley rats before and after starvation for 80–96 h are shown in Table 1. The rats were kept singly in cages and had previously been fed on standard 'Purina' laboratory chow. Water *ad lib.* was provided during the starvation period. Pre-starvation body-weights were 155–170 g; after starvation, weights ranged from 100 to 121 g.

Mean levels of hæmoglobin, hæmatocrit and red blood cell count before starvation are comparable with other published values on 'normal' laboratory rats[2,3]. The hypochromia and microcytosis of the erythrocytes may be indicative of relative iron-deficiency, though iron (3 mg/10 g food) was included in the rat diet. High red blood cell, platelet and reticulocyte counts are a striking feature of the normal rat hæmogram. The intense reticulocytosis in the Sprague–Dawley rats before starvation is higher than that noted in an early investigation of normal albino rats by Orten and Smith[2]. Their figures reveal a mean of about 8 per cent reticulocytes in rats of comparable size.

The polycythæmia and hæmoconcentration seen after starvation (Table 1) are probably a reflexion of the degree of dehydration in the starved animals. Similar changes occur in rats given food but no water.

Inanition was accompanied by leukopænia which varied in individual rats from 1,600 to 4,300 white blood cells per cubic millimetre of blood. Differential leukocyte counts were not averaged because of marked variation from one animal to another. Granulocytes were generally less than 20 per cent of peripheral white cells in normal rats and no real differences were found in the starved animals.

Specific nutritional deficiencies known to influence hæmatopoiesis were not delineated in the starved rats. It seems possible, however, that part of the response of the starved rats to anæmic plasma, and indeed the slight erythropoietic effect of normal plasma or saline, may be the result of factors other than a specific hormonal erythropoietic factor. The suggestion has been made that polycythæmia plasma contains an inhibitor to erythropoiesis[4]. Any such inhibition is apparently easily overcome by anæmic plasma and even to some extent by normal plasma or saline. Reticulocytes in the starved rats increased from a mean of 0·8 per cent on the fourth day of starvation to 3·0 per cent 24 h after normal saline (3·0 ml.) alone was given intravenously.

Table 1. MEAN HÆMATOLOGICAL VALUES IN 88 SPRAGUE–DAWLEY RATS BEFORE AND AFTER STARVATION

	Before starvation	After starvation
Body-weight (g)	155–170	100–121
Hæmoglobin (g/100 ml.)	12·8	17·9
Hæmatocrit (%)	46	58
R.B.C (million/mm^3)	6 86	9·18
Reticulocytes (% of R.B.C.)	19	0·8
W.B.C. (per mm^3)	19,400	3,680
Platelets (direct count/mm^3)	934,000	669,000

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PATHOLOGY

Pathological Proliferation of the Trophoblast Homograft in Women

ABOUT 1 g of trophoblast tissue, obtained in the course of legal interruptions in the second and third month of pregnancy, was grafted into the abdominal wall (mm. recti abd.) of: (*a*) 5 elderly women suffering from incurable cervical cancer; (*b*) 25 elderly women suffering from incurable cervical cancer and treated daily with 5 mg of œstrone acetate 3 weeks prior to grafting and consecutively until the removal of the graft; (*c*) 3 patients suffering from endogenous hyperœstrogenism.

5–7 weeks later the grafts were removed and examined histologically, about one half of the tissue being reserved for the estimation of chorionic gonadotropin (HCG).

The grafts from the cancer patients not treated with œstrogens were found, according to expectation, to have degenerated (Fig. 1), and no HCG could be detected by the Galli Mainini test. The grafts from œstrogen-treated patients showed no homograft reaction, were viable, and in addition marked proliferation with polymorphism of the nuclei and metachromasia were also present (Fig. 2). In some instances syncytial cells were found dispersed in the connective tissue (Fig. 3). Similar findings were obtained in cases suffering from endogenous hyperœstrogenism (Fig. 4).

The results demonstrate clearly that a high œstrogen-level suppresses the reaction of the host to homologous trophoblast tissue, and permits this not only to proliferate but also to synthesize HCG. It remains to be seen whether this action of œstrogen

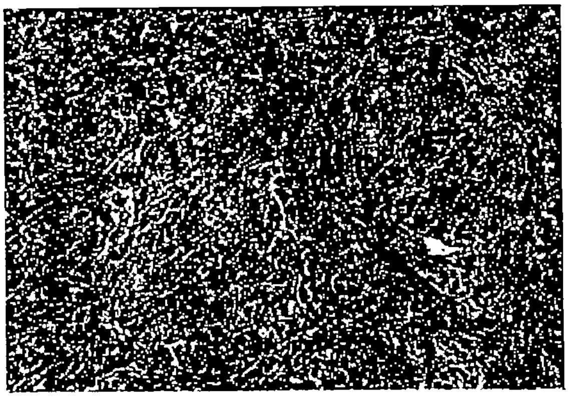

Fig. 1. Trophoblast implanted without œstrogen treatment. Degenerated villi, inflammatory infiltration. (Hæmatoxylin–eosin stain. × c. 150)

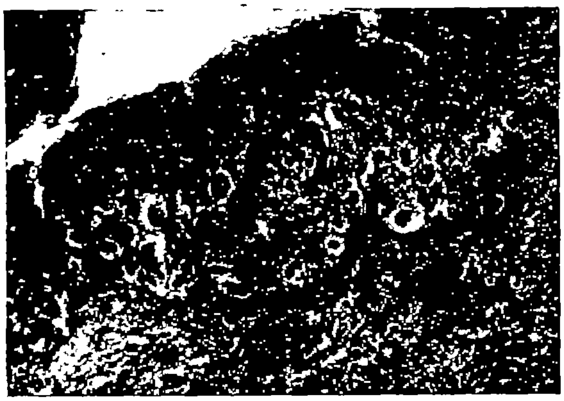

Fig. 2. Adenomatous proliferation of polymorphous Langhans cells after œstrogen treatment. (Hæmatoxylin–eosin stain. × *c*. 150)

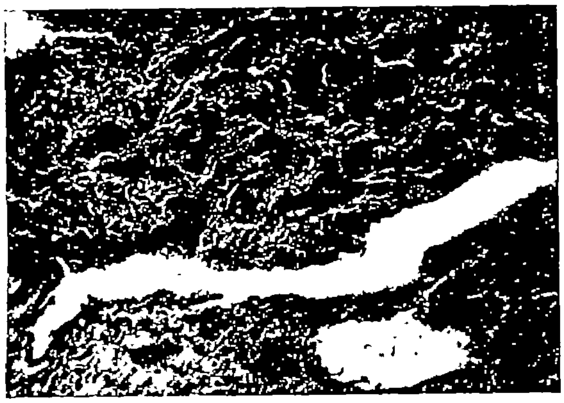

Fig. 3. Invasion of the trophoblast epithelium into connective tissue after œstrogen treatment. (Hæmatoxylin–eosin stain. × *c*. 150)

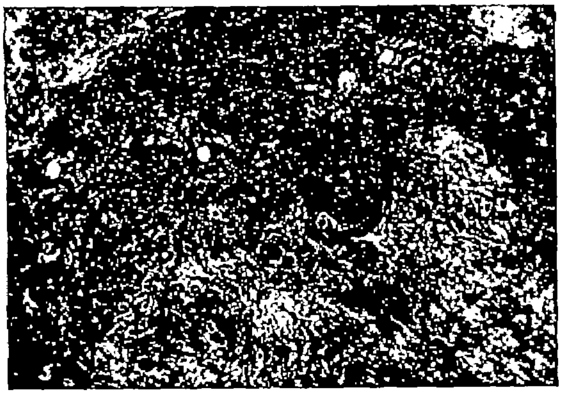

Fig. 4. Invasive growth of the trophoblast epithelium in endogenous hyperœstrogenism. (Hæmatoxylin–eosin stain. × *c*. 75)

is mediated by the hypophysis as some observations seem to indicate[1,2], or whether it is of a more direct nature; though, according to our experimental investigations, the œstrogen did not cause any synthesis of HCG *in vitro*.

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Viability of Ehrlich Ascites Tumour Cells exposed to Sodium Chromate labelled with Chromium-51

Rajam *et al.*[1] were the first to report the binding of $Na_2{}^{51}CrO_4$ by Ehrlich mouse tumour cells, the viability of which was demonstrated by solid tumour formation after subcutaneous inoculation. Selecki[2], using a much greater isotope concentration (10 μc. per 10^7 cells), also reported subcutaneous growth. In view of the higher radiation exposure involved, it became necessary to confirm the viability of such preparations grown in the ascitic phase, in which gain in weight and subsequent transplantability can be compared directly with controls.

Ascitic fluid was pooled from 3 tumorous donor mice, counted in a hæmocytometer chamber, and divided into 3 portions. One was incubated with $Na_2{}^{51}CrO_4$ at a concentration of 10 μc. per 10^7 cells, washed with normal saline 5 times after Selecki's method[2], and adjusted finally to a concentration of 11×10^6 cells per ml. The second portion was incubated with normal saline alone, washed as before and adjusted to the same concentration. The third was stored for the same period, adjusted to the same cell concentration, but otherwise was undisturbed. Fourteen mice were inoculated with the first preparation, 7 with the second, and 6 with the third; the dose in each case was 0·3 ml.

Fig. 1 shows the similarity of gain in weight, due to ascites, in the three groups of mice; none of the differences is significant ($P > 0{\cdot}5$). To test the viability of treated cells further, serial replicate passage was originated from each of these mice and later from a similar preparation. Growth was vigorous through 9 and 8 transplant generations respectively before elective termination. Cytogenetic analysis of two mice in the first transplant generation

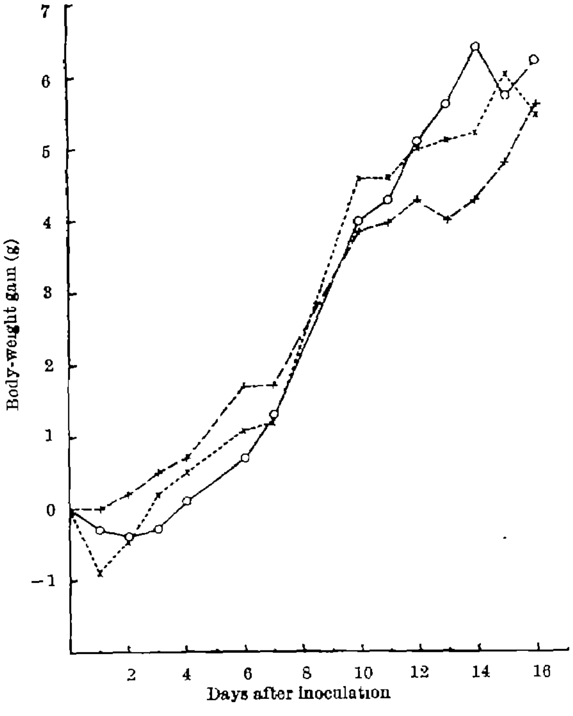

Fig. 1. Plot of weight gain due to tumour growth against days after inoculation. O, $Na_2{}^{51}CrO_4$ treated cells; + and ×, untreated control cells

showed no departure from the modal $2n = 43$ typical of the hyper-diploid tumour line maintained in this laboratory.

These findings confirm previous reports of the viability of Ehrlich ascites tumour cells incubated with $Na_2{}^{51}CrO_4$ even at high concentration. Grown in the more sensitive ascitic phase, a direct comparison of labelled and unlabelled cells in terms of weight gain, transplantability and—to a limited extent—chromosome complement has been possible.

I thank Dr. P. L. T. Ilbery, School of Public Health and Tropical Medicine, University of Sydney, for help with the chromosome analysis.

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Experimental Production of Mesothelial Tumours of the Pleura by Implantation of Dusts in Laboratory Animals

IN view of the possible association between the development of mesotheliomas of the pleura and exposure to asbestos dust in people living in the Cape asbestos fields[1,2], a preliminary experiment was undertaken to see if it was possible to produce mesotheliomas, in animals, by injecting various forms of asbestos into the pleural cavities. 110 young rats, mice, and guinea pigs were used. The experiment has not yet been concluded as 45 of the guinea pigs, and four of the rats, are still alive thirty months after the start of the experiment. This is a preliminary report of our positive findings in 106 rats which have either died or have been killed.

The rats used were pure-bred Wistar rats, from the South African Institute for Medical Research stock; they were six weeks old at the time of inoculation. These animals were divided into eleven groups of ten animals; five groups were male, and six female.

The following dusts were used: (1) three samples of crocidolite dust prepared from virgin fibre obtained from mines in the southern and northern regions of the Cape asbestos fields and from a mine in the Pietersburg district of the Transvaal; (2) three samples of dust obtained from the mills of crocidolite mines in the same geographical distribution as those described in (1); (3) two samples of chrysotile dust prepared from virgin fibre—the first obtained from a mine in which the asbestiform serpentine occurred as an ore-body in basic rock, and the other dust from a deposit where the serpentine was found in dolomite; (4) a sample of amosite prepared from virgin fibre; (5) a sample of 99·9 per cent pure silica dust; (6) a sample of pure carbon black. (5) and (6) were used as controls.

The dusts were ground to a fine powder, and were suspended in normal saline at a concentration of 50 mg/ml. These materials were injected into the pleural cavities of the rats. The details of the preparation of the dusts and the method of inoculation will be described in a further publication. Dr. J. S. Harington analysed all these samples for the presence of oils and 3 : 4-benzpyrene[3]. These substances were found in all the crocidolite samples, very slight amounts in the amosite sample, but none in the chrysotile, silica or carbon black materials.

Bronchiectasis, a common feature among laboratory rats after the age of 6 months, was found in almost every animal. Indeed, this condition, with associated abscess formation or empyema, was the most usual cause of death. Seven animals with breast adenomata were all killed at a stage when these tumours had become so large that they were causing the animals marked discomfort. Eight animals developed reticulum-celled sarcomas and four mesotheliomas. These rats appeared to have died of these malignancies.

Two female rats died of large pelvic abscesses, and in one small group of animals no obvious cause of death could be found.

Pleural mesothelial tumours were observed in two animals inoculated with crocidolite fibres; one sample of the crocidolite being obtained from the Pietersburg area and the other from the northern portion of the Cape asbestos fields. Of the other two, one occurred in an animal inoculated with chrysotile fibre from the ore body, and one among the rats receiving the pure silica.

So far as can be ascertained from the literature[4], tumours of this type do not occur naturally in rats of the Wistar strain.

A further rat, a survivor of a dusting experiment in which animals were exposed to a fine chrysotile dust for 120 days, died 450 days after removal from the dust room. At necropsy, this animal was found to have a pleural mesothelioma. Macroscopically and histologically, the tumours in these animals were similar to those seen in human cases. In addition, hyaluronic acid secretion was demonstrated histochemically in four of these tumours, while in one animal, Dr. Harington was able to determine its presence chemically in the pleural fluid. The presence of hyaluronic acid has been an aid to the diagnosis of the tumours in human cases[5].

Fibro-adenomata of the breast have been frequently observed among female laboratory rats, and Ratcliffe[4] observed them to be the commonest tumour among stock Wistar rats. Reticulum-celled sarcomas were also not infrequently observed among these animals.

Table 1. OCCURRENCE OF TUMOURS IN RATS AFTER INTRA-PLEURAL INOCULATION OF 25 MG OF DUST

	Type of dust						
	Crocidolite fibre	Crocidolite ore	Chrysotile	Amosite	Silica	Carbon	Total
No of rats	30	30	20	10	10	10	110
Mesotheliomas	2		1		1		4
(Months after inoculation)	17 and 23		15		17		
Adenomas of the breast	2	4	1				7
(Months after inoculation)	11 and 18	19, 21, 23, 23	22				
Reticulum-cell sarcoma	3	3	1	1			8
(Months after inoculation)	16, 17, 17	16, 16, 22	16	22			
Sex of rats	20 F 10 M	30 F	10 F 10 M	M	M	M	
Survival time of group following inoculation (months)	11–25	7–30 (2 survivors)	11–30 (1 survivor)	14–30 (1 survivor)	11–24	12–22	

In view of these findings it was, therefore, accepted that the occurrence of these tumours was not the result of the experimental procedures.

It has been demonstrated that it is possible to produce tumours which appear to be arising from the mesothelial cells of the pleura by inoculating certain dusts into the pleural cavities of rats. One further tumour was obtained by exposing an animal to an asbestos dust cloud. The fact that these neoplasms were produced by silica and chrysotile asbestos as well as crocidolite fibre complicates the interpretation of these preliminary observations, and indicates the need for further investigations.

I thank members of the Unit who assisted in this experiment, those who provided the dust samples used, and especially Dr. H. Palmhert and Mary Hart, who helped in the animal inoculations.

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An Early Test for Possible Skin Carcinogens in the Mouse: Effect of Different Doses of 3-Methylcholanthrene in Benzene Solution

AN early test for possible skin carcinogens in the mouse has been reported[1,2]. The test is based on a tetrazolium reduction method. It was demonstrated that the test could reveal the carcinogens in a blind test of 21 substances of which 7 were carcinogens.

The present communication gives the values obtained with different doses of 3-methylcholanthrene in benzene solution applied to a circumscribed area of the skin of hairless mice. The results are given in Table 1.

Table 1. TETRAZOLIUM TEST WITH DIFFERENT DOSES OF 3-METHYLCHOLANTHRENE

μg applied	% solution in benzene	No of mice	Test result
0·4	1/128	16	0·898
0·8	1/64	16	0·792
1·6	1/32	16	0·818
3·1	1/16	32	1·107
6·3	1/8	32	1·168
25·0	1/2	32	1·361
50·0	1/1	16	1·410

Terracini, Shubik and Della Porta[3] found a step-like increase in the tumour yield after single applications to mouse skin of different doses of DMBA. Studies are in progress at our Institute to find out if such a step exists for hairless mice exposed to single applications of 3-methylcholanthrene. The preliminary results ten months after the single painting are shown in Table 2.

Table 2. SKIN PAPILLOMATA OCCURRING TEN MONTHS AFTER A SINGLE SURFACE APPLICATION OF 3-METHYLCHOLANTHRENE IN BENZENE TO HAIRLESS MICE

% solution	No. of mice	Papillomata
1/64	30	1
1/32	30	1
1/16	30	20
1/8	30	22
1/1	30	34

These results demonstrate that such a well-known carcinogen as 3-methylcholanthrene gives negative results with the tetrazolium test when very small doses are given. The testing of unknown substances should therefore be performed with relatively strong, but non-ulcerative, doses. The possible specificity of the test, however, is given some further support by the preliminary results reported in Table 2. These seem to be in accordance with the conclusion of Terracini, Shubik and Della Porta[3]: "A critical dose level exists at which single application of carcinogens becomes fully effective".

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Mineral Phase in Osteoporotic Bone

IN a survey of the incidence of fractures[1], particularly those of the head and neck of the femur, expressed in terms of population at risk, the distribution found made it quite clear that some must be of pathological origin. Such bones appear osteoporotic, and there has been a widespread assumption that the tissue component at fault is the calcium phosphate.

Should a change occur in the mineral, or in the calcium- and phosphate-level of its surroundings, one would expect to find changes in composition. Traditional methods of chemical analysis for calcium and phosphate cannot be profitably used, because the main need is to distinguish between a number of closely related calcium phosphates which might be present as mixtures. A further complication is that the mineral crystallites in bone have a very large surface area, so that adsorbed ions could produce misleading results. At present the most sensitive method for demonstrating individual components, which can be interpreted unambiguously, is X-ray diffraction.

A satisfactory method for distinguishing the various calcium phosphates involves taking photographs at room temperature, and then after heating to 1,000° C (ref. 2). At room temperature possible compounds are hydroxyapatite, octacalcium phosphate, monetite, brushite and calcium carbonate. After heating: apatite remains as apatite; apatite mixed with the other calcium phosphates, or apatite with phosphate adsorbed on to the crystal surfaces, reacts to form β-tricalcium phosphate, the proportion depending on the amount of excess phosphate present; calcium carbonate is transformed into calcium oxide.

One example where a difference is known in the tissue fluids surrounding the mineral in bone is in infants. These children have a higher serum phosphate than older children and adults. X-ray diffraction examination of bone from infants gave the hydroxyapatite pattern when unheated, and hydroxyapatite mixed with a little β-tricalcium phosphate after heating. Bones from the older age-groups gave hydroxyapatite only. In this case the different ionic concentrations in the surrounding fluids have been mirrored in the mineral composition.

To investigate possible differences in the mineral composition in osteoporotic bone the upper ends of the femur were used. At least three separate subjects were taken from each decade, and twice as many from the sixty-plus age-groups. All the bones taken from subjects aged below fifty-five looked normal. Above that age, approximately half looked normal, while half had sustained pathological fractures of the head or neck of femur. From each bone specimens were taken from cancellous bone in the head and neck, and compact bone from the top of the shaft.

On examination of the X-ray diffraction patterns it was found that bones from all age-groups, other than infants, gave hydroxyapatite lines only, both before and after heating, whether the specimens were normal or osteoporotic. This unchanged mineral composition suggests very strongly that the mineral crystallites have a purely passive role in any changes which may occur in osteoporosis. Further, the lack of adsorbed phosphate indicates that the calcium/phosphate ratio in the fluids immediately surrounding the mineral remains normal in osteoporotic bone.

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Bilirubin Interference in the Diphenylamine Reaction

IN the course of analysis of human bile for the presence of mucosaccharide and deoxyribose-reacting material by the diphenylamine reaction[1,2], an unusual peak at 650 mμ was found. It was thought possible that this peak might be due to the yellow-green colour of bile, resulting from the presence of high concentrations of bilirubin and other pyrrolic breakdown products. This possibility was checked using pure bilirubin, deoxyribonucleic acid (DNA), and a mixture of bilirubin and DNA (Fig. 1). It can be seen that a solution of bilirubin (after heating at 90° C with 5 per cent trichloroacetic acid (TCA)) produces a peak at 650 mμ on boiling for 20 min with the diphenylamine reagent. DNA gives a peak at 600 mμ under the same conditions, whereas a mixture of bilirubin and DNA gives a peak at 620 mμ. The extinctions of DNA and bilirubin appear to be additive. Bilirubin also interferes in the Burton[3] procedure for deoxyribose, producing a strong green colour.

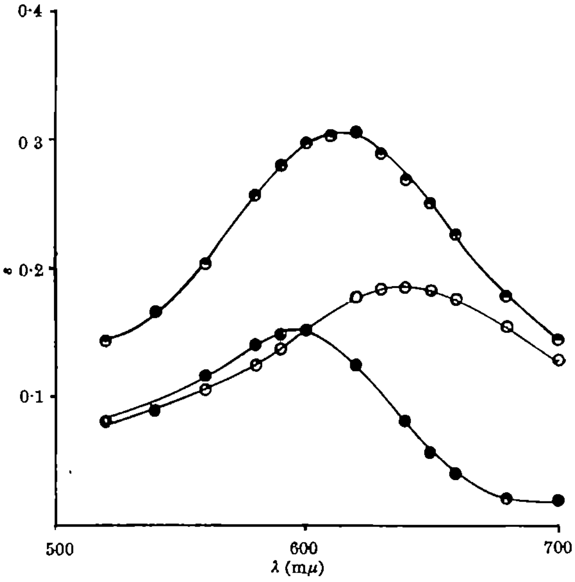

Fig. 1. Absorption spectra of the colours produced by heating bilirubin and DNA with the diphenylamine reagent. ●, 14 μg DNA in 2 ml. was heated in a boiling water bath with 4 ml. of diphenylamine reagent (ref. 1) for 15 min ; ○, 53 μg bilirubin dissolved in chloroform was mixed with 2 ml. 5 per cent trichloroacetic acid and warmed to evaporate the chloroform, 4 ml. of diphenylamine reagent was then added and the mixture heated on a boiling water bath for 15 min ; ◒, 14 μg of DNA and 53 μg bilirubin, treated as here, were heated for 15 min with 4 ml diphenylamine reagent

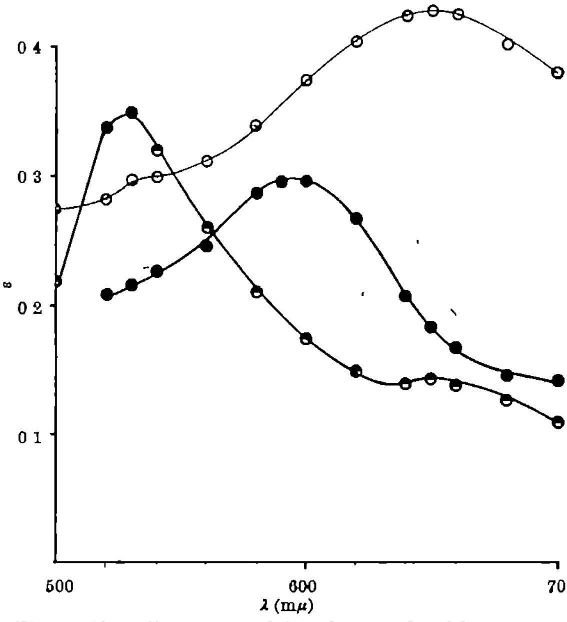

Fig. 2. Absorption spectra of the colours produced by heating. ○, Bile; ●, rat liver extract; ◒, jaundiced serum with diphenylamine reagent. 0·5 ml. of a 1 : 10 rat liver suspension, 0·5 ml. bile and 0·5 ml. of serum were each heated at 90° for 20 min with 4·5 ml. 5 per cent trichloroacetic acid; after cooling, the mixtures were centrifuged and 2 ml. of each supernatant was heated for 15 min in a boiling water bath with 4 ml. diphenylamine reagent. After cooling, the absorption spectra were measured using a Unicam *SP*-600

To determine whether this effect has any significance in physiological systems, we have studied the diphenylamine reaction in the serum of cases of obstructive and hæmolytic jaundice, in the drainage bile of a case of obstructive jaundice (cholangio-carcinoma of the hepatic duct), and in rat liver and rat serum 5 days after common bile duct obstruction. In such cases, after hot TCA extraction and centrifugation, the protein pellet is markedly blue in colour due to adsorption of a greater part of the bile pigment. The subsequent interference by bile pigment in the diphenylamine reaction probably depends on the relative proportions of bile pigment and protein and the affinity of the pigment for protein during the hot TCA extraction. With bile, for example, where the ratio of bilirubin to protein is high, the TCA extract itself is coloured by the bile pigment; however, in liver and serum where the protein content is much greater, the TCA extract is colourless even with high initial bilirubin-levels. Fig. 2 shows three representative curves for human drainage bile, human jaundiced serum and rat liver 5 days after common bile duct obstruction. It can be seen that bile shows intense interference in the diphenylamine procedure for deoxyribose reacting compounds, jaundiced serum much less, although a distinct shoulder is apparent at 650 mμ, and rat liver shows no obvious shoulder at 650 mμ. However, in rat liver, the decrease in extinction between 600 and 650 mμ is less than is found with TCA extracts of normal liver or with pure DNA (cf. Fig. 1).

It is apparent that in experimental work where a high bilirubin content is likely, that is, obstructive or hæmolytic jaundice, erythroblastosis fœtalis, etc., care must be exercised in quantitative interpretation of mucosaccharide and DNA-levels obtained by the diphenylamine reaction.

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IMMUNOLOGY

An Agar-Gel Diffusion Technique for demonstrating Antibody Production by Lymph Node Cells in Tissue Culture

Many previous investigations on antibody production in tissue culture have utilized indirect systems for antibody detection[1,2]. Others have examined the incorporation of isotope-labelled amino-acids into antibody by tissue-cultured cells[3]. There are few reports concerning the production of precipitating antibodies to protein antigens by cells in tissue culture for more than 1–2 days, and these are often unconvincing. Preliminary experiments in our laboratory with fluid media from cultures of immune lymphoid tissue fragments were not successful in demonstrating precipitins. However, it was thought that the combination of the sensitivity of the agar-gel diffusion technique with the continuous antibody production of cells in tissue culture possessed possibilities of demonstrating precipitating antibody production. Accordingly, the following techniques were developed to reveal anti-bovine serum albumin antibody production by immunized lymph node cells in tissue culture.

New Zealand white rabbits ranging in weight from four to six pounds were immunized with bovine serum albumin by means of intravenous injections of 2 ml. alum-precipitated bovine serum albumin, 1 mg/ml., five days a week for one week, followed by three additional weeks of daily intravenous injections of 3 ml. bovine serum albumin. Precipitin titres were then carried out on the serum of these rabbits, and the animals with highest titres were given intradermal injections of 0·5 c.c. bovine serum albumin, 25 mg/ml. in Freund's adjuvant, into the foot-pads of both hind legs. 3–4 days later the popliteal lymph nodes draining the injected foot-pads were removed and fragments of the minced node were placed in tissue culture with Eagle–HeLa media and 20 per cent rabbit serum. Two types of tissue culture containers were utilized in these experiments, one a thistle tube with expanded superior portion and the other a narrow glass tube 5 mm in diameter and 6 cm in length. The bottoms of the narrow portions of both tubes were filled with 0·2 c.c. of 0·6 per cent agar mixed with 0·2 c.c. of bovine serum albumin in solution at a concentration of 1·0 mg/ml. Layered above the bovine serum albumin in agar was a 2 cm band of plain agar. Tissue culture media with lymph node fragment was placed on the superior surface of the layer of plain agar. The tissue cultures were incubated at 37° and, when necessary, additional tissue culture fluid was added to the media. Observation of the plain agar column for bands indicative of an antigen–antibody reaction was made, and when these bands occurred, cells were removed from the cultures for histological examination in representative experiments. At the same time, tissue culture fluid was removed and qualitative precipitin and ring tests were set up with appropriate dilutions of bovine serum albumin. These were uniformly negative. Controls consisted of normal non-immunized lymph node cells with the same tissue culture media, heated immunized cells in tissue culture, and normal and heated anti-bovine serum albumin serum.

Negative results were obtained with media alone, with media plus non-immune lymph node cells and with heated immunized cells. Immunized lymph node cells in the thistle tubes produced faint bands at 3–7 days, with gradual development of opacity and increasing band width during the next seven days. The two tubes on the right in Fig. 1 represent normal rabbit cells in tissue culture, and tissue culture media alone, both of which result in no bands or opacities in the agar. The second tube from the left demonstrates the multiple bands in plain agar which occur when anti-bovine serum albumin serum is placed in the thimble portion of the tube. Heated anti-bovine serum albumin serum gave bands similar to unheated serum. Immunized lymph node cells in tissue culture for fifteen days are present in the tube on the left, which demonstrates bands in the neutral agar layer. In addition to two distinct bands, there is a diffuse opacity present in the superior portion of the plain agar just below the tissue culture. It is probable that the bands occur so near the antibody-producing cells because of the large antigen excess present in the system. It is noteworthy that the bands occur in the central portion of the neutral agar when anti-bovine serum albumin serum is placed in the top of the tube.

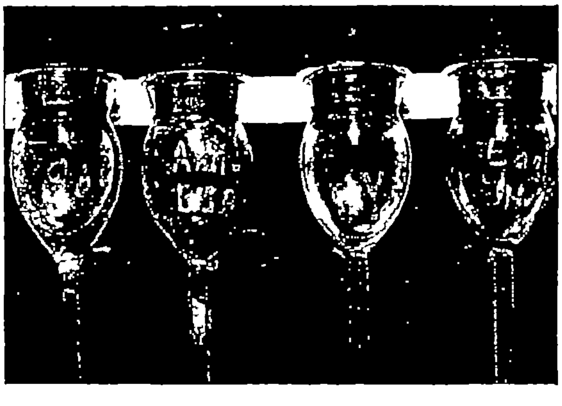

Fig. 1. Thistle tubes with plain agar in central portion and bovine serum albumin in agar at bottom of tubes. Note bands in agar below immune cells in tube on left, no bands in tube with normal cells second from right. Anti-bovine serum albumin serum gave multiple bands in central portion of agar column

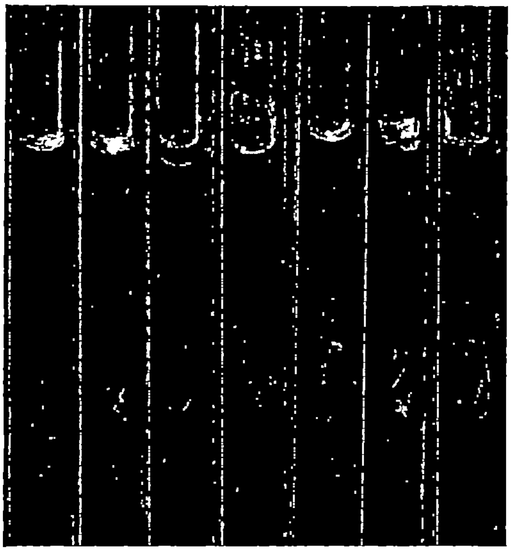

Fig. 2. Seven tubes with immune cells in tissue culture above neutral agar, with bovine serum albumin agar at bottom Multiple bands are present in all tubes except No 5 (from the left)

Fig. 2 shows the results of similar experiments performed with the smaller diameter glass tubes with no expanded superior portion. In these tubes the bands first became visible at 2–4 days, with gradual development of multiple bands in most of them by 10–12 days. The photograph was taken fourteen days after the lymph node fragments were placed in tissue culture. One tube in this group of experiments did not develop bands and it is assumed that the cells were not viable or had become undifferentiated into fibroblasts. Smears of representative tissue cultures associated with band production revealed mononuclear cells and cells resembling fibroblasts.

Some explanation must be given for the appearance of multiple bands in an agar-gel system utilizing bovine serum albumin and an anti-bovine serum albumin serum. It is possible that the variations in temperature which occurred when cultures were removed from the incubator and observed could account for this phenomenon, as has been previously described[4]. It is more probable that the multiple bands are a result of contaminants present in the commercial bovine serum albumin used in our experiments, thus resulting in the production of several antibodies by the host. It is possible that band formation is a result of preformed antibody release, rather than synthesis in tissue culture. The fact that heated anti-bovine serum albumin serum, but not heated immunized cells, gave bands in agar indicates that antibody synthesis by immunized cells in tissue culture has occurred.

The principal advantage of this method for studying antibody production *in vitro* is that it appears to offer greater sensitivity[5] in the detection of precipitating antibodies than the ring test or qualitative precipitin test applied to tissue culture fluid, since such fluids removed from tubes with band formation gave negative results when reacted with bovine serum albumin. Furthermore, the agar-gel tissue culture method is extremely simple to set up, easy to observe, and does not necessitate intermittent withdrawal of tissue culture fluid for antibody determinations. Finally, it has the advantage of giving some idea concerning the relative complexity of the antigen–antibody reactions occurring in the system.

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Immunotolerance to Heterologous Heart in the Rat

WHILE seeking to demonstrate the organ specificity of cardiac tissue[1,2], an attempt was made to produce a specific antiserum in the rats using the phenomena of immunotolerance[3,4]. New-born rats received 18 daily intraperitoneal injections of homogenates of pooled organs (lung, liver, spleen, kidney and serum) of bovines, rabbits or guinea pigs during 3 weeks, followed by 3 weekly injections, a total of 220–250 mg protein being given to each rat. Other groups were given homogenate of heart or of heart and organ-pool and an untreated control group was included in each series.

At 7 weeks of age, the rats were test-bled and given weekly injections of 10 mg protein of heart homogenate of the same species as before. Blood was taken after 2, 3 and 4 injections : this communication refers to results obtained with serum from the 4th bleeding.

Sera were examined against the appropriate heart in the tanned red cell hæmagglutination test and by capillary precipitation and complement fixation tests using the methods previously described[1,2]. Table 1 shows that the pre-treatment with heart and/or organ-pool homogenates significantly decreased the production of antibodies to heterologous heart injections.

Table 1. TANNED RED CELL HÆMAGGLUTINATION TESTS IN IMMUNOTOLERANT RATS INJECTED WITH HEART HOMOGENATE

Tolerant to: (species)	Pre-treatment with organ-pool	pool+heart	heart	Controls	*S.D.* (*P*)
Rabbit	41/53*	13/19	15/31	38/38	< 0·001
Guinea pig	12/15	—	4/18	18/19	< 0·001
Bovine	22/32	24/28	26/29	26/28	0·05

*No. rats with titre > 1 40/total in group.

In the passive hæmagglutination test, immunotolerance was most marked in respect of organs of rabbit and guinea pig, the heart homogenate being more active than the organ pool. No differences could be demonstrated between the specificity of the antibodies produced by animals pre-treated with heart or with organ-pool. In the case of rats given injections of bovine tissues, the immunotolerance produced was much less marked, and organ-pool was more active than heart.

Similar trends were obtained in the precipitation and complement fixation tests although the proportion of controls producing antibodies was much smaller in the groups treated with rabbit and guinea pig organs (Table 2). There was no correlation between production of the different types of antibody by a specific animal nor did the production of a high titre in one test necessarily imply a positive reaction in another. Rats pre-treated and later challenged with bovine antigens showed dissociations between

Table 2. COMPLEMENT FIXATION (C.F.T.) AND PRECIPITIN TESTS (P.T.) IN IMMUNOTOLERANT RATS INJECTED WITH HEART HOMOGENATE

Tolerance to: (species)		Pre-treatment with: organ-pool	pool+heart	heart	Controls	*S.D.* (*P*)
Rabbit	C.F.T.	0/37*	—	0/31	13/30	<0·001
	P.T.	3/37	—	4/29	18/30	<0·001
Guinea pig	C.F.T.	4/15	—	0/18	10/20	<0·01
	P.T.	4/15	—	1/19	15/21	<0·001
Bovine	C.F.T.	3/30	0/29	0/29	26/28	⋞0·001
	P.T.	2/32	1/28	1/29	26/28	⋞0·001

*No. rats positive/total; C.F.T. ≯ 1·20; P.T. ≯ 1 mm. precipitate.

the results of the passive hæmagglutinations and the other tests; immunotolerance in the latter being established to a much more significant degree. Moreover, a number of pre-treated rats already showed circulating antibodies in sera taken before the course of immunizing injections. This dissociation between the results of different tests, interesting in itself, has been noted by other workers[5] and will be discussed elsewhere.

In the cases of rats injected with rabbit and guinea pig tissues, a similar degree of immunotolerance was found in respect of direct hæmagglutination of the appropriate erythrocytes. An unexplained finding was a drop in titre in the complement fixing and direct agglutinating antibodies of certain animals between, for example, bloods taken after the second and fourth injection, while the titres in the tanned red cell passive hæmagglutination test continued to rise.

We have previously postulated[1,2] the existence of organ-specific antigen in cardiac tissue and the present experiments provide further support for this view. In theory, one might expect the tolerance produced against individual organs of a foreign species to be obscured by the general immunotolerance to the species. In fact, it has been possible to produce immune tolerance, for example, to human erythrocytes in rabbits but not to blood-group antigens[6].

However, the present successful production of organ-specific immunotolerance may be associated with immunological properties peculiar to cardiac tissue and the giving of much larger amounts of antigen than usual[7,8] during the pre-treatment phase.

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RADIOBIOLOGY

Modification of Radiation Effect on Mammalian Tumour Cells by Pharmacological Agents

IN an earlier communication it was demonstrated that the effect of X-irradiation on the reproductive capacity of mammalian tumour cells could be modified by the incorporation of relatively small amounts of halogenated pyrimidine deoxyribosides into the DNA of these cells[1]. Additional work has now been completed in which the time and dose relationships of administration of the drugs and variation in the ionization density of the radiation have been examined. The P–388 lymphocytic leukæmia of the $DBA/2$ mouse and its use by serial dilution assay to examine cell reproductive capacity have been reported in detail[2]; the TD_{50} is defined as the mean number of cells necessary to produce 50 per cent tumour 'takes' in a group of recipient animals of the same strain, and the 'surviving fraction' is the result of control TD_{50} divided by experimental TD_{50}.

Table 1. EFFECT OF X-RAYS ON TUMOUR CELLS TREATED WITH 5-IODODEOXYURIDINE

Treatment	Mean TD_{50} Tumour cells/mouse	Surviving fraction
Control	2 7 (2·3–3·0)	
IUdR, 100 mg/kg/day, 4 days	3·1	
IUdR, 100 mg/kg/day, 35 days	3·0	
1940 rads, 3-MeV X-rays	170	0·016
1940 rads, 3-MeV X-rays, IUdR, 100 mg/kg/day, 4 days	840	0 003
1940 rads, 3-MeV., X-rays, IUdR, 100 mg/kg/day, 35 days	1,320	0 002

In the earlier investigations, 5-iododeoxyuridine (IUdR) was administered daily to tumour-bearing donor animals by single intraperitoneal injection for 4 days prior to irradiation and/or assay to determine cell reproductive capacity. The mean doubling time of cells of this tumour is approximately 15 h[2], and it was felt that no significant additional incorporation of the halogenated DNA precursor would result from more prolonged contact with the drug. Table 1 shows the results of an experiment designed to test this supposition, in which tumour cells were exposed to 35 daily injections of IUdR at a dose of 100 mg/kg body-weight. The P-388 tumour is routinely transplanted on the 7th day following inoculation of 10^6 cells intraperitoneally, hence this investigation involved the daily exposure to the halogenated analogue through five transplant generations of the tumour. This course of administration of the drug did not raise the TD_{50} for unirradiated tumour cells, and the potentiation of radiation effect noted in these drug-treated cells was only slightly greater than that observed when the tumour cells were exposed to the halogenated analogue for only 4 days prior to irradiation, paralleling the *in vitro* observations of Kaplan *et al.*[3] that in excess of 4–6 cell doublings in the presence of the halogenated analogue no significant increase in drug incorporation can be made by further prolonged contact.

Szybalski has suggested that one mechanism by which the halogenated pyrimidine deoxyribosides act is to render the DNA of those cells which have incorporated them more fragile, hence subject to damage by ionizing events which alone would transmit insufficient energy to produce this damage[4]. It seemed worth while, therefore, to examine the effects of densely ionizing radiation (fission-spectrum fast neutrons) on cells the DNA of which contained halogenated analogues, as the dose-response to these radiations is 'single-hit' in type[5], that is, the passage of any one particle is sufficient to cause loss of reproductive capacity of the cell. In Fig. 1, the dose-response to fission-spectrum fast neutrons is shown for tumour cells exposed to IUdR, 300 mg/kg/day for 4 days prior to irradiation; no significant difference is observed between the response of these cells and that

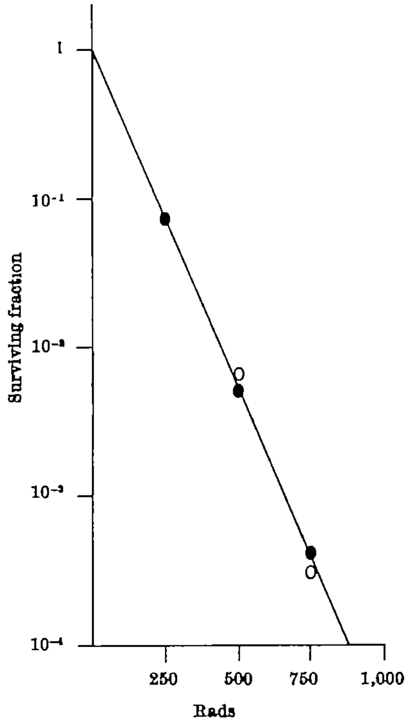

Fig. 1. Dose-response, fission-spectrum fast neutrons. ●, P-388 leukæmia, *in vivo*; ○, P-388 leukæmia, *in vivo*, previously treated with IUdR, 300 mg/kg/day × 4

of cells which had not been pre-treated with the halogenated analogue.

Finally, tumour-bearing donor animals were treated with the third of the halogenated pyrimidine deoxyribosides, 5-chlorodeoxyuridine (CUdR), in a similar manner to earlier investigations with the iodo- and bromo-analogues. The results are shown in Table 2. This compound presents less similarity to the normal precursor, thymidine; the size of the chlorine atom is somewhat smaller than the methyl group which it replaces[6]. Direct toxicity of the drug on reproductive capacity of these tumour cells was observed at the low dose of 100 mg/kg day × 4 days, in contrast to results with BUdR, where toxicity appeared at 300 mg/kg/day and IUdR, where no significant difference between control and drug-treated tumour cells was noted at a dose of 500 mg/kg/day × 4 days. With CUdR, additivity of radiation and drug effect was noted in almost all groups, but no significant potentiation (as determined by comparing control TD_{50} irradiated TD_{50} with control plus CUdR TD_{50} irradiated plus CUdR TD_{50}) of the radiation effect was noted. This result is similar to that observed at higher drug dosages with bromodeoxyuridine[1].

Table 2. EFFECT OF COBALT-60 γ-RADIATION ON CELLS TREATED WITH 5-CHLORODEOXYURIDINE

Treatment	Mean TD_{50} Tumour cells/mouse	Surviving fraction
Control	2 5 (1·0–4·0)	
CUdR, 100 mg/kg/day, 4 days	4·3 (3·7–4·9)	
CUdR, 300 mg/kg/day, 4 days	5·3 (4·5–6·1)	
CUdR, 500 mg/kg/day, 4 days	4·8 (4·4–5·2)	
1,500 rads, ^{60}Co γ, anoxic, 115 rads/min	135	0·019
1,500 rads, ^{60}Co γ, CUdR, 300 mg/kg/day, 4 days	240	0 022
2,000 rads	490	0 005
2,000 rads CUdR, 100 mg/kg/day	350	0·012
2,000 rads CUdR, 300 mg/kg/day	1,000	0 005
2,000 rads CUdR, 500 mg/kg/day	1,200	0 004
3,000 rads	13,700	0·00018
3,000 rads CUdR, 100 mg/kg/day	32,900	0 00013
3,000 rads, CUdR, 500 mg/kg/day	32,000	0·00015

It has been shown that IUdR, and to a certain extent BUdR, can potentiate the effect of X-irradiation delivered under anoxic conditions on tumour cell reproductive capacity. High drug dosage with BUdR and CUdR at all dose-levels studied acted only additively with X-rays in the P–388 mouse leukæmia. The finding that incorporation of an amount of iododeoxyuridine into these tumour cells sufficient to cause major modification of the dose-response to X-rays fails to modify their response to densely ionizing fission-spectrum fast neutrons gives support to the surmise that these compounds act by rendering the DNA of cells containing them more fragile and hence liable to damage by single ionizing events less energetic or less numerous than those which ordinarily would result in the reproductive death of the cell.

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Mechanism of X-ray Inhibition of the Sodium Pump in Human Erythrocytes : Adenotriphosphatase Activity of Stromata

PREVIOUS results have shown that X-irradiation of human erythrocytes decreases the active efflux of sodium (sodium pump)[1]. However, 890 rads and 4,450 rads, which are effective in inducing a 45 and 73 per cent decrease of the active sodium efflux, respectively, do not inhibit glycolysis nor produce any effect on concentration of adenotriphosphate in red blood cells[2]. The lack of inhibition of the cellular system furnishing energy to the ion pump led us to investigate the biochemical mechanism in the membrane which underlies the active movement of sodium across the membrane.

Dunham and Glynn[3] and Post *et al.*[4] have shown that the ATPase activity of human red cell stromata consists of two components. The first component does not require the presence of alkali metal ions and is not inhibited by cardiac glycosides. The second component, on the contrary, requires sodium and potassium and is inhibited by cardiac glycosides,

Table 1

No. of exp.	Additions to the basal medium†	X-ray dose (rad)	ATPase activity (mM P/l cells/h)	ATPase activity requiring Na and K (mM P/l cells/h)	Inhibition (per cent)	No. of exp.	Active sodium efflux (sodium pump)* (mM/l cells/h)	Inhibition (per cent)
10	None	0	2·52 ± 0·047					
	Na and K	0	3·80 ± 0·049	1·28 ± 0·011	—	14	1·1 ± 0·048	—
9	None	890	2·58 ± 0·110					
	Na and K	890	3·25 ± 0·111	0·67 ± 0·043	48	14	0·6 ± 0·039	45
6	None	4,450	1·78 ± 0·043					
	Na and K	4,450	2·16 ± 0·050	0·38 ± 0·035	70	12	0·3 ± 0·021	73

Arithmetic mean ± standard error of mean.
*Data from ref. 1.
† The basal medium of incubation was: *tris* buffer *p*H 7·05 (115 mM) ATP neutralized with *tris* before addition (1·5 mM); magnesium chloride (0·5 mM); cysteine (1 mM). The medium containing sodium and potassium was obtained by addition of sodium chloride (16 mM) and potassium chloride (16 mM) to the basal medium.

which inhibit the sodium pump without interfering with the glycolysis and ATP synthesis of the cell[5]. This second component of the ATPase activity of red cell ghosts is, therefore, intimately linked with the sodium pump.

Human red cell ghosts were prepared by Herbert's method[6]. The suspension of stromata was divided in two parts, one of which was X-irradiated by a Dermopan apparatus with beryllium window, operating at 50 kV and 25 m.amp with 1 mm aluminium as a filter. The thickness of the irradiated suspension was 0·3 cm. Dose-rate was 12·5 rads/sec. Measured amounts of the stromata suspension were incubated in small flasks containing pre-warmed solutions at 37° C in a water bath and shaken by a motor during the 45-min period of incubation. Blanks for medium and cells were run in parallel. At the end of incubation, ice-cold 6 per cent perchloric acid was added to the stromata suspensions (1 : 1) and, after spinning at + 4° C, orthophosphate was determined in the supernatants by King's method[7]. The ATPase activity was referred to the volume of erythrocytes. The volume of cells in the flasks was obtained by comparison of the hæmoglobin content of a known quantity of the original suspension with that of a standard hæmolysate obtained by lysing a known amount of cells packed by centrifuging at 1,500*g* for 1 h, in a known amount of water.

Table 1 shows that X-irradiation with 890 rads and 4,450 rads inhibits the ATPase activity of stromata which requires sodium and potassium by 48 and 70 per cent, respectively. After the same doses of X-rays the ATPase activity, which is not dependent on sodium and potassium, decreases only by 4 and 29 per cent, respectively. The ATP-splitting activity requiring sodium and potassium is, therefore, particularly sensitive to X-rays, and the magnitude of this inhibition is in agreement with the decrease of sodium active efflux. In fact, 890 rads and 4,450 rads induce a 45 and 73 per cent respective decrease of the sodium pump. These results show that X-rays interfere with the mechanism of the pump in the cell membrane.

The investigations of Hokin and Hokin[8,9] led to the hypothesis that the formation of orthophosphate from ATP by membranes is actually the result of a metabolic process which consists of two steps by which sodium is actively transported across the membrane. The first step, which is assumed to occur at the inner surface of the membrane, consists of two reactions:

$$\mathrm{ATP}^{\equiv} + \mathrm{DG} \xrightarrow{\text{DG-kinase}} \mathrm{PA}^{=} + \mathrm{ADP}^{=}$$

$$\mathrm{PA}^{=} + 2\,\mathrm{Na}^{+} \longrightarrow \mathrm{Na}_2 - \mathrm{PA}$$

and the second step on the other site of the membrane:

$$\mathrm{Na}_2 - \mathrm{PA} \xrightarrow{\text{PA-phosphatase}} \mathrm{DG} + \mathrm{HPO}_4^{=} + 2\,\mathrm{Na}^{+}$$

where DG = diglyceride and PA = phosphatidic acid. PA would thus act as the carrier for the active transport of sodium. Therefore, the inhibitory effect of X-rays on the ATP-splitting activity of stromata which requires sodium and potassium has to be seen as an inhibition of the metabolic cycle of the sodium carrier.

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BIOLOGY

Capacity of Fragments of Leaf Primordia to produce Whole Leaves

For a number of years both microsurgical and sterile culture techniques have been brought to bear on problems of leaf development in the vascular plants. Among the phenomena which have been investigated is the elusive process of leaf determination whereby the pattern of leaf morphogenesis is imposed on tiny outgrowths or primordia on the flanks of the apical meristem. Partial isolation of younger primordia by variously placed vertical incisions has led, in some cases, to the development of centric leaves, that is, organs which are determinate in their growth, but radial rather than dorsiventral in their symmetry[1].

In the fern *Dryopteris*, such partial isolation may result in the development of the youngest three primordia as shoot buds, that is, outgrowths which are both indeterminate and radial[2]. Similarly, it has been found that in the fern *Osmunda cinnamomea* L. any among the youngest nine primordia may develop as shoots, and ultimately whole plants, after excision and explantation to a sterile nutrient medium of simple composition. The development of such explants into leaves increases in frequency in progressively older primordia, and the tenth (P_{10}) and all subsequent primordia have always given rise to leaves[3].

The sum total of these experiments would suggest that leaf determination is a progressive process, possibly involving several steps, and that once it has become irreversible it imparts to a primordium a considerable measure of autonomy in subsequent development. Cutter[2], using surgical methods, concluded that in *Dryopteris* determination coincides with the establishment in a primordium of a distinct apical cell. It has been demonstrated that no such correlation with the setting off of an apical cell exists in the determination of primordia in *O. cinnamomea*. Rather, evidence, which has been described in full elsewhere[3], derived from an examination of the early development of primordia after excision, has led to the postulation that the fate of a particular primordium might depend on its content of a specific leaf-forming substance at the time of excision. It was felt that a combination of surgical and cultural techniques might be used to test, and possibly to refine, this hypothesis.

Subdivision of leaf primordia and the explantation of fragments might reveal whether development as a leaf is dependent on some configuration of cells in the intact primordium. If fragments of primordia, which have not been irreversibly determined as leaves, can produce whole leaves then the concept of a leaf-forming substance operating within the primordium would receive support. A considerable number of such subdivisions have been carried out and the results of these studies will be published in full elsewhere. The purpose of this communication is to report the outcome of one series of experiments in which it has been demonstrated that fragments derived from the vertical longitudinal splitting of primordia have the capacity to develop as whole leaves.

The organism used in the work recorded here was *Osmunda cinnamomea* L. and the methods utilized are essentially those previously used in sterile culture of excised fern leaves[4]. Sterilization procedures consisted of a surface treatment of the whole bud in a 7 per cent (by weight) solution of 'Pittchlor' (a commercial product containing 70 per cent calcium hypochlorite, manufactured by the Columbia-Southern Chemical Co., Pittsburgh, Pa.). Starting with P_{10} (10th oldest primordium) leaf primordia down to P_4 were identified and split sagittally with micro-scalpels made from razor blade fragments. Left and right halves were excised and planted in separate 1-oz. square tablet bottles, coded so that they could be compared later, on a medium which contained a modified Knudson's solution of mineral salts[5], 2 per cent sucrose, and 0·8 per cent agar. A number of unsplit control primordia were also included in the experiment. The explants were maintained in a culture room for a period of about three months at a temperature of 24° ± 1° C and an illumination of 600 ft. candles supplied by a combination of cool white fluorescent tubes and incandescent bulbs.

Excised half primordia of leaves of *O. cinnamomea* are able to undergo growth and development in sterile nutrient culture to produce whole leaves. At the times of excision, the half primordia consisted of nothing more than small inverted half-cones of meristematic tissue, the largest and oldest of which was less than 1 mm in height. At the end of the three-month growing period, they have undergone sufficient growth and development to produce leaves which consist of a rather broad base which is surmounted by a pinna-bearing rachis. In spite of the trauma imposed by the micro-surgical techniques used, leaves were not produced any less frequently than in unsplit primordia.

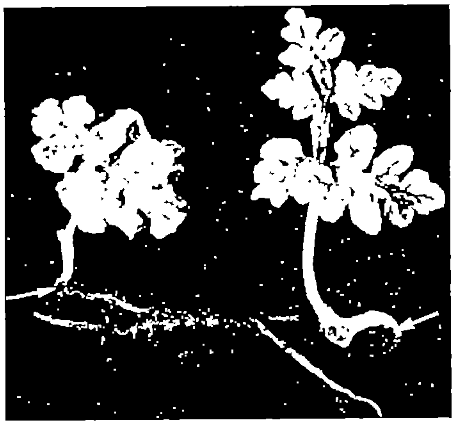

Fig. 1. Left and right halves of a split P_5 three months after excision. Each leaf has been turned 90° outward on its axis so that the scar of the longitudinal split (arrows) is visible. (× 3)

At the end of the three-month growing period, it is possible to take the left half and the right half of a particular primordium and to put these two halves together. In this way the two end-products may be compared closely and the developmental pattern of each of the original half primordia reasonably defined. This is possible throughout the series of primordia used in these experiments. Fig. 1 shows such a recombination of the halves of an original P_5 three months after excision. In most cases, although differences in size are common, the two halves of any primordium both develop either as leaves or as shoots. In a few instances, however, one half primordium produced a leaf while the other gave rise to a shoot. The development of a shoot from a half primordium corresponds closely to the pattern previously described for whole excised primordia[3].

Repeated observations of excised leaf primordia of *O. cinnamomea* have shown that it is possible to ascertain the original plane of dorsiventrality, even after considerable growth has occurred, from the position of the excision scar. It is important to be able to do this since the plane of dorsiventrality of a leaf produced by an excised primordium does not always correspond to that of the original explant. Half primordia from the older end of the series appeared to develop without any significant change from the plane of dorsiventrality of the original primordium. By contrast, halves of P_4 or P_5 give rise to leaves which face the scar of the longitudinal split (Fig. 1), suggesting that the plane of dorsiventrality has undergone a re-orientation.

It has been suggested that a leaf-forming substance contained within the primordium at the time of excision, rather than a specific structural pattern, may be responsible for the development of the primordium as a leaf. These experiments, which have shown that it is possible for a fragment of a primordium to produce a whole leaf, lend support to the idea of a leaf-forming substance rather generally distributed in the primordium. It is hoped that further work, including a detailed investigation of the stages of development of explanted leaf fragments of the type described in this report, together with an investigation of other types of fragments, will permit a better characterization of the process of leaf determination.

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Mechanism of the 'Climacteric' Rise in Respiration in Banana Fruits

During the past 40 years various theories have been advanced to explain the large rise in rate of respiration, that is, the 'climacteric' rise, which accompanies ripening in certain fruits[1-8]; but no hypothesis has yet been accepted as proved.

We have used specific enzymatic methods[9-11] to determine the changes during the ripening of bananas (variety Lacatan) in the contents of fructose-6-phosphate, glucose-6-phosphate, fructose-1,6-diphosphate, phosphoenolpyruvate and malate. The changes in citrate and α-keto-acids (pyruvate, α-ketoglutarate and oxaloacetate) were estimated by conventional chemical methods. Associated with the 4- to 5-fold increase in rate of output of carbon dioxide during ripening, there was a 20-fold increase in the content of fructose diphosphate (Figs. 1 and 2) and increases of some 3- to 6-fold in the amounts of the α-keto-acids and of malate.

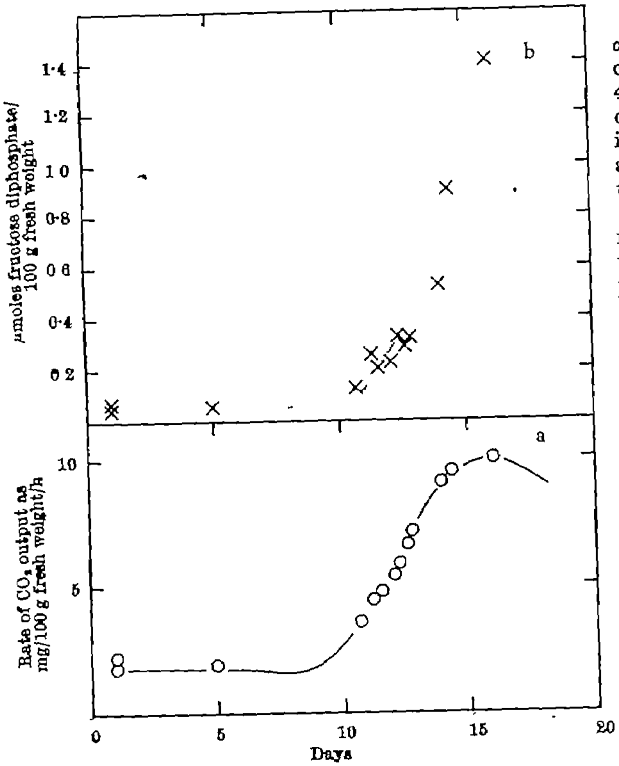

Fig. 1. *a*, The continuous line curve shows the change in rate of output of carbon dioxide of a single finger ripened at 18° C. The circles show the rates of respiration of other single fingers taken for analysis at different stages of ripeness. The circles are plotted with their respective rates on the continuous line so that the time scale for them is arbitrary. *b*, Changes in content of fructose diphosphate of the single bananas of which the rates of output of carbon dioxide are shown in Fig. 1*a*. The time-scale is that of Fig. 1*a*

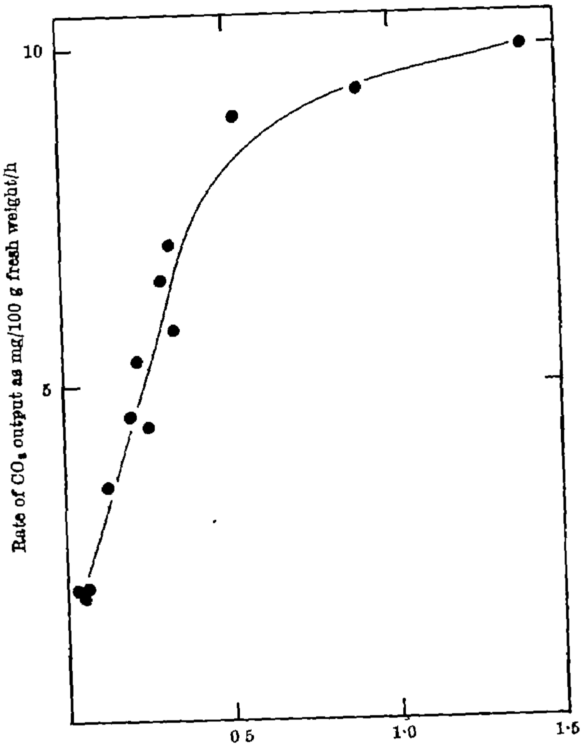

Fig. 2. Relation between rate of output of carbon dioxide and content of fructose diphosphate

The results support the view that the increase in rate of respiration during the 'climacteric' rise is due to the increase in the concentration of fructose diphosphate. Since the contents of glucose-6-phosphate and of fructose-6-phosphate were increased by only 2- to 3-fold, the rise in fructose diphosphate may be related to an activation of phosphofructokinase as suggested for animal muscle[12].

Treating bananas with ethylene induced changes of the same pattern as those mentioned here.

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Room-Temperature Storage of Coconut Pollen

Pollen introduction and exchange have been suggested as a means of broadening the scope of breeding programmes designed to produce disease-resistant and high-yielding coconuts[1-3]; and at a recent meeting of the Food and Agriculture Organization Technical Working Party on Coconut Production it was stressed[4] that the most important material question to be solved concerned the mode of transportation of pollen. It is clear that present methods of storage of coconut pollen[5-8], though useful, are inconvenient for routine pollen exchanges over long distances.

J. R. King[9-11] has reported prolonged room-temperature storage of freeze-dried pollens in sealed containers under an inert gas, and more recently under vacuum[12]; and early results of work on storage and dispatch initiated in this Department show that coconut pollen similarly treated can be stored for long periods at room temperature.

In one experiment, unopened male flowers from inflorescences of six 'Jamaica tall' palms (A–F) were placed in separate newspaper envelopes and oven-dried at 40° C (for approximately 2 days). Pre-treatment in this way has resulted in greatly increased quantities of pollen[13]. Initial pollen viability estimates were made from counts of at least 400 grains after germination on a sucrose gelatine medium (10 per cent sucrose, 10 per cent gelatine).

The equipment used for freeze drying consisted of a 6-port ampoule header connected by a U-tube to a high-vacuum pump. Ampoules containing pollen were attached and pre-frozen by immersion in dry ice and acetone before drying under vacuum (2–5 mm mercury). The U-tube surrounded by freezing mixture served as a moisture trap.

Duplicate batches of 6 ampoules after 3 h freeze drying were heat-sealed under vacuum.

After one month's storage at room temperature, samples A_1, B_1 and C_1 were opened and viabilities estimated immediately, after 2 and 4 h, and after one day at relative humidity 70 per cent. Samples from D_1, E_1 and F_1 were similarly tested after two months and replicates D_2, E_2 and F_2 were then packed in light-weight polystyrene containers and separately dispatched on a round trip to New Guinea. Viability was again tested *in vitro*, approximately 4 months after collection. The results obtained are given in Table 1.

The results show that freeze drying is a workable technique for the treatment of coconut pollen, since viability obtained after prolonged room temperature storage is in excess of that normally required for pollination. Further samples A_2, B_2 and C_2 still remain to be tested at 6 months, and the results of a number of controlled pollinations and further experiments to determine the optimum period of freeze drying will be reported later.

Table 1. VIABILITY ESTIMATES OF FREEZE-DRIED COCONUT POLLEN AFTER STORAGE AT ROOM TEMPERATURE

Sample	Initial viability (%)	Storage period	Viability per cent. Pollen tested after different periods at room temperature and 70 per cent relative humidity* 0	2 h	4 h	Approx. one day
A_1	82	1 month	18	16	12	12
B_1	49	,, ,,	7	14	10	10
C_1	83	,, ,,	nil	9	8	6
D_1	80	2 months	51	51	42	31
E_1	66	,, ,,	34	35	33	35
F_1	34	,, ,,	30	22	18	22
D_2	80	4 months	2	14	21	—
E_2	66	,, ,,	27	22	31	—
F_2	34	,, ,,	20	24	23	—

*Preliminary experiments indicated improved viabilities after 2 and 4 h.

The ability to store coconut pollen for prolonged periods at room temperature will be an asset to coconut breeding and seed production, and, in particular, will facilitate introduction and exchange.

I thank Dr. J. L. Plummer, of the University College of the West Indies, for help with the freeze-drying technique. Thanks are also due to Mr. T. A. Jones, director of this Department, for advice, to Mr. A. E. Charles of the Department of Agriculture, Keravat, New Britain, in the Territory of Papua and New Guinea, who co-operated by labelling and returning the samples, and to Mrs. M. Hall for technical assistance with the pollen viability tests.

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A Technique for inoculating Wheat with Rusts for Glass-house and Test-tube Culture

The conventional method of inoculating wheat with rusts is to plant wheat seed in pots in a glass-house and smear the first leaf with spores of rust when the seedlings are 6–7 days old. The pots are incubated for 24 h in a moist chamber and then transferred to the benches of the glass-house. This technique takes about 15–20 days from the date of seeding to the date of the appearance of the rusts, depending on the species of the rust, temperature and light conditions in the glass-house being favourable for the development of the different species of the rusts.

For inoculating wheat with rusts by this technique for glass-house and test-tube culture, wheat seeds are germinated in a Petri plate on a moist filter paper in a room. After 60–72 h, when the plumule has grown about half an inch, the seed is picked up by hand. Meanwhile, a spore suspension of rust is made in sterile water containing a trace of detergent. Using a fine needle in a hypodermic syringe, the spore suspension is injected a little above the point from where the plumule and radical are differentiating. The test of successful inoculation is the appearance of a drop or the ejection of a fine squirt of water from the tip of the plumule. If this drop is taken on a slide and examined under the microscope, it will be found to contain the rust spores. With a little practice, it is possible to inoculate about

120 seedlings in 1 h with 3–4 ml. of the spore suspension.

The inoculated seedlings are then transplanted in 6-in. pots in the glass-house and irrigated with water. Leaf and stem rusts appear after 7 and 10 days respectively from the date of transplant on the first leaf with 100 per cent infection in the seedlings.

To obtain the culture of rust in a glass test-tube by this technique, the inoculated seedling is transferred to a glass test-tube containing 10 ml. of 0·5 per cent water agar. A few drops of water are added to the surface of the agar to maintain good humidity in the tube. The tubes with open mouth may be kept in the glass-house or in a room receiving direct sunlight. The seedling continues to grow in the tube and rust pustules appear on the first leaf, and in some cases on the second, third and fourth leaf, as well as on the first tiller.

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Occurrence of Unusual Bodies in a Marine Pennate Diatom

DURING an investigation of the marine phytoplankton in a shallow Rhode Island coastal salt pond[1], an abundant benthic diatom flora occurred throughout the water column on May 3, 1961. This community was probably roiled up from the bottom during the vertical mixing characteristic of the water-mass at that time (Table 1); a distinct halocline persisted prior to this date. The water-mass was 1·5–2·0° C warmer than in mid-April and while the bottom (6 ft.) salinity decreased by 2·5 parts per thousand, the mean water-column salinity decreased by only 1 part per thousand. Similarly, gross changes were not observed in other environmental factors studied: dissolved oxygen, nitrate, nitrite, silicate and phosphate.

Table 1. PHYSICAL CONDITIONS AND PENNATE DIATOM ABUNDANCE ON MAY 3, 1961

Depth (ft.)	*Gyrosigma* sp. cells/l.	All pennate diatoms cells/l.	Temp. (°C.)	Salinity (‰)	Density σt
0	360	255,000	9·2	26·42	20·41
3	440	235,000	8·7	26·55	20 58
6	2,600	210,000	8·7	26·60	20·68

Included among these tychopelagic diatoms was the sudden appearance of a *Gyrosigma* sp., represented almost exclusively by empty, broken frustules and moribund individuals in the preserved 52-ml. samples examined microscopically from each depth in the phytoplankton census. One intact specimen was encountered, however, which differed from the other *Gyrosigma* specimens and all other pennate diatoms previously examined. This specimen contained 200–250 spherical, green bodies measuring 3·5–7·5μ in diameter, and distributed in a random, though clumped, manner throughout the intact cell. The periphery of these bodies was slightly thickened. Neither flagella, pyrenoids nor a nucleus were observed. Apart from these bodies, the specimen was devoid of its usual complement of cytoplasmic material and chromatophores.

Subdivision of the protoplast into atypical, non-flagellated bodies has been observed in three other species of pennate diatoms[2–4]. Recently, the occurrence of biflagellate swarmers within the cells of the marine pennate diatom *Amphiprora hyperborea* has also been reported[5]. However, the *Gyrosigma* bodies in no way resembled the *Amphiprora* swarmers. While they did possess a somewhat thickened wall, their general structure and number suggest that they were not similar to the spore-like bodies noted in the limnetic pennate *Surirella spiralis*[2]. Kolbe[4] observed the frequent occurrence of 6–16 naked, pigmented, pyrenoid-containing bodies in motile specimens of the brackish water pennate *Nitzschia vitrea*. He concluded that these rounded, pigmented bodies were formed by subdivision of the chromatophores, a phenomenon he designated as 'Hypertomie'. Kolbe suggested this might also be the origin of similar bodies observed in the limnetic diatom *Eunotia lunaris*[3]. The green pigmentation of the *Gyrosigma* bodies suggests a possible derivation from the original cell chromatophores, but their lack of a pyrenoid and great abundance distinguish them from those observed by Kolbe. Finally, the *Gyrosigma* bodies were morphologically distinct from the microspores commonly observed in centric diatoms[6].

While the *Gyrosigma* bodies bear certain resemblances to the non-flagellated bodies noted on rare occasions in other pennate diatoms, they appear to represent a new type. It is unknown whether they represent a reproductive stage of the life-cycle, an unusual decomposition of a moribund specimen, or are symbionts. The nature and function of the unusual bodies reported in the other pennate diatoms also remain an enigma[5,7].

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ENTOMOLOGY

Laboratory Colonization of the Horn Fly, *Hæmatobia irritans* (L.)

THE horn fly is a blood-sucking insect (*Hæmatobia irritans*) the only host of which, for all practical purposes, is cattle. The adult remains on cattle almost continuously, leaving momentarily to lay eggs in the freshly dropped fæces. Horn flies have been reared on a caged steer at Kerrville since 1947, but it was only in 1961 that this colony could maintain itself without the addition of field-collected flies. Some attempts at colonization of horn flies away from a bovine host have been briefly reviewed by McLintock and Depner[1]. Many workers have reared adults from the egg stage and maintained the flies on laboratory diets (citrated bovine blood) but the production of fertile eggs has not been reported. On the theory that micro-organisms in citrated bovine blood were detrimental to the flies and/or that bovine blood did not contain all the essential nutrients for reproduction, a series of laboratory diets was tested. Flies taken as pupæ from the caged-steer colony were fed bovine blood containing antibiotics, and attempts were made to fortify the blood with other materials. These experiments led to the establishment of a laboratory colony that reproduced without contact with a host.

For each test diet, 480 ml. of blood was drawn from a steer into a vacuum transfusion bottle containing 120 ml. of ACD* anticoagulant solution. The blood was mixed immediately with the other ingredients and the unused portion stored under refrigeration. For each test diet, 10 male and 10 female flies were placed in a cylindrical plastic cage about 3 in. tall and 1½ in. in diameter, covered at bottom and top with light-weight unbleached muslin. To avoid handling the flies, each cage was fitted horizontally with a sliding sheet-metal divider so that the flies could be trapped in one part of the cage while the other part was cleaned. The flies fed through the muslin cover from absorbent cotton pads soaked in the diet mixture and placed on top of the cage. The top of the cage was covered with a plastic dish to retard evaporation. The pads were usually replaced twice a day; once a day the cage was cleaned and the muslin covers changed. If eggs had been laid, they were collected at cleaning, placed on moist paper towelling in a vial, and held at 80° F for hatching. The cages used for colony rearing were similar, except that they were 6 in. in diameter and the bottoms were made of plastic screen, which was placed over cow manure on which the flies could oviposit. The manure was collected fresh and frozen for a few days to avoid insect contamination.

The reproduction data obtained with some representative diet mixtures are presented in Table 1. The first diets tested were blood only and blood in combination with various antibiotics. The best results were obtained with 1 mg streptomycin plus 1,000 units penicillin plus 250 units mycostatin/ml. of diet (referred to in Table 1 as 'standard antibiotic mixture'). Flies receiving blood plus this antibiotic mixture lived longer, but very few viable eggs were laid.

Table 1. REPRODUCTION OF HORN FLIES FED LABORATORY DIETS (10 MALES AND 10 FEMALES PER TEST)

Diet mixture	No. of eggs laid	Percentage of hatch
Flies held at 80° F		
1 part blood, 1 part beef juice + 1 mg streptomycin and 250 units mycostatin/ml.	199	16
Flies held at 90° F		
1 part blood, 1 part beef juice + 1 mg streptomycin and 250 units mycostatin/ml.	874	25
Blood only	182	13
Blood + standard antibiotic mixture	139	25
4 parts blood, 1 part physiological saline + standard antibiotic mixture + 20 mg yeast extract/ml.	248	45
1 part blood, 1 part beef juice	228	17
1 part blood, 1 part beef juice + standard antibiotic mixture	793	40

The next ingredient added was 'beef juice', a liquid obtained by centrifuging fresh ground beef muscle tissue for about 15 min at 3,000 r.p.m. When a diet of 1 part blood, 1 part beef juice, and the standard antibiotic mixture was fed, sufficient viable eggs were obtained to keep the colony in existence for five generations, although numbers did not increase from one generation to the next. Until the sixth generation the flies were held at 80° F. On the same diet, but with the temperature at 90° F, the number of flies increased from 125 in the sixth generation to 887 in the seventh, and the preoviposition period decreased from 6 to 4 days after eclosion. With testing and rearing conducted at 90° F, the colony, now in its tenth generation, is flourishing. Differences in the number of eggs laid and percentage hatch when the flies were reared at the two temperatures are shown in Table 1.

Continuous fluorescent lighting was used for testing and rearing. The humidity was not controlled, but because the cages were enclosed by plastic dishes covering the moist blood pads, humidity inside the cages was probably higher than that in the room.

Several materials more readily available in standardized form than the centrifuged beef juice, notably a commercial yeast extract and a prepared beef extract formulation, have been tested as alternative additions to the blood and antibiotics, but none has proved superior in promoting reproduction.

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* Acid Citrate Dextrose—National Institutes of Health Solution *B*. Each 100 ml. contains 1·32 g sodium citrate U.S P., 0 44 g citric acid U.S.P., and 1 47 g dextrose U S P.

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Effect of Phenylthiocarbamide on Viability of *Drosophila melanogaster* Strains with High and Low Tyrosinase Activity

Kroman and Parsons[1] have reported that the mutant ebony, *e*/*e*, in *Drosophila* is less viable than *e*/+ and +/+ on medium containing phenylthiocarbamide (PTC). They suggest[2] that poor viability of *e*/*e* on PTC medium may be correlated with the low tyrosinase activity reported for this genotype[3]. Experiments were performed to test this suggestion using several other strains of flies, and the results indicate that factors other than those associated with low *in vitro* tyrosinase activity account for poor viability on medium containing PTC.

Siblings were allowed to lay approximately 100 eggs in vials on normal medium containing 0·04 per cent PTC (experimental) and on normal medium (controls). For each strain tested, there were 7–10 experimental vials and 3–8 control vials. Cultures were maintained at 25° C. and were observed daily. In no case did PTC in the medium interfere with egg-laying or with hatching. In all instances development on the PTC medium was delayed after the first instar stage. All cultures which developed to the third instar stage gave rise to viable adults; in cultures from which no adults emerged, all individuals had died before the third instar stage. Adult survivors eclosed several days later and were smaller in size and in number than in control cultures.

The results are shown in Table 1. Activity is reported in DOPA oxidase units[4] since DOPA is the

Table 1. VIABILITY OF VARIOUS STRAINS OF FLIES ON MEDIUM CONTAINING PTC

Strain	Enzymatic activity in extracts of adults of control cultures	Survival on medium containing 0·04% PTC		
		Second instar	Third instar	Adult
1. *Hi*-3 (selected Samarkand)	950	+	—	—
2. *Lo*-3 (selected Samarkand)	250	+	—	—
3. *Hi*-4 (selected Samarkand)	900	+	+	+
4. *Lo*-4 (selected Samarkand)	250	+	—	—
5. *Hi*-5 (selected *Cy*/*Pm*)	750	+	+	+
6. *Lo*-5 (selected *Cy*/*Pm*)	300	+	—	—
7. Canton-*S*	150	+	+	+
8. *Lo*-2 (*a*-1)	70	+	+	+

convenient substrate used in routine assay, but the same relationship holds when tyrosine is used as substrate. The results indicate that fitness of flies raised on PTC medium is not related to the level of tyrosinase activity which has been found to be characteristic of these lines[4]. PTC inhibits tyrosinase activity *in vitro* by combining with the copper atoms of tyrosinase molecules. Perhaps the failure of strains 1, 2, 4 and 6 to survive on PTC medium may be due to low levels of other copper-requiring enzymes, whereas strains 3, 5, 7, and 8 may have high levels of other copper enzymes. A second possibility is that tyrosinase essential for a critical second instar process may not be quantitatively related to *in vitro* measurements in extracts of adults.

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Pyridoxine Requirements of *Agria affinis* (Fall.)

House[1], using a chemically defined diet and aseptic rearing technique, showed that larvæ of the parasitic dipteron, *Agria* [= *Pseudosarcophaga*] *affinis* (Fall.), would grow on an apparently pyridoxine-free diet. Recent reviews of Levinson[2], Lipke and Fraenkel[3], Friend[4], and House[5] indicate that *A. affinis* is peculiar in this respect.

There are several possible explanations for this peculiarity: House's diet may contain some impurity with vitamin B_6 activity; sufficient vitamin may be carried over from the female parent for one generation; the larvæ may be able to synthesize the vitamin; or pyridoxine or its derivatives may play no part in the metabolic processes of the insect. In the work recorded here these possibilities were limited by analysing the diet and larvæ for vitamin B_6 and by comparing the effects of lack of the vitamin on the transaminase systems of *A. affinis* and *Musca domestica* L., which last, as House and Barlow[6] found, will not pupate when grown on essentially the same deficient medium.

Agria affinis were obtained from a colony maintained on pork liver[7], and *Musca domestica* L. from a colony maintained on Richardson's[8] medium. Techniques of aseptic rearing were described by House[9] and the basic chemically defined diets by House and Barlow[6,10] for *A. affinis* and *M. domestica*, respectively. Vitamin B_6 analyses were performed for us by the Vitamin and Nutrition Section of the Department of National Health and Welfare, Tunney's Pasture, Ottawa, using *Saccharomyces carlsbergensis* 4228. Nitrogen analyses by Lang's micro-Kjeldahl method[11], and glutamic-oxalacetic transaminase determinations by the method published by the Sigma Chemical Co.[12] were performed on homogenates of whole larvæ.

All means are given with their standard error; differences between means are compared by t tests and are taken to be significantly different when $P < 0{\cdot}05$.

The vitamin B_6 assays were not precise because the amount of material which could be supplied was small: the very young larvæ dissected from gravid females and the larvæ reared individually on the supposedly pyridoxine-free diet were scarcely sufficient for a single determination. It can only be said that there was sufficient vitamin B_6 activity in the diet to account for several times its increase in the larvæ during growth.

The comparison of the effects of the pyridoxine-free diet on transamination in *A. affinis* and *M. domestica* brought out the following facts. First, there is a significant difference in transaminase activity between larvæ of the two species taken from their colonies. House-fly larvæ contained 29·9 ± 3·7 and *A. affinis* 20·9 ± 3·1 U/mg wet weight. There is little probability that this was due to a difference in metabolically active tissue because the nitrogen contents of the two species were the same (4·2 ± 0·3 per cent). Thus the enzyme activities based on nitrogen content, 0·49 ± 0·07 U/μg nitrogen for *A. affinis* and 0·71 ± 0·13 U/μg nitrogen for *M. domestica*, were still significantly different. Second, chemically defined diets are far from nutritionally adequate, judged on the proportion of nitrogen in the larvæ. Reared on the chemically defined diet with pyridoxine, *A. affinis* larvæ were only 0·66 ± 0·14 per cent nitrogen and *M. domestica* larvæ 0·94 ± 0·15 per cent. Lack of pyridoxine did not alter the nitrogen content. With the reduction in nitrogen there was a smaller reduction in transaminase so that it was somewhat higher than in stock-reared larvæ, on the basis of nitrogen content, *A. affinis* having 1·33 ± 0·32 U/μg nitrogen, *M. domestica* 0·84 ± 0·07 U/μg nitrogen. Third, lack of pyridoxine reduced transaminase contents significantly in both species, but most seriously in *M. domestica*. In this species the transaminase-level fell from 0·84 ± 0·07 to 0·30 ± 0·06 U/μg nitrogen; in *A. affinis* the decrease was from 1·33 ± 0·32 to 0·96 ± 0·34 U/μg nitrogen.

It is curious that larvæ reared on the chemically defined diet increased in size though the concentration of nitrogen in their bodies was thereby reduced to about one-sixth that of larvæ from the colony and that they were able to develop into an adult under such conditions. As the larvæ grow and develop while their nitrogen concentration is decreasing, the latter would be the best criterion for improving the diet until these larvæ are similar to stock-reared larvæ. Rate of development and/or an increase in total weight are probably the criteria that have been used most in nutritional studies. Though De Groot[13], Sedee[14], and others used nitrogen content, Sedee found that the nitrogen content of *Calliphora erythrocephala* (Meig.) corresponded so well with their dry weights that the nitrogen determination was discontinued. Apparently, no one has used nitrogen concentration, which would seem to be the most valuable criterion in this case.

It is now possible to draw some limited conclusions with regard to the pyridoxine requirement of *Agria affinis*. First, the supposedly pyridoxine-free diet contains enough impurity with vitamin activity to account for the accumulation of pyridoxine during growth. No assumption need be made of vitamin carried over from the female parent, of synthesis of the vitamin, or of lack of a metabolic role for it. Indeed, the results show that a dietary supply of pyridoxine is required to maintain the level of transaminase activity. Although lack of pyridoxine is not a growth-limiting factor for *A. affinis* reared on the present chemically defined diet, it could be so if protein metabolism were improved.

The fact that *M. domestica* larvæ, to develop, require additional pyridoxine in this diet while *A. affinis* do not, indicates an important difference between these species. Perhaps the form of the vitamin present in the 'pyridoxine-free' diet is usable by *S. carlsbergensis* and *A. affinis* but not by *M. domestica*; such differences have been reported[15]. Alternatively, *A. affinis* may be able to sequester the vitamin more efficiently than *M. domestica.* It is axiomatic that metabolic differences between animals are as great as the morphological differences generally used in taxonomy. Exploitation of these could lead to development of insecticides as discriminating as taxonomy. Evidence of this is already present in the development of chemotherapeutics, antibiotics, fungicides, and herbicides with specificity of a broad nature, and each instance of such metabolic differences deserves extensive investigation.

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MICROBIOLOGY

Ability of a *Vibrio* grown on Benzoate to oxidize *para*-Fluorobenzoate

THE utilization of aromatic compounds by micro-organisms for growth in mineral-salts media is well established; pathways by which such substances are broken down by micro-organisms have been recently reviewed[1]. A number of micro-organisms can also use halogen-substituted organic acids as growth substrates in mineral-salts media[2], the organically bound halogen being liberated as the halide ion during growth. An enzyme preparation, obtained from a pseudomonad grown in the presence of monochloroacetate, has been described[3] which catalyses the conversion of monochloroacetate into glycollate and chloride ions.

In this communication we would like to point out that the possibility exists that a micro-organism, grown in the presence of a non-halogenated aromatic acid, may be capable of metabolizing, albeit partially, simple halogen analogues of the aromatic growth substrate. Similar situations with regard to normal metabolites and simple halogen, especially fluorine, analogues are well known. For example, the synthesis of a monofluorocitrate from monofluoroacetate (for review see ref. 4), the synthesis of monofluoromalate from monofluoroacetate involving the enzyme malate synthetase[5,6] and the synthesis of bacterial cell-wall mucopeptide precursors linked to 5-fluorouracil instead of uracil[7].

Vibrio 01 (ref. 8) can be grown readily (for example, overnight) at 30° C with forced aeration, in a medium composed of sodium benzoate 0·005 M; potassium dihydrogen phosphate, 2 g; ammonium sulphate, 1 g; crystalline magnesium sulphate, 0·4 g; distilled water, 1 litre; and sufficient sodium hydroxide to adjust the initial *p*H of the medium to 7·2. The crystalline magnesium sulphate was added aseptically to the rest of the medium after *p*H adjustment and sterilization. No growth occurred over a period of 48 h in a medium in which an equimolar amount of *p*-fluorobenzoate was substituted for benzoate, even when such a medium was inoculated with cells previously adapted to benzoate.

Washed suspensions of benzoate-grown cells will, however, oxidize *p*-fluorobenzoate, typical results being shown in Fig. 1. In the case of benzoate, oxygen uptake greater than that in the endogenous ceases after the uptake of about 11 atoms of oxygen per mole of benzoate initially present; with *p*-fluorobenzoate as substrate, the corresponding figure is 8–9 atoms of oxygen. In contrast *p*-chlorobenzoate and *p*-bromobenzoate are but slightly oxidized, 1 atom of oxygen per mole of substrate initially present being taken up in each case. The *m*-fluoro analogue (not shown in Fig. 1) is oxidized much more slowly than *p*-fluorobenzoate by benzoate-grown cells with the uptake of about 2 atoms of oxygen per mole of substrate initially present.

Using the zirconium-alizarin method, as described by Feigl[9], fluoride ions could be detected in reaction

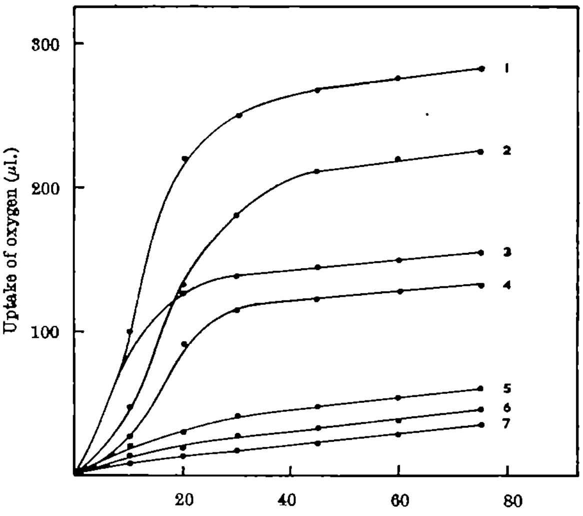

Fig. 1. Oxidation of various substrates by washed suspensions of *Vibrio* 01 grown with benzoate. Conventional Warburg apparatus was used, each flask contained 0 2 ml. (20 per cent w/v) potassium hydroxide in the centre well, and 1 ml. of bacterial suspension and phosphate buffer, *p*H 7·2, to a total volume of 3 ml Side bulbs contained: (1) 2 μmoles benzoate; (2) 2 μmoles *p*-fluorobenzoate; (3) 1 μmole benzoate; (4) 1 μmole *p*-fluorobenzoate, (5) 2 μmoles *p*-chlorobenzoate; (6) 1 μmole *p*-chlorobenzoate; (7) endogenous. The oxidation of *p*-bromobenzoate (2 μmoles and 1 μmole) is practically identical to (5) and (6) respectively

mixtures at the end of experiments in which cells had been oxidizing *p*-fluorobenzoate. In order to obtain some quantitative data on the amount of free fluoride produced, four flasks were set up (*A*, *B*, *C* and *D*) each containing 0·015 M phosphate buffer, *p*H 7·2; *A*, *B* and *C* contained both *p*-fluorobenzoate and benzoate at final concentrations of 0·002 M and 0·001 M, respectively. *D* contained benzoate but none of the fluorobenzoate. Washed benzoate-grown cells, collected from a fully grown 1·5-litre culture, were resuspended in 30 ml. of phosphate buffer, this suspension being divided into three 10 ml. portions. Flask *A* was inoculated with one portion, flask *D* with another; the third portion was heated at 56° C for 30 min and then added to flask *B*. No cells were added to flask *C*. The final volume in each flask was 100 ml. The contents of all flasks were strongly aerated at 30° C. After 3 h, cells were removed from the contents of each flask by centrifuging; the supernatants were then analysed for their free fluoride content[10]. Phosphates, which interfere in this method, were first removed by precipitation as the silver salt by adding sufficient half-saturated silver nitrate solution; excess silver ions were removed by adding the minimal amount of a 0·1 per cent (w/v) sodium chloride solution. Precipitates of silver phosphate and silver chloride were removed by centrifuging.

Free fluoride ions were detected in the supernatant obtained from flask *A*, the amount liberated being 60–65 per cent of the organically bound fluorine initially present. No fluoride ions could be detected in the supernatants obtained from flask *B* (inoculated with heat-treated cells), from flask *C* (uninoculated) or from flask *D*, which contained no *p*-fluorobenzoate.

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Production of Opacity in Egg-Yolk Medium by *Listeria monocytogenes*

SEVERAL micro-organisms are known to cause opacity when growing in fluid or on solid culture media containing native, non-heated egg-yolk[1–13]. This egg-yolk reaction can help in the classification of the bacteria and in some species it is associated with the virulence (especially toxicity) of the organisms. We have observed that *Listeria monocytogenes* strains regularly produced opacity in egg-yolk agar medium hitherto not reported in the literature.

Testing 50 strains of *Listeria monocytogenes* on egg-yolk agar plates (with 5 per cent non-heated, native egg-yolk content) after an incubation of 6 days at 37° C all the strains produced opacity in the medium. Two forms of reaction by the egg-yolk were observed. The majority of the strains (45) caused only slight opacity in the medium under and around the macrocolonies with a weak or marked clearing in the centre of the zone. The remaining 5 strains, on the other hand, formed strong and dense precipitates in the medium without clearing like the strains of *B. cereus*.

These characteristics showed no tendency to vary during an observation period of one year and remained stable even after serial transfers on egg-yolk agar plate. The cultures seemed to be homogeneous in this respect, as no aberrant colonies could be detected after examining thousands of isolated colonies of each strain. No correlations between the serotype, age, origin of the strains and the egg-yolk reaction type have been found. The only pattern was the hæmolytic activity, which showed some agreement with the egg-yolk reaction characteristics. All *Listeria* strains producing strong precipitate in the egg-yolk medium were strongly hæmolytic on blood-agar plate. The weak opacity producers exerted generally weak hæmolytic activity; only two strains caused marked hæmolysis on blood-agar plate.

The egg-yolk reaction in different bacterial species is often associated with phospholipase (lecithinase, cephalinase) activity; in other cases lipase action or proteolysis have been suggested as the cause of the disturbance in the stability of the medium[9,14,15]. The reactions produced by *Listeria* strains are due to the action of macromolecular substances, probably enzymes, since the changes failed to develop when the strains were cultured on egg-yolk agar plate covered with a sterile 'Cellophane' membrane. Further investigations are in progress to elucidate the mechanism of the egg-yolk reactions in *Listeria monocytogenes* strains and to examine the probable role of these characteristics in the virulence.

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Susceptibility of *Proteus mirabilis* and its Stable *L*-Forms to Erythromycin and Other Macrolides

Proteus spp. and other Gram-negative rods have been reported to be highly resistant to macrolides[1], but nothing is known about the mechanism of this natural resistance. Using *Proteus mirabilis* I have compared the susceptibilities of the normal bacillary forms and the so-called stable *L*-forms. The latter are distinguished by the absence of the cell walls of the parent strain. The absence has been demonstrated by the lack of phage receptors[2,3] and by chemical analysis[4]. In this respect they are similar to the protoplasts of Gram-positive bacteria produced by the action of lysozyme[5]; but, unlike these, they are able to grow well on solid media and may also be adapted to cultivation in broth. Like protoplasts, however, they do not revert to the normal rod forms, but grow indefinitely as *L*-forms even in the absence of penicillin, which was used for their induction in the first place.

Earlier studies on the susceptibility of *L*-forms to antibiotics were made by Ward *et al.*[6] and other workers[7]. However, in most cases use was not made of stable *L*-forms but rather the unstable *L*-forms or spheroplasts or mixtures of these forms, all of which possess cell walls or fragments thereof. With *Proteus mirabilis* it is possible to investigate the behaviour of the cell as a whole and of the 'cell contents' (protoplast) in the presence of substances such as antibiotics.

In my experiments, erythromycin, carbomycin (magnamycin), spiramycin, oleandomycin, leucomycin and angolamycin were tested by an agar diffusion method using double-layer plates, the upper layer being inoculated with either the normal rod forms or the stable *L*-forms. The antibiotics were added to holes (0·05 ml.) to produce zones of inhibition after diffusion. The concentrations of the test solutions were 1,000, 100, 10 and 1 μg/ml. Table 1 shows the results.

Table 1. ACTION OF MACROLIDES ON *Proteus mirabilis* (STRAINS VI AND 52) AND THEIR STABLE *L*-FORMS

(Figures represent the minimal inhibitory concentrations in μg/ml.)

	VI	52	*L*-form VI	*L*-form 52
Erythromycin	≈ 1,000	≈ 1,000	< 1	< 1
Magnamycin	> 1,000	> 1,000	< 1	< 1
Leucomycin	> 1,000	> 1,000	< 1	< 1
Spiramycin	> 1,000	> 1,000	< 10	< 10
Oleandomycin	> 1,000	> 1,000	< 10	< 10
Angolamycin	> 1,000	> 1,000	< 10	< 10

It can be seen that the macrolides act selectively on the stable *L*-forms while the parent strains are virtually unaffected.

For comparison, some other antibiotics have been tested by the same method. Netropsin, neomycin, kanamycin, paromomycin, streptomycin, novobiocin, chloramphenicol, chlortetracycline and oxytetracycline inhibited at relatively high concentrations both the bacillary forms and the *L*-forms, the latter being commonly slightly more sensitive. Grisein and actidione, as well as mycostatin, trichomycin and some other polyene antibiotics, did not affect any of the forms of *Proteus*.

The strikingly selective action of the macrolides as a group may be due to the absence of the cell walls in the *L*-forms. In contrast to many other antibiotics, the molecules of these drugs are evidently prevented by the walls from freely entering the cell contents of the normal rod forms. It is unlikely that the observations can be explained by basic differences in the metabolism of *Proteus* and its *L*-forms because the reactions so far investigated have been shown to be essentially the same[4]. Furthermore, the possibility of selective enzymatic inactivation of the macrolides by the rods was excluded—prolonged incubation of the antibiotics with *Proteus* rods did not lead to specific loss of activity.

It is suggested that within the commonly used range of concentrations the marked natural resistance of *Proteus mirabilis* to macrolides is due to the inability of the substances to penetrate the cell walls of the normal rods in effective amounts and is not due to insensitivity of the metabolism of *Proteus*.

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PSYCHOLOGY

Counter-injection: a New Technique for the Analysis of Drinking

A METHOD of regulating separately the sensory and osmotic or other physiological factors in ingestion is of importance in the analysis of satiation and reward. Oesophageal fistulation has frequently been used for this purpose; but it removes many of the sensory consequences of ingestion in the upper intestinal tract along with the elimination of absorption factors. Further, the animal after the operation, which is difficult in small animals, cannot feed normally and is not easy to maintain in a good state of health. Preloading has also been reported; but one of the disadvantages of this method is that it introduces critical delays between the preload and actual drinking.

Briefly, the technique described here is to inject into the stomach by way of a chronically implanted fistula small quantities of fluid (such as hypertonic saline) in proportion as the animal itself drinks, say, water. In this way, the tonicity of the fluid arriving in the animal's stomach can be rigidly controlled, however much the animal drinks of some other concentration. So far we have used this technique to make water drunk by the rat result in a slightly hypertonic saline in its stomach.

This method requires a precise means of regulating the amount of saline injected into the stomach with respect to the amount of water being ingested by the animal. To accomplish this, a simple experimental technique was developed and tested. Drinking took place in a round wire cage with a plastic cover; the animals were tested singly and were required to lap a glass nozzle through a hole in the plastic top. Each lap activated the relay of an electronic drinkometer, a more sensitive development of the one described by Hill and Stellar[1]; this relay, in turn, activated the stepping motor of a Davis vertical liquid pump, fitted with a 50-c.c. syringe filled with water. As the motor advanced with each lap of the rat, water was pumped from the syringe through a piece of polyethylene tubing down into the drinking nozzle; the pump motor was so regulated that, with each lap of the rat against the nozzle, a lap's worth of water was deposited on its tongue. Furthermore, the pumping apparatus was modified in the following way: a smaller syringe was taped rigidly to the barrel of the 50-c.c. syringe (a small plastic wedge was placed between them to keep the barrels of the syringes parallel to each other), and a small piece of plastic was affixed to the stepping motor, so as to extend underneath the plunger of the second syringe, as well as the first—thus adapting the pump to drive both syringes. The smaller syringe was filled with hypertonic saline (or water, depending on the experimental condition) and was attached to the stomach fistula by way of polyethylene tubing. By means of this mechanism, each lap of the nozzle caused a small amount of water to be delivered to the rat's mouth, and, simultaneously, a proportionally smaller amount of hypertonic saline to be injected into the stomach. The ratio of these two amounts was precisely determined by the ratio of the internal diameters of the two syringes, since their plungers were moved through equal distances by the pump.

A preliminary experiment with this technique was carried out on 4 albino rats. One-hour drinking tests on each rat were conducted every other day for three weeks, with counter-injections into the stomach taking place in the first 30 min only. After each hour's test, water was left available to each rat for an additional hour in a different cage.

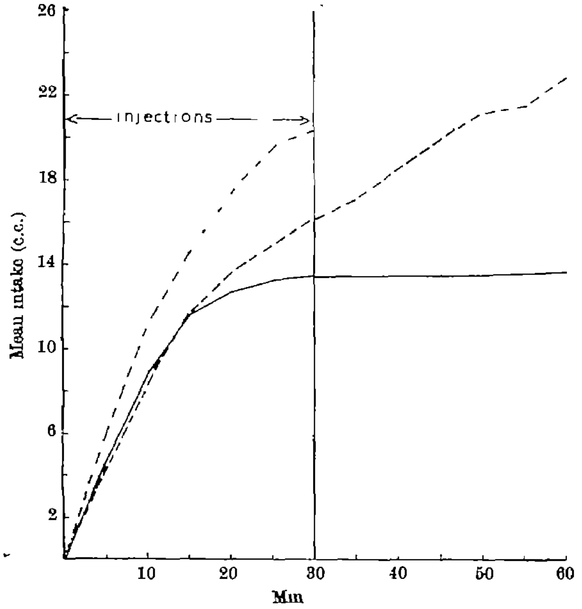

Fig. 1. ——, Water drunk, water injected, 0·1 : 1·0, ----, water drunk, 10 per cent saline injected, 0·1 : 1·0, —·—·—, 0·6 per cent saline drunk, no injection

Measurements of water ingested were taken at 5-min intervals during the test hour by reading directly from the scale on the 50 c.c. syringe, and the number of laps were obtained also from a counter, attached to the drinkometer. There were two experimental conditions: (1) the rats were allowed to drink water and counter-injections of hypertonic saline into the stomach rendered the concentration slightly above 0·9 per cent (operationally, for every c.c. of water drunk by the rat, 0·1 c.c. of 10 per cent saline was injected); (2) the rats were allowed to drink water and counter-injections of water (0·1 c.c. per c.c. drunk) were made into the stomach (control).

Cumulative drinking curves obtained from these tests show that the amount of drinking done in the first 15 min (when rats do most of their drinking following deprivation) is identical under both conditions, indicating that post-ingestional factors do not have an effect during this time. To test the possibility that these rates of drinking in the first quarter-hour might be the maximum possible for the rat, the same animals were allowed to drink 0·6 per cent saline in the same situation, though without injections. Under this condition, their rate of drinking was immediately greater than under previous conditions, and in 30 min they had ingested, on the average, more than they had in an hour under experimental condition (1). A replication of these preliminary tests is now being carried out, incorporating this third condition, in which hypotonic saline is drunk, while counter-injections render the solution of the same tonicity in the stomach as in the previous experiment.

Observations of the animals while drinking, with and without injections, showed that drinking patterns are not disrupted in any way by the technique; the rats seemed not to notice the injections at all, and very quickly adapted to the presence of the tubing connected to the fistulas behind their necks. Their general health was good.

This technique should prove applicable to a wide variety of experimental situations involving drinking behaviour in the rat.

This work reported was supported by U.S. Public Health Service grant *M*–4563 and National Science Foundation grant *G* 21376, while one of us (H. L. B.) was a Woodrow Wilson Fellow at Stanford.

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A Significant Facet of Attention in Human Mental Activity

In the initial stages of observation, even with energy phenomena, instrumentation for measuring such a novel finding may not be available. At that time only the verbal formulation can be used to describe the event[1].

I have made numerous observations in the area of the parent–child (love) relationship or 'emotional-dependency-drive', or human-attention-seeking. About 15,000 cases were examined neuro-psychiatrically[2,3], which examination included in each case a review of historical data, the application of a variety of psychological tests, neuro-endocrine inspection, interview with parents and a psychiatric examination of the subject (juvenile). In addition, more than 1,000 cases of adults were diagnosed (examined) and were treated for neuro-psychiatric symptom-sign-complexes such as are found in a clinical neuro-psychiatric practice. Over the span of twenty years, tentative hypotheses[4,5] were formulated which now have been crystallized into a relatively comprehensive biological concept as will be expressed here in brief.

A dog is completely weaned (no attention needed) from its parent animal at 21 days. Weaning in infra-human animals indicates no further 'dependencies upon' or 'turning to' the parent animal. However, I have observed that if an alert and potentially aggressive species of dog, such as the German shepherd, after it has been weaned, is placed in a human environment, it becomes 'attached' mainly to one of the human objects. The dog actively turns-to or seeks-'attention'-from the human object-source, in competition with the attention-seeking-human-subjects of that same object-source. The dog demonstrates signs of jealousy and rivalry which may attain serious proportions even unto that which allegedly obtained between Cain and Abel. This observation regarding a special type of dependency or 'attention-seeking' probably classifies within the phenomenon of tropism (biological turning to)[6].

Implied in the foregoing sentences is a significant dimension which has not been formulated in the descriptions available in the literature[7,8] on the attention or in-attention components in the mental modality of the human nervous system.

Moreover, the fact that the dog's nervous system becomes involved in essentially a human neurophysiological event suggests that the semantic and symbolic phases of psychology are not required in the activation of this neural phase of attention-seeking or attention-response. Seemingly, in this essentially human dependency affair or motivation an effect is contaged[9] rather than communicated. Ostensibly, the fact that a dog becomes involved in this special area of functionality suggests that the evolutionary *anlage* for the process which becomes so magnified in the human is already established in the infrahuman animal.

The degree to which this phenomenon operates through fixed-action-systems (unconsciously) universally in the human being can only be determined by the extent to which each human being's behaviour reveals the human-attention-seeking-tendency. Psychosomatic symptom-signs (coronary spasm, vascular hypertension, gastro-intestinal ulcerations) which may become so intense as to cause death may also be part of the fixed action-driving-force operating in this entire complex, and suggests in part the violent intensity which Nature has invested in this attention-seeking-motivation.

In the human adult this element of attention-seeking appears to be a continuation of a process so evident in the human infant and child where the parental-attention or parental-type-of-love-effect is sought through: (*a*) extended crying[10] where no causative organic or physical disturbance can be found; (*b*) through excessive wakefulness where the parent continues to go back-and-forth to observe the infant; (*c*) later, through refusal to eat, especially where the parent pressures in that direction; (*d*) through temper tantrums; and (*e*) still later through positivistic (conforming) or negativistic (reactive) ('rebellious') behavioural patterns. As determinable from the legend, Did Cain kill Abel because of jealousy or rivalry for the limited attention available from Eve, their immature mother, who in turn through reactiveness was seeking a parental-type of love attention from her husband, Adam, as is evidenced by her' eating the apple when asked not to ?

Subserving the physiology in this essentially 'human-subject to human-object' tropistic phenomenon must be a real attribute of psycho-neuronal energy. Perhaps Freud's formulation of cathectic and super-cathectic energy[11] will be found related to a phase of the energy concept offered here. If such a relationship does exist then we have the beginning of a bridge between the Freudian psychoanalytic approach to mental therapy and the more recent biochemical (pharmaceutical) one as might be inferred from the succeeding paragraph.

As all physiology is subserved by biochemistry, ostensibly, this specialized psycho-neuronic physiology which the author is describing must be subserved by a specialized biochemistry. Is it then probable that the psycho-neuronic or emotional-dependency-motivation in the human is an evolutionarily acquired quality, or property of the human psychoneuronic apparatus, and that it is potentially adjustable through biochemicals[12,13] ? The answer to this question may be crucial to man's survival[14-16].

I am not describing a new natural principle; I am formulating a very important one, old as man, on a physiological, scientific basis. The human psycho-neural-dependency-appetite or motivant ('the need for parental love') proceeds as a type of mutual tropism (turning) between child and parent. A so-called rapport-tie, relationship, or bridge becomes established between them. This rapport-tie is a physical invisible bridge between human object and subject[5]. Relative to this theme, over the bridge, which is of variable strength, the parent, object, or donor transfers to the child that amount of psycho-neural-maturing-attribute ('love') it has available. The biopsycho-neural maturing process has qualitative, quantitative and temporal dimensions. The donor remains happy while supplying the maturing factor (psycho-neural attribute of energy) so long as the demand for it does not exceed the donor's available supply. The human infant, child, or subject is unwittingly, intensely driven by naturally established psycho-neural mechanisms to seek for the psycho-neural maturing factor from its attending objects or parental sources. The subject shows external and internal evidence of happiness, pleasure, or optimum biological functioning to the extent it actually obtains psycho-neural maturing factor through existing rapports. To the extent that a psycho-neural dependency need is unmet or to the extent that a psycho-neural functional maturation is incomplete, to that extent from birth to death does a subject, by various provocative or symptomatic ways, continue to seek further maturing effects and/or to seek relief from the stress of biopsycho neural functional immaturity. The biopsycho neural maturing factor is an attribute of energy developed in and by the (human) psycho-neural cortex[12,13].

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, October 15

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5 30 p m.—Mr. J. Muir, Mr. J. Norris and Mr. K. C. Overton: "Determination of MCPA and Related Acids by Gas Liquid Chromatography".

University of London (at Queen Elizabeth College, 61–67 Campden Hill Road, London, W.8), at 5.30 p m.—Sir Harrie Massey, F.R.S.: 'Space Research" (Inaugural Lecture).

Institution of Mechanical Engineers, Railway Engineering Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m. Discussion on "Noise and Railways".

Institute of Metal Finishing (at Northampton College of Technology, St John Street, London, E. C. 1), at 6.15 p.m.—Mr. A. Nicholson "Maintenance of Electroplating Plant".

Tuesday, October 16

Institution of Agricultural Engineers (at the Royal Society f Arts, John Adam Street, Adelphi, London, W.C.2), at 10 a.m.—pecial General Meeting of Corporate Members. 10.30 a.m.—Mr. ameron Brown: Presidential Address, followed by Scientific Papers.

University of London (in the Anatomy Theatre, University ollege, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. J. Maynard mith. "The Biology of Patterns".*

University of London (in the Beveridge Hall, Senate House, ondon, W.C.1), at 5.30 p.m.—The Rt. Hon. Lord Todd, F.R.S. Phosphates in Vital Processes".* (First of eighteen lectures on The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Society of Chemical Industry (joint meeting of the London ection and Aslib Chemical Group, at 14 Belgrave Square, London, W.1), at 6.30 p.m.—Dr. G. M. Dyson. "Research at Chemical bstracts".

Wednesday, October 17

Institute of Navigation (at the Royal Geographical Society, Kensington Gore, London, S.W.7), at 4.30 p.m.—Annual General eeting. Dr. G. E. R. Deacon, F.R.S.· "Research and Navigation" residential Address).

Royal Meteorological Society (at 49 Cromwell Road, London, W.7), at 5 p.m.—Mr. D. H. Johnson "Rain in East Africa"; . P. Graystone: "The Introduction of Topographic and Frictional ffects in a Baroclinic Model".

British Nuclear Energy Society (at the Institution of Civil ngineers, 1–7 Great George Street, London, S.W.1), at 5.30 p.m.—. R. Hurst: "Dounreay Fast Reactor Operating Experience".

Institution of Electrical Engineers, Electronics Division Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. A. Ratcliffe, R.S.. Chairman's Address.

British Institution of Radio Engineers, Computer Group the London School of Hygiene and Tropical Medicine, Keppel reet, Gower Street, London, W.C.1), at 6 p.m.—Discussion on: tandard Logical Elements".

Oil and Colour Chemists' Association, London Section (at Royal Society of Tropical Medicine and Hygiene, Manson House, Portland Place, London, W.1), at 7 p.m.—Dr. S. Cotson: "Education and Training for the Surface Coatings Industry".

Royal Institute of Chemistry, London Section (joint meeting th the British Society for the History of Science, at the Science useum, South Kensington, London, S.W.7), at 7 p.m.—Dr. A. ow "The Chemical Industry before Muspratt".

Thursday, October 18

University College, London (in the Anatomy Theatre, Gower eet, London. W.C.1), at 1 15 p.m.—Prof. P. E. Corbett "The d and the Ladder—Pets, Beasts and Monsters in Greek Art".*

Society of Chemical Industry, Road and Building Materials oup (at 14 Belgrave Square, London, S.W.1), at 2.30 p.m.—Meeting "Problems in Sampling Road and Building Materials".

nstitution of Mining and Metallurgy (at the Geological iety, Burlington House, Piccadilly, London, W.1), at 5 p.m.—neral Meeting.

innean Society of London (at Burlington House, Piccadilly, don, W.1), at 5 p.m.—Dr. P. B. Tomlinson: "The Vegetation of Florida Everglades"; Prof. C. F. A. Pantin, F.R.S.: "A Relict ine Fauna".

niversity of London (at the London School of Hygiene and pical Medicine, Keppel Street, Gower Street, London, W.C.1), .30 p.m.—Prof. D. A. K. Black· "The Alimentary Tract and y-Fluid".* (Second of eighteen lectures on "The Scientific Basis Medicine" organized by the British Postgraduate Medical Federa-".

oyal Society of Tropical Medicine and Hygiene (at Manson se, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. A. J. gan. "A Survey of Sleeping Sickness in Northern Nigeria, from Earliest Times to the Present Day" followed by a Discussion. rman· Sir George McRobert.

Thursday, October 18—Wednesday, October 24

World Congress of Underwater Activities (at Church House Westminster, London, S.W.1).

Friday, October 19

British Society for Parasitology (in the Meeting Room of the Zoological Society of London, Regent's Park, London, N.W.1), at 10.30 a.m.—Meeting on "The Application of New Techniques to Parasitology". Chairman· Sir John Randall, F.R.S.

Photobiology Group (at the Institute of Ophthalmology, Judd Street, London, W.C.1), at 2.30 p.m.—Meeting on "Physiology and and Photochemistry of the Eye".

University of London (at Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 5 30 p.m.—Prof. H. Mohr (University of Freiburg): "The Control of Plant Growth and Development by Visible Radiation".* (Further lectures on October 22 and 23.

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. S. F. Singer (Washington). "A Satellite's View of the Weather".

Saturday, October 20

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. W. K. Ford. "Tropical Insect Life".*

Monday, October 22

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5 30 p.m.—Discussion on "Earthing Arrangements for Electrical Equipment, with particular reference to Double Insulation".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5 30 p.m.—Dr F. G. Heath: "The Theory and Practical Value of Ternary Systems".

Royal Geographical Society (at 1 Kensington Gore London, S.W.7), at 8.30 p.m.—Mr. Brian J. Moser and Mr. Donald Taylor: "Lost Tribes of the Piraparana".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned.

Lecturer (with a special interest in teaching and research in applied mechanics) in the Department of Mechanical Engineering—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (October 18).

Lecturer (competent in physiological psychology and/or experimental psychology) in Psychology—The Registrar, The University, Leicester (October 20).

Lecturer (with a good honours degree or equivalent) in Chemistry, to teach all branches to G.C.E. (A), and Physical Chemistry and/or Organic Chemistry to degree and Grad.R.I.C. level—The Principal (HE.4/C/2806/10), L.C.C. Norwood Technical College, Knight's Hill, London, S.E.26 (October 20).

Chair of Mathematics—The Registrar, University of York, The King's Manor, York (October 22).

Chief or Senior Technician in the Department of Zoology—The Secretary to Council, Westfield College (University of London), Hampstead, London, N.W.3 (October 22).

Experimental Officer or Senior Experimental Officer in the Department of Mechanics, for duties which will include the design and construction of electronic and optical apparatus for studying turbulence, aerodynamic noise, and flows at very high speeds including their interaction with magnetic fields—The Registrar, The University, Manchester 13 (October 24).

Lecturer or Assistant in Clinical Chemistry in the Department of Pathology, Queen's College, Dundee, and the Royal Infirmary, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (October 27).

Assistant Experimental Officer (with a sound knowledge of genetics and chemistry, and a degree in either of these subjects) in the Department of Applied Genetics—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (October 31).

Lecturer or Assistant Lecturer (preferably with a special interest in biogeography or surveying) in the Department of Geography, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (October 31).

Statistician (with a first- or second-class honours degree in mathematics, with statistics) with the Agricultural Research Council Statistics Group, School of Agriculture, for the application of statistics to agricultural research, and computer programming—The Secretary, School of Agriculture, Cambridge (October 31).

Director (honours graduate, preferably in mathematics, with postgraduate training, and previous experience in a computing laboratory), to take charge of the newly instituted Computer Unit which is to be developed within the university over the next few years—The Secretary, The University, Edinburgh (November 1).

Lecturer or Senior Lecturer in Zoology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1, (Australia and London, November 1).

Lecturer or Assistant Lecturer in Agricultural Biochemistry, Mathematics, and Physics (two vacancies) at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 8).

Professor of Inorganic Chemistry—The Registrar, University Registry, Oxford (November 8).

ASSISTANT LECTURER IN PHYSICAL CHEMISTRY—The Secretary Queen's University, Belfast, Northern Ireland (November 10).

GRADUATE PROGRAMMER IN THE COMPUTATION LABORATORY, for duties which will include advising users in programming and operating a Ferranti Pegasus computer to which magnetic tape is to be added—The Secretary and Registrar, The University, Southampton (November 10).

LECTURER OF ASSISTANT LECTURER IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY—The Secretary, The University, Edinburgh (November 14).

LECTURER (Grade II) IN PHILOSOPHY—The Registrar, The University, Bristol 8 (November 15).

LECTURER or ASSISTANT LECTURER IN PHARMACOLOGY at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 15).

PROFESSOR OF PATHOLOGY—The Registrar, University Registry, Oxford (November 15).

ASSISTANT (preferably with a degree in horticulture or botany) AT THE ROSEWARNE EXPERIMENTAL HORTICULTURE STATION, Camborne, Cornwall, for research on time of heading and quality in winter cauliflower—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

PHYSICIST, PHYSIOLOGIST or PHYSICAL CHEMIST, to assist in research on pulmonary function—Dr. M. L. Thomson, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1.

POST-DOCTORAL RESEARCH ASSISTANT (graduate, preferably with research experience in X-ray crystallography), to investigate the structure of liquids and solutions by X-ray diffraction methods—Dr. R. Mason, Department of Chemistry, Imperial College of Science and Technology, London, S.W.7.

READER (with appropriate research experience) IN PHYSICS—The Registrar, Department 26/I, Bradford Institute of Technology, Bradford 7.

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UNIVERSITY FREEDOM AND THE ACADEMIC DEFENSIVE

THE firmness and dignity with which the free universities of South Africa opposed the policy of the South African Government towards the universities, culminating in the Separate University Education Act of 1959, won the respect and admiration of the entire academic world, as was manifested at a notable meeting in London in November 1957. It was at a general assembly of the University on April 16, 1959, when the Bill was in its final stages in the South African Parliament, that the University of the Witwatersrand, after affirming the principle that a university is a place where men and women without regard to race and colour are welcome to join in the acquisition and advancement of knowledge, pledged itself to continue faithfully to defend this ideal against all who have sought by legislative enactment to curtail the autonomy of the University. Accordingly, the assembly dedicated itself to the maintenance of this ideal and to the restoration of the autonomy of the University.

To commemorate in every third year this occasion, the University established a Chancellor's Lecture, the first of which was delivered on April 4, 1962, by Sir Eric Ashby. Entitled *Universities under Siege*, this lecture has now been published by the University*. In thought and language it is a heartening and inspiring paper. Without commenting on the policies of the South African Government which are so widely regretted, Sir Eric sets forth not merely the argument for the free university but also the nature of its responsibilities in a world when in all universities so many of the values they cherish are endangered. Taking, as it were, the torch from one passage in the essay, "The Open Universities of South Africa", in which five years ago, at the height of the controversy, the university set forth with clarity and dignity its function in the Union of South Africa, Sir Eric illumines that ideal, expounds its relevance in this changing world and points to the opportunities as well as the problems which confront us in applying it fully in our times.

"Experience shows that a society, however successful it may have been in the past, will not long survive if it cannot cope with the tasks of a new era. For this reason every civilized society tends to develop institutions which will enable it to acquire, digest and advance knowledge relevant to the tasks which, it is thought, will confront it in the future. Of these institutions, the university is the most important."

From that text Sir Eric expounds the uniqueness of universities among social institutions, emphasizing first the dual loyalty which springs from their universality. Because they deal with concepts which command the assent of educated men irrespective of race, colour, religion, language or politics, they cannot fully serve the society which maintains them unless that society grants them certain privileges so that they can also be loyal to the international brotherhood of universities. They are within the State, yet they must preserve an individuality apart from the State. They cannot exist except in the matrix of politics, yet they must remain out of the reach of politics.

The university's claim to these privileges which are not granted to other institutions in society can be based on tradition, precedent and statute, often of centuries standing, but Sir Eric wisely does not rely solely on this legalistic approach alone. Precedents and traditions are not immutable: indeed, Dr. Emil Brunner remarked in his Gifford Lectures that in a world in which democracy, in the sense of equality, has become a kind of religion, tradition can neither be kept nor be formed. This, he continued, is one of the main causes of the instability of Western society during the past two centuries, and although the Union of South Africa is notably conservative and traditional in outlook, it is somewhat paradoxical that so sharp a threat to the university ideal should have emerged there. However, even in Britain it is obvious that the climate of opinion is increasingly bringing under examination such institutions as the University Grants Committee and that the new universities are likely to need all the help which they can receive from such co-operative institutions in their developing and formative years.

Sir Eric Ashby fully recognizes that precedents and traditions are for ever being eroded by research and reflexion on social problems and that the history of academic freedom records many setbacks. These setbacks, however, have strengthened, not destroyed, the concept of a university, and from this he argues that the defence of academic privilege is best based on the empirical observation that without its privileges a university cannot fully serve the society which maintains it. Academic privilege is not just a tradition: it is a necessity.

In developing his argument here, Sir Eric is arguing fundamentally for the free society, for, although it is above all in the university that new ideas are nurtured and tested, controversy develops, and out of controversy deeper understanding, the process is not confined to the university. The university is the principal instrument of society for adventurous and constructive thinking, the forum for dialectic over intellectual affairs, and no outside prejudice or interest should be allowed to hinder the adventure of thinking, the clash of minds, the search for truth. Sir Eric Ashby said frankly that a nation where thinking is rationed cannot survive in the world of to-day, and he pointed out that university privileges are preserved in all intellectually healthy countries, even though the universities in these countries have long since lost their defences against the State. In Holland, Western Germany, France, and the British Commonwealth, in the United States (apart from a few private universities) the universities now depend on the State for finance. In none of these countries is there any effective formal defence against invasion of their traditional privileges, and yet in all their privileges to select students and staff, to plan courses and curricula, are respected and honoured.

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* Universities under Siege. (The Chancellor's Lecture.) By Sir Eric Ashby. Pp. 19. (Johannesburg: University of the Witwatersrand, 1962.)

These privileges, Sir Eric Ashby argues, are thus retained not by power but by popular consent, because it is recognized that the privileges have been shown to be necessary. The possessions which matter most in a university are not buildings, not even its library and equipment, but its ideas, and quality of ideas depends on quality of staff. First-rate staff will not work under conditions fixed by those who are afraid of ideas. Moreover, it is probably true to say that the widespread concern with which the disagreement between the Government and the University Grants Committee over university finance was viewed in Britain this spring was due to the fear not so much that university expansion would be checked as that the universities would be unable to attract and retain sufficient staff of high quality.

Turning then to discuss the duty of a university, Sir Eric addresses some heartening words to the open universities of South Africa. Referring with deep understanding to their present difficulties, he points out that those universities have not betrayed their privileges from within, and that the way in which the University of the Witwatersrand is resisting the challenge from without commands the admiration of the academic world. Such is an inspiration not merely to older institutions in Cambridge and Harvard, Berlin and Leyden, but also to the much younger institutions in Dakar and Ibadan, Khartoum and Salisbury, and, not least, the two at present under siege in Ghana. Some of the universities in tropical Africa have, in fact, particular reason to be grateful to the University of the Witwatersrand for its declaration of April 16 because their integrity too is threatened by racial discrimination. The stand which Nigerian intellectuals are taking to keep their universities truly cosmopolitan, to prevent them from being mere staff colleges for African nationalists, would have been much more difficult, Sir Eric states, if the open universities of South Africa had succumbed without protest to the legislation of 1957.

Universities, he continues, are all members of a supra-national community and serve their local communities by supplying an international commodity—scholarship, science, technology, medicine—on international standards. They cannot fulfil even their national purposes unless they are loyal to cosmopolitan traditions of what universities represented. If a few of the great universities were to compromise on level of attainment among students and level of scholarship among staff the integrity of all universities would be endangered, and that is equally true of compromise over privileges and immunities. That is why the stand which the University of the Witwatersrand is making against the violation of one of its privileges is a defence not only of itself but also of universities everywhere. A university which is unfaithful to its own traditions in order to appease the State or to gain temporary advantage debases the currency of its scholarship and betrays its own academic staff.

Lastly, Sir Eric Ashby looks to the tasks of the future. Turning back to the passage in his text, "a society will not long survive if it cannot cope with the tasks of a new era", he reminds us that universities have become key instruments in a continual adjustment of society to its environment, and this is nowhere more evident than in Africa. A university must be responsive to its environment, but in responding it must not break faith with the supra-national brotherhood of universities and the traditions it inherits from them. To strike the balance, over standards and privileges, he reiterates, the university must be firmly uncompromising; over the currents of thought which run through it and the pattern of government it adopts, the university must be relevant to its own society.

Probably no part of Sir Eric's lecture was more stimulating to his audience than the following passage in which he sought to interpret what this meant in terms of the curricula and statutes and regulations of a South African university. Its relevance for the universities of the British Commonwealth generally is no less apparent, whether in what he had to say about problems of self-government in modern universities or about the problem of responsiveness to currents of thought. It is not simply that he extends the spectrum of subjects of study. His comment on the social mimicry of European education in the universities of tropical Africa, especially in the faculties of arts and social studies, is not for those universities alone. It has deep implications for the universities of Great Britain and should be pondered by the Commonwealth Educational Conference as well as by the Department of Technical Co-operation and those bodies, like the Nuffield Foundation, which have been seeking to foster the study of the questions to which he refers and on which the fate of the African continent may well depend.

If questions like the transfer of ideas from Ibo to English, the impact on the African mind of European ideas of justice and equity, how systems of social welfare replace the obligations of the extended family, or what tensions occur in the African when he becomes urbanized and has to discipline himself to a routine and work either with Europeans or like Europeans are to be rigorously and impartially examined, an immense amount of help must be forthcoming. Some of it is already being organized from the United States, but here is a responsibility which should be accepted primarily by Britain and towards which the Commonwealth universities and the Overseas Research Council could and should be making a significant contribution.

Sir Eric Ashby primarily challenges the University of the Witwatersrand and other universities in multiracial communities with their unique opportunities to study this hurricane of culture-change and to ensure that every student is persuaded to think systematically about the problem as a basic ingredient of his or her higher education. It is essentially a problem in the humanities, and as these universities respond to the challenge, new ideas are likely to result from which the great dilemma of South Africa itself may be solved. But just as it must mean much to a university under siege to know that its refusal to compromise on standards and privileges earns it the respect and support of universities generally, so its attempt to observe in scholarly manner, to understand, and perhaps in a small way to influence the course of Westernization in Africa, it should be sustained by the knowledge that those problems are also thought worthy of study by the universities of the Commonwealth, and that the universities of the United Kingdom in particular are engaging strenuously in that task. Admirable projects have indeed already been undertaken by some universities in Great Britain, but much more generous support could well be forthcoming to the young universities of Africa through the Overseas Research Council in the critical study of the greatest human problem of our age: the peaceful co-existence of people of different races. Universities generally may be grateful for Sir Eric's noble words; the University of the Witwatersrand will the sooner be able freely to bring the black and white peoples of South Africa together in that adventure of scholarship in the measure in which Britain herself discharges her own responsibilities in that same field.

Since the lecture was delivered the Sabotage Act has become law and the area in which resistance is effective has been further narrowed. Something of how much those defending freedom generally in South Africa appreciate the support of the outside world may be sensed from the statement Chief Lutuli, Nobel Peace Prizewinner for 1960, wrote before the Act became law. Publication of that message has been prohibited in South Africa, and it would be premature to deduce from the publication of the Chancellor's lecture that there has yet been any weakening in the Union Government's assault on civil liberties generally or any pause in its tragic pursuit of apartheid. Sir Eric remarks trenchantly that the only convincing evidence for apartheid existing in a university would be the inferiority intellectually of black men to white.

EVOLUTION: BIOLOGICAL AND HUMAN

Evolution and Man's Progress

Edited by Hudson Hoagland and Ralph W. Burhoe. Pp. 181. (New York and London: Columbia University Press, 1962.) 4.50 dollars; 36*s*.

THIS unusual and stimulating book includes contributions from two dozen men eminent in genetics, palæontology, evolution, systematics, general biology, social and cultural anthropology, psychiatry, psychology and sociology. It is clearly impossible to review it in detail: all I can do is set down some of the reflexions to which it has given rise.

In the first place, it is a symptom of the present crisis in 'man's progress'. Neither the physicochemical sciences and their application in technology, nor the traditional ideas of the political or economic 'sciences', nor the pseudoscientific ideologies like Marxism, turn out to provide a satisfactory basis for human thought and action in regard to progress. Accordingly, thinking men are turning towards the biological and the human sciences in an attempt to reach some new and more satisfying synthesis.

This book is a welcome contribution to this effort. However, it does not go far enough in the direction of what one of the contributors terms 'synthesism', but which I would prefer to call simply integration. So far as I can see, the new synthesis must grow out of the concept of evolution as an operative process. We need to have in mind not merely the genetic basis of evolution, whether biological or human, not merely the results of small-scale evolution of populations or species, but the process as a whole, with special reference to how it produces its extraordinary results, like highly complex animal organization, mind (behaviour accompanied by subjective awareness), human societies, and science itself.

This means considering human evolution as a continuation of biological evolution, but operating with the aid of quite new (psychosocial or sociocultural) mechanisms, and producing quite novel kinds of result. Until very recently the social anthropologists and psychologists and sociologists have fought shy of thinking in evolutionary terms. So far as I know, the Darwin Centennial at Chicago in 1959 was the first occasion when a number of them were prevailed on to join with their biological colleagues in doing so. In 1960, Sahlins's and Service's symposium on *Evolution and Culture* continued the process. And the paper by Steward and Shimkin in the present volume takes an important further step in this direction. They write, "our treatment of culture and its evolution rests upon nine heuristic concepts which constitute a mixture of hypothetical postulates and real but tentative observations". This is already half-way to the method used by Darwin in regard to biological evolution. What seems to me missing is a central concept, something analogous to Darwin's principle of natural selection, which provided the directive agency in the biological process.

Doubtless we shall in due course be able to formulate the principles of 'psychosocial selection'*. Meanwhile, a biologist may suggest that one essential step towards a general theory of evolution in its psychosocial phase should be to consider actual societies and cultures, existing and historical, from a functional–evolutionary point of view—how do they work, and how did they, their genetic bases, and their psychosocial organs, like science, religion or art, originate historically?

This will bring out the cybernetic character of all evolutionary processes, the share of feedback in their mode of operation. This is becoming increasingly recognized (for example, by Waddington) in biological processes, both ontogenetic and phylogenetic. The recognition of its importance in cultural evolution will invalidate not only all semi-mystical theories, like Spengler's or Toynbee's, but also all those involving a rigid predeterminism, like Marxism.

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* Since writing this, I find that Hoagland himself (*Clark Alumni Univ. Mag.*, No. 264; 1962), following H. A. Murray, has stressed that "ideas may be considered to social evolution what genes are to biological evolution", and that therefore "social environmental selectivity"—in other words, psychosocial selection—operates between competing ideas. See also my introduction to *The Humanist Frame* (Allen and Unwin, 1961)

This sort of approach would make it impossible for social scientists like Prof. Skinner (p. 133) to consider "governmental, religious, economic, educational and therapeutic institutions . . . simply as behavioural technology" aimed at "the control of human behaviour", and to treat their evolution as "part of the history of technology".

Nor, if a truly integrative approach and method is adopted, will it be possible for so many sociologists, psychologists, and even biologists to continue the ostrich-like reductionism which manages to avoid looking the facts of mind and its evolution in the face by sticking its head into the arid sands of behaviourist materialism. 'Mind' and 'matter', the subjective and the objective, are in fact integrated in actual evolving organisms, and the mental or subjective component eventually becomes dominant in man. If I may coin a new term, we need an integrative *eductionism*, which studies processes with reference to their outcome and results instead of only analytically with reference to their origins and component elements.

The next main task of the biological and social sciences will be to study this integration and all its operative implications. Prof. Maslow (p. 162) rightly says that this involves a new philosophy of science, which envisages understanding as its goal rather than control, and relies on intuition and empathy as well as on logical analysis and controlled experiment. In this connexion, it is good to find that distinguished evolutionist, G. G. Simpson, writing (p. 109) "as a man's mind is more than the sum of neuron minds, so the collective mind of mankind is clearly a very different thing from man's individual mind". This 'collective mind' is what Teilhard de Chardin rather loosely called the "nöosphere"; a better term might be 'nöosystem'. We would then find that different grades of cultural evolution were characterized by differently organized nöosystems.

In the biological section of the book, Prof. Muller's plea for an effective eugenic programme is a vivid reminder of the need for man to think seriously about his future destiny before it is too late.

Prof. Crow has made a valuable contribution to human evolutionary genetics. But he continues to use the unfortunate terminology of the population geneticists, who define 'fitness' "in a strictly Darwinian [*sic*] sense", in terms of differential reproductive success. This, I feel sure, is bedevilling the whole Darwinian theory, of evolution by natural selection. I would prophesy that within the next few years evolutionists would distinguish sharply between two kinds of selection, which may be called survivance selection and reproductive selection, the former operating through the differential survival of individual organisms, the latter through their differential fertility. Biological evolution operates mainly by survivance selection. Reproductive selection only comes into play as a major factor in exceptional cases like that of display in polygamous-promiscuous birds. Darwin must have been aware of the distinction between the two types of selection when he set up sexual selection as something essentially different from natural selection.

In man, survivance selection, though operating strongly in early stages of human evolution, is now largely in abeyance. But in man (as R. A. Fisher stressed 32 years ago in his classic *Genetical Theory of Natural Selection*), owing to his unique variability in effective fertility, reproductive selection is playing an increasingly important, and on the whole unfortunate, part. During biological evolution, the survivance type of selection operated to produce a steady improvement of organisms, both singly and collectively. The resultant general trend is not merely one towards adaptation in the usual limited sense, but deserves the title of advance or progress, and can be objectively defined in such terms as more efficient exploitation of environment, greater self-regulation and independence of environmental changes, fuller awareness, more flexible behaviour and greater capacity for profiting from experience.

Improvement and advance are always relative to what Darwin called the conditions of life; these include not only the particular environment of single species, but the global conditions of life as a whole.

A study of evolutionary improvement, both large-scale and long-term as well as small-scale and short-term, would seem to be a necessary prerequisite for a fully fruitful discussion of the subject of this book, *Evolution and Man's Progress*. For example, such a study would make it impossible to discuss eugenics in terms of some supposedly "ideal population" (p. 59): the only tenable ideal for eugenics is one of continuing improvement of the human stock, by raising the level of a number of desirable genetic qualities. It would also make it possible for sociologists and human psychologists to think in truly scientific and adequately comprehensive terms about human evolution, whether cultural or genetic, in the psychosocial phase. Let us hope that Profs. Hoagland and Burhoe will organize a second symposium and discussion on "Man's Improvement". Meanwhile, we are grateful for the valuable results of this first meeting of minds on the subject. JULIAN HUXLEY

TRANSMISSION IN CLOSED WAVEGUIDES

Electromagnetic Waveguides and Cavities

By Prof. Dr. Georg Goubau, in collaboration with Prof. Dr. Richard Honerjäger and Prof. Dr. Rolf Müller. (Physics and Technology, Vol. 5.) Pp. xvii+656. (London and New York: Pergamon Press, 1961.) 100*s*. net.

THIS book is a translation of a work by Prof. Goubau and his former colleagues in the University of Jena, first published in 1955. It covers the work they carried out at Jena prior to 1945, but was not published until 1955 due to difficult post-war conditions. Most of the manuscript was written between 1945 and 1947, but some changes were made at a later date. However, as the work is largely theoretical, the delay between the original work and its present publication in translation does not detract from its merit.

The book deals with the theory of transmission in closed waveguides, of fields in cavities, and coupling to cavities and the theory of networks of elements connected by waveguides. Surface waves and the type of surface waveguide associated with Prof. Goubau's name are not dealt with in this book.

It is unfortunate that while the text has been translated the mathematical symbols have not been changed from the original Gothic type, which is not readily recognized by most English readers, especially when not clearly reproduced. In the text some of the Gothic symbols have been set in Roman type, while others have been left in the original type, which is confusing on a first reading. The figures, also, have not been clearly reproduced.

The book will be of interest to those familiar with basic waveguide theory and concerned with the more advanced aspects of the theory. Throughout the book the general mathematics of the different problems are considered first and then the application of the theory to more practical situations. The book may also be of interest for comparing the separate development of this type of problem in Germany and in Great Britain and the United States during the war years.

The translation of foreign works into English is to be encouraged so that developments abroad are readily accessible to English-speaking readers. It would be unfortunate if the deficiencies in mechanical processes used should lead to a more limited use of these translations than might otherwise occur.

P. A. MATTHEWS

SAFETY IN NUCLEAR ENGINEERING

Technology, Engineering and Safety

Edited by C. M. Nicholls. Vol. 3: Pp. viii + 448 + xii. 105*s*. net. Vol. 4: Pp. viii + 523. 140*s*. net. (Progress in Nuclear Energy Series, Series 4.) (London and New York: Pergamon Press, 1960 and 1961.)

IN reviewing the two latest volumes in the series edited by C. M. Nicholls and comparing the contents with those of Volume 2, published in 1960, one is struck by the considerable reduction in the number of reactor types which are discussed. Thus, the later volumes concentrate predominantly on gas- and water-cooled systems and there is only occasional mention of other coolants. This trend is not unexpected or, indeed, unwelcome, since the large majority of power reactors being developed or constructed are either gas-cooled ('*Magnox*', '*A.G.R.*', '*H.T.R.*') or water-cooled ('*P.W.R.*', '*B.W.R.*' and variants).

Volume 3 is divided into four chapters under the headings of engineering, technology, nuclear safety–criticality and nuclear safety–reactor safety. The 15 articles comprising these chapters are all written by experts in their respective fields and generally reach a high standard. An article on steam as a reactor coolant is of particular interest at the present time. It forms a valuable introduction to the subject and highlights some of the problems to be overcome before successful superheating reactors can be commercially exploited. Rather more than half the book is devoted to various aspects of safety and there are five articles on criticality, some of which make fascinating, if sobering, reading.

Volume 4, the latest in the series, follows the same general layout as its predecessors. There is a chapter under the heading process technology, containing four articles reporting American experience on the gas–solid and liquid–liquid chemical engineering operations involved in uranium processing and the reprocessing of nuclear fuels. Other noteworthy contributions include reviews on various aspects of high-temperature gas-cooled reactor technology. This subject is particularly appropriate at a time when widespread attention is being given to the ultimate development of the gas-cooled reactor, from Calder Hall on one hand, to the *Dragon* Project at Winfrith in Dorset and similar high-temperature reactor projects in the United States and Germany, on the other. Water-cooled reactor technology is covered by an article on the important subject of boiling stability and by an informed discussion on recent developments in zirconium alloy corrosion. There is also an article in the reactor safety section on recent developments in the study of metal–water reactions. This is still an open subject, but the present review goes a long way towards clarifying the issues involved.

The chief criticism of this series is that it attempts to cover too wide a range of subjects; for example, the various aspects of safety could well be the basis of a separate series. With this reservation, the present books constitute a valuable fund of information, well presented and indexed, which should be of special interest to those already having some experience in this field. Some of the articles contain a wealth of references (one in Volume 4 has 241 and another 136) which the reader will find of considerable assistance in following up subjects in which he has a particular interest.

D. ENGLISH

INFORMATION THEORY

Information Theory

Papers read at a Symposium on Information Theory held at the Royal Institution, London, August 29th to September 2nd, 1960. Edited by Colin Cherry. Pp. xi + 476. (London: Butterworth and Co. (Publishers), Ltd., 1961.) 95*s*.

THIS volume contains the thirty-six papers and discussion presented at the fourth London Symposium on Information Theory. The expositions extend beyond pure information theory and are classified under the headings of: coding and detection theory and statistical theory; telecommunication systems; human reaction to information; sensory information and biological models; learning mechanisms and other artefacts; classification theory, syntactics and semantics. The papers were presented by active research workers, and the subjects range from mathematics (for example, the ten papers under the first two headings) to experimental psychology (such as most of the eleven papers under the third and fourth headings).

A general comment on the content of the symposium may be of interest. The former, fairly well defined, boundaries of such traditional subjects as applied mathematics, psychology, physiology and information handling (documentation, bibliography and general library procedures) are rapidly being swept away, and concepts and methods of one discipline are making deep inroads into neighbouring territory. This process is nowhere illustrated better than in the present volume on information theory. The successful research workers in this field will, in future, find it necessary to be able to speak with the psychologist, physiologist and librarian on his own territory, and this will particularly apply to the applied mathematician who sets out to contribute to one or more aspects of the subject. This, it is clear, is likely to have a profound effect on the structure and content of undergraduate courses in the universities. It will not be enough for the applied mathematician to learn only about the applicable parts of pure mathematics and their use in physics and engineering; nor will the psychologist and physiologist be able to forego, somewhere in his upbringing, an introduction to the ideas of probability, statistics, classification theory (in a mathematical sense, such as expounded in the papers by Golomb and by Rescigno and Maccacaro), and the experimental techniques of the physical sciences. For the librarian, interested in the development of new methods for information handling (storage, retrieval, classification and so on) rather than in the operation of an existing system, the position is even more difficult. He will need a training embracing a much wider field than that covered in the existing courses in librarianship.

It is evident that we are in the middle of a scientific revolution, concerned not so much with content in the various fields but with the very strong interactions which are developing between these fields. This, it is safe to forecast, will mean that the next few decades will produce scientists having a very different orientation from those of recent decades. To anyone concerned with these developments the papers of the fourth Symposium may be recommended as indicating in a vivid way some of the problems which will have to be faced in drawing up the new structure. L. S. GODDARD

VEGETATION OF THE SCOTTISH MOUNTAINS

Plant Communities of the Scottish Highlands

A study of Scottish Mountain, Moorland and Forest Vegetation. By Dr. Donald N. McVean and Dr. Derek A. Ratcliffe. (Monographs of the Nature Conservancy, No. 1). Pp. xiii+445+26 plates. Over 60 Tables. (London: H.M. Stationery Office, 1962.) 77*s*. 6*d*. net.

THIS is the first of the monographs to be produced by the Nature Conservancy, and its aim is to describe and classify the vegetation of the Scottish mountains. Previous works, limited to regional studies of Highland vegetation, have been made by ecologists since 1900 onwards, but the aim of the authors is to extend the surveyed areas and provide a wider outlook. It does not claim, however, to be complete, and large areas have still to be surveyed. The authors were faced with the problem of arrangement, whether to base the contents of the monograph on an ecological or a systematic system or on the life-forms of the dominant species. They have chosen rightly the third method of approach as the main basis for classification. It is similar in effect to one based on altitudinal zonation. A standard treatment is adopted for each community or type of the vegetation and full analysis of the floristic composition of each is given in sixty tables. The geographical distribution, habitats, relationship to other communities, and very full information about soils and other environmental features are provided in the tables. There are also very useful distribution maps of the various types of communities and large folding maps of the present distribution of the oak, pine and birchwoods and their distribution during this climatic period.

The methods of collecting and arranging data are more or less those used by Poore and Dahl. It became clear to the authors as the investigation proceeded that the various types of vegetation found on the Scottish mountains form a continuous series, that there may in places be sharp discontinuities between types, but that in wider areas a complete series of intermediates can usually be found. It was therefore necessary to make a selection of a sufficient number of points in this continuous series as reference units which would adequately give an idea of the whole range. The term *nodum*, coined by Poore for reference points in a continuous series, is therefore used, and the names of associations and noda have been based on the names of their most prominent or dominant constituents. Tables 1 and 2 give a list of noda with their characteristic constants and dominants.

After the introduction and description of methods used in the survey follow chapters dealing with the vegetation from the forest up through the dwarf shrub heath, grass heath, herb and fern meadows to the moss heaths of the higher altitudes. There are chapters on bogs, mires, springs and flushes.

A comparison is made with Scandinavian vegetation, and close similarities are evident between the Scottish noda and their equivalents in Norway. The authors conclude that Scottish mountain vegetation must be regarded as an oceanic extension of that of north-west Europe. The similarity in climate and flora results in the same altitudinal zonation and appearance of the vegetation in Scotland and Norway. The differences that exist must be due to greater exposure to Atlantic gales, higher summer and winter temperatures and more extensive grazing by farm animals in Scotland. There is a very strong similarity between the plant communities round springs and in flushes where there is little or no grazing and where the controlling factor, wetness of soil, is largely independent of climate and altitude. The effects of climate on the vegetation, particularly those due to snow cover and solifluction, are carefully considered.

More than half the book consists of tables, including sixty-four lists of species occurring in the selected noda, and the geographical distribution of these noda in the localities surveyed. There are also thirty-four vegetation distribution maps and numerous tables to indicate the distribution of vegetation and soil types in the principal areas. There are twenty-six photographs illustrating some of the characteristic facies of the vegetation, a usefu bibliography and excellent idex.

The Conservancy and the authors are to be congratulated on producing a book, well written and excellently illustrated, which gives not only a wealth of useful information, but also will undoubtedly stimulate further research on Scottish vegetation It will be of value not only to ecologists, who will be able in the future to detect changes if they occur in the vegetation as the result of succession or change of climate, but also to those interested in the preservation of Nature or who are concerned with the utilization of Scotland's natural resources. Since 195 the investigations have been in progress, and the authors have covered a wide area of the Highland in all weathers and made profuse notes in the field from which these printed records are compiled Nothing like this has been hitherto accomplished in the British Isles. It approaches the type of survey for which the ecologists and plant geographers o Montpellier and Toulouse are well known, and should be made available to all libraries and botanica departments.

The planning and printing of the book and quality of the tables, maps and illustrations, leave little t be desired. JOHN WALTON

STUDYING THE EVER-CHANGING SEA

By Sir ALISTER HARDY, F.R.S.

The following is an abbreviated version of only part of the Evening Discourse given at the British Association meeting at Manchester on September 8. Its title there was "Man and the Beneficent Sea". I am here using a different heading which is more appropriate for the piece I have selected for publication in *Nature*: that mainly describing some new and recent work of the Edinburgh Oceanographic Laboratory. The first section of my lecture dealt in general terms with the natural economy of the sea and my conviction that man will have to get more from it in the future to feed his increasing populations. After emphasizing the differences between the basic plant production on the land and in the sea, and its effect on animal life, I referred to the possible future use in the sea of various methods akin to farming; these I had previously discussed at a symposium in Section D (Zoology) of the Association's meeting at Cardiff two years ago when they were also reported in *Nature* (188, 91, 1960). The entire lecture will be published in *The Advancement of Science*.

THERE will no doubt be many new ways in which we shall be able to increase the harvest of the sea when we come to have a greater knowledge of it. The scientists of the different Government laboratories in Europe have already built up an enormous body of information about the lives of our fish and their environment: a knowledge won from the sea under great difficulties. Linked with their work is that of the independent laboratories such as those of Plymouth and Millport, and the National Institute of Oceanography in Great Britain, where research work into the more fundamental background conditions are undertaken. While all these laboratories have done wonders with the resources available, the resources are not enough. The ocean is vast: it is a hidden world, and its exploration is expensive. Compared with what we are spending on physical, or on agricultural research, we are not yet, I believe, putting an adequate proportion of our available revenue into the work which will, if sufficiently supported, yield so much more of the sea's beneficence for the future of mankind.

I have just mentioned the difficulty of the ocean being a hidden world; there is another great difference between the sea and the land that I particularly want to emphasize: the fact that it is always on the move. The sea is a dynamic system; it is useless to think of it as we think of the terrestrial world. While the sea-bed is fixed, the planktonic pastures which nourish its life are for ever changing; conditions in one year may be very different from those of last year or the year before that. If these changes affect the life of the sea-bed, as surely they must, still more will they influence fish like the herring which feed directly on the plankton; moreover, all the bottom-living fish spend a vital part of their young lives up in the plankton which may be favourable for them in one generation but not in the next.

For many years now I have been urging the need for studying the changes in the ocean in the same sort of way as we study those of the atmosphere. A great deal of my time before the War was spent, when a professor at Hull, in developing a method of recording planktonic changes over wide areas. I shall describe here some of the results now coming from the work I started just thirty years ago; they are those obtained by the splendid band of enthusiasts who have carried on the work since I left off. They have now extended the survey of monthly observations right across the Atlantic to America and northwards to the Arctic Circle. It is their achievement, and not mine, that I want to discuss; I should, however, briefly sketch its beginnings.

The continuous plankton recorder, on which this survey is based, has frequently been described; I need only indicate its nature. Somewhat torpedo-shaped and fitted with diving planes, it will swim below the surface at a constant depth when towed behind a ship; as it goes along it continually samples and preserves the plankton on a long gauze banding wound on to a spool by a propeller turned by its passage through the water. For every mile of sea traversed a small section of the gauze winds on; at the end of a run, of perhaps 400 miles, the spool is taken out, unwound below a microscope and the plankton estimated. The first machine was used on the 1925–27 *Discovery* Expedition to the Antarctic; on my return from this voyage and appointment to Hull, I modified it for use on commercial ships crossing the North Sea. With the kind co-operation of a number of shipping lines we first ran from Hull to a number of Continental ports and so covered the southern North Sea with a network of lines of observation; monthly charts of the main changes in the plankton began to emerge. In 1937 H.M. Treasury, on the advice of the Development Commission, increased its support to enable us to open another laboratory at Edinburgh to run similar lines from Leith to cover the northern part of the North Sea; we also began a line into the Atlantic towards Iceland.

The North Sea is affected by an influx of oceanic water which rounds the north of Scotland to pass down the east coast of Britain and by a lesser flow up the English Channel through the Straits of Dover; the force of these may vary considerably. The spread of certain plankton organisms characteristic of these waters can be used to indicate their relative strength and extent. From our monthly charts we could see areas of plant and animal plankton change their positions, wax and wane with the passage of time; as the years came to be compared we could see striking differences between them. An examination of the changing plankton is indeed very like charting the weather; we are studying moving centres, not of high and low pressure, but of high and low plankton production, and of many different kinds. As in meteorology we can only understand these changes by having many observations made over a wide area at the same time, and repeated at intervals; fortunately the waters move much more slowly than the atmospheric disturbances.

The War brought a temporary halt to the activities; but when peace came, the team came together again, first under Dr. C. E. Lucas, who had been with me from the beginning; I had now become Linacre professor at Oxford. Dr. Lucas then became director of the Scottish Fishery Laboratory at Aberdeen and was followed by Dr. K. M. Rae until he took a professorship in the United States in 1957; now Mr. Roland Glover is in charge of the enterprise. The Edinburgh Laboratory, much enlarged as the Oceanographic Laboratory under the ægis of the Scottish Marine Biological Association, has now

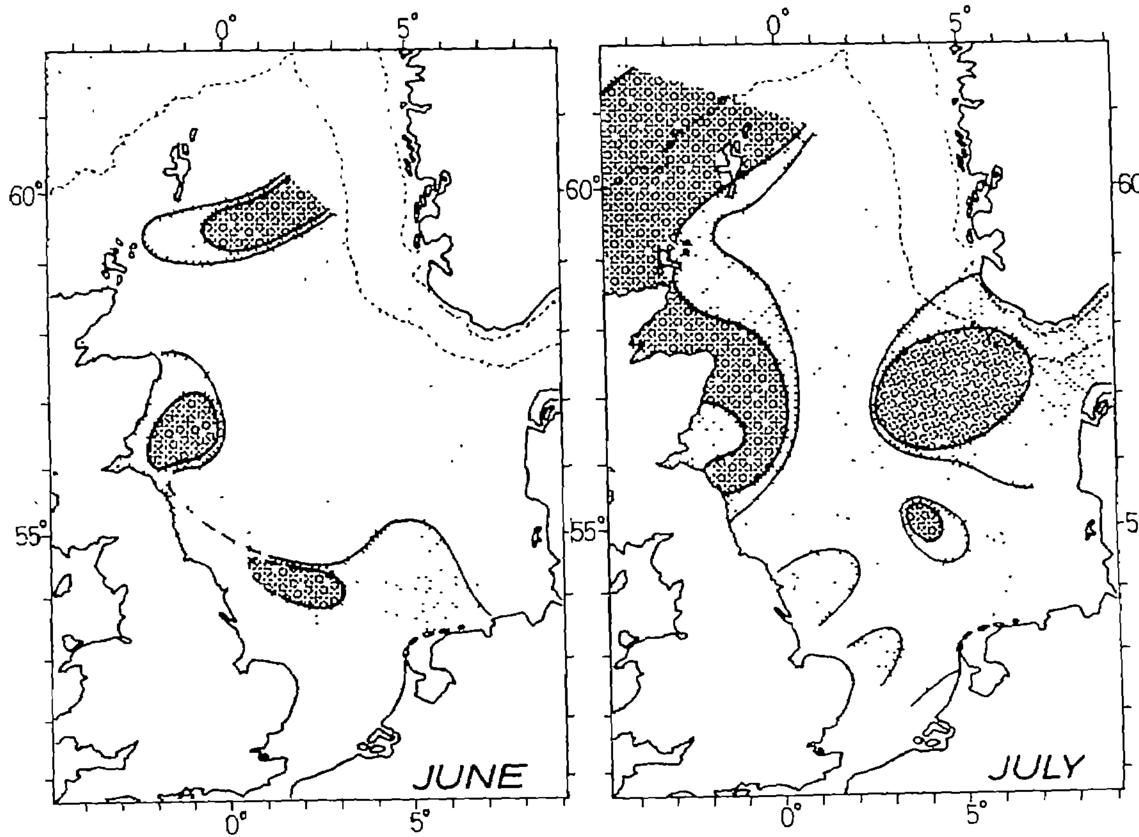


Fig. 1. Charts showing how the distribution of the total phytoplankton in the North Sea has diverged from a 18-year average during June and July 1961. The heaviest and lightest shadings represent, respectively, values of more than 150 and less than 50 per cent of the norm for the month

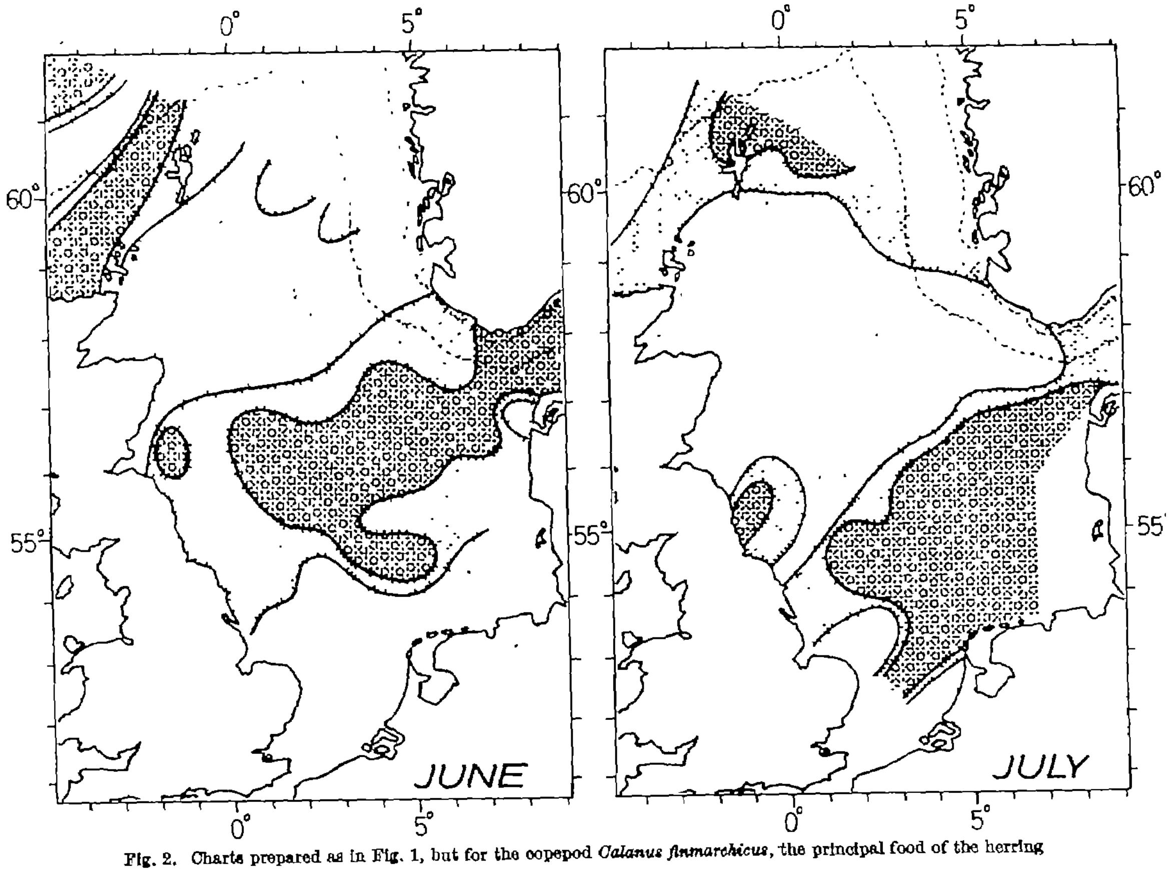
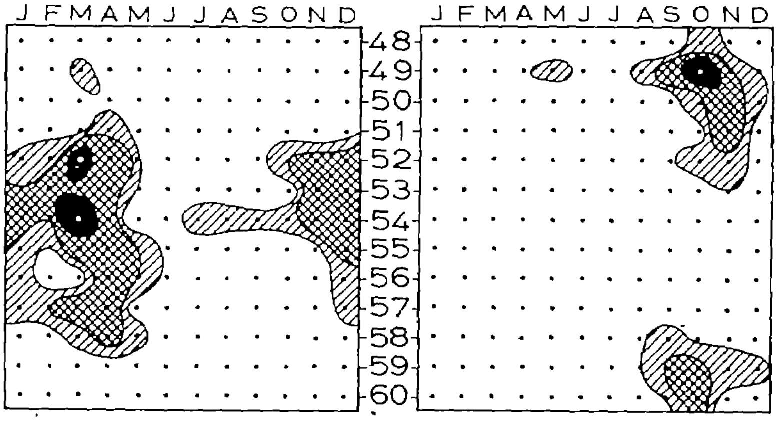


Fig. 2. Charts prepared as in Fig. 1, but for the copepod *Calanus finmarchicus*, the principal food of the herring

become the headquarters of the survey. It is some of the work of these leaders who followed me, and of their staff, that I want to present.



Thalassiothrix longissima

Corycaeus anglicus

Fig. 3. Diagrams showing the varying degrees of abundance of two plankton organisms, the diatom *Thalassiothrix longissima* and the copepod *Corycaeus anglicus*, in the North Sea over the months and years of 1948–60

After the War the work gained the valuable addition of lines into the Atlantic and to the north run by Norwegian, Dutch and British weather ships on their way out to and from their stations. This greatly increased the significance of the work, for it had long been recognized that the conditions in the North Sea must to a large extent be determined by still earlier events in the western ocean, that is, farther back in the track of the North Atlantic current before it rounds the north of Scotland. Quite recently the Edinburgh Laboratory published[1] its first *Atlas* of this area showing the distribution of no less than 80 of the more important plankton species based on the average of all the month's results for the past ten years. The charts represent the 'normal' distribution for the organisms concerned; they form a basis to which patterns in different years may be compared to see how at any one time the particular organism may have departed from its 'norm'. As the work proceeds and the 'norm' becomes based on a longer series of years, it will increase in reliability; the period is now fourteen years.

The charts in Figs. 1 and 2 show, as examples, such divergences demonstrated in the North Sea for the total phytoplankton and the copepod *Calanus*, the most important food of the herring, for June and July of last year (1961). They are prepared in the following way. The North Sea is divided into a grid of squares, and for each important organism a set of 13 such grids is made, one for each month of the year; each is then filled in to show, square by square, the 12-year average quantity of the organism in question for the particular month concerned. Thus month by month we can see how far the occurrence of each organism is diverging from its norm; the values are now filled in in the squares on a chart for each month as percentages of the norms, and so maps of such deviations can be produced. In the examples shown the contour lines are drawn at levels of 75 and 150 per cent of the normal; the heaviest shading represents quantities over 150 per cent, the medium shading those between 75 and 150 per cent (that is, near the normal) and the lightest shading indicates values below 75 per cent.

The teams at the Edinburgh Laboratory have over the years given us many interesting correlations of events in the plankton; they are now providing the material for quite a new understanding of the sea. Their long and patient work is now being rewarded with a splendid achievement. They now have the means of showing how any one area in any one month departs from the long-term mean; the charts reproduced are just one example of how such data can be used. It is the first time these have been published. I think I am not exaggerating when I say that I feel their gaining the ability to do this marks a really important break-through in the means of investigating this changing hidden world. They are now beginning to have their hands on the pulse of the sea, the flowing changing streams of the plankton which are the link between the inorganic world and the fortunes of the fisheries. A much longer time, of course, must elapse before the mean is based on a sufficient number of years to enable one to think of predicting the effect of such changes on a fishery; but progress in that direction is well under way. By the use of computers, the Edinburgh oceanographers are now treating a large number of different species to give us an idea of how the whole plankton community is changing with its environment.

They can also express the yearly differences in plankton occurrence in a number of ways. Fig. 3 gives an example of one such method; here we see diagrams showing the relative abundance of two organisms in a large area of the North Sea ranging over the months from left to right (January–December) in each year as we pass down the diagram from top to bottom (1948–60). Each value used represents the average for the whole area for the month concerned; contours of the changing values with time are then drawn. As experience grows we shall learn more of what these patterns mean in relation to changing hydrology on one hand and fluctuations in the fisheries on the other.

Now a further phase of expansion is just beginning. In recent years, since 1959, they have pushed farther westward with an experimental line from Iceland to Newfoundland on a ship sailing between Reykjavik and New York. This has shown a much richer plankton in the area to the south of Greenland than had been expected and the presence of a great many of the young of the fish *Sebastes marinus* (the red fish or Norway haddock), which indicates the presence of a great stock of this species waiting to be fished as soon as a satisfactory method of mid-water trawling can be devised. The results from this line have created much interest in North America where the authorities have encouraged an extension of the survey in the western Atlantic, particularly in relation to their fisheries in what is known as the 'ICNAF' area (International Commission for the North-west Atlantic Fisheries). The Edinburgh Laboratory has been awarded a contract by the U.S. Office of Naval Research which, together with increased financial support from the British Treasury, will permit the establishment of a trial survey linking the European and American continents. Last year (July 1961) the first new route was started with this international

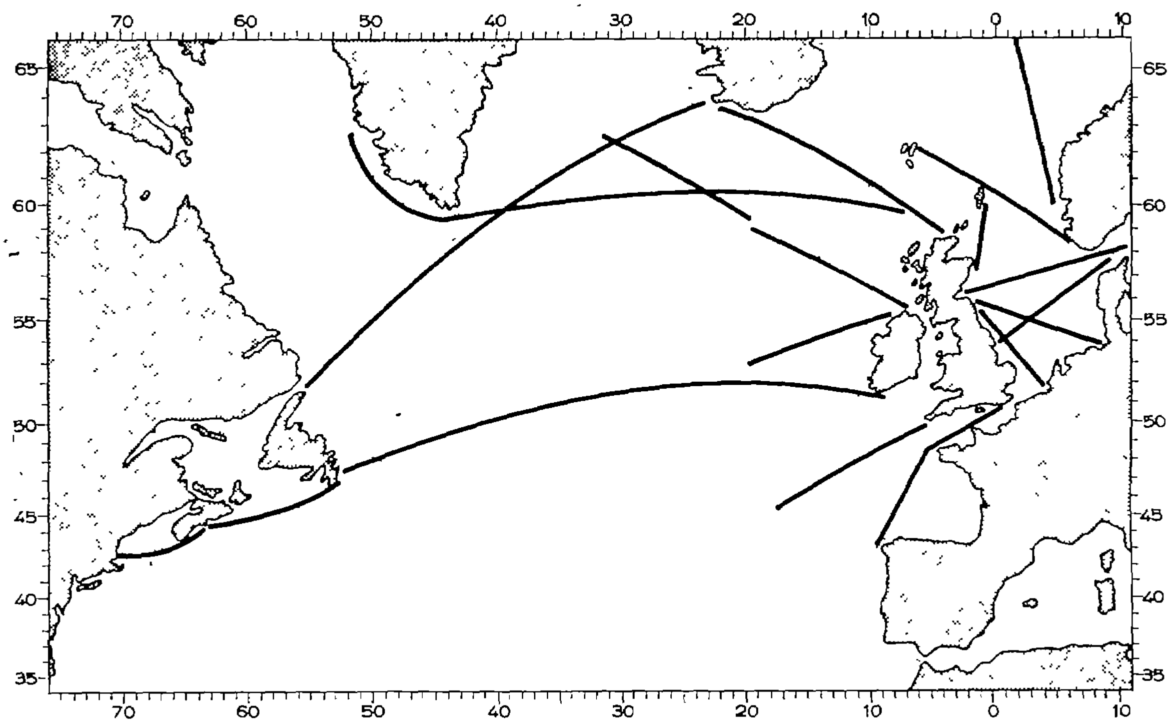


Fig. 4. A map of the monthly plankton recorder routes covered by the Edinburgh Oceanographic Laboratory in its survey during 1962

support; it runs across the ocean between Liverpool and Newfoundland and then down the continental shelf to Halifax (Nova Scotia) and Boston (Massachusetts). In Boston, American scientists kindly help to service the gear aboard the ship. Another new route was started early this year (1962) with the help of the Royal Greenland Department of Trade; this is sampling between Scotland and Greenland. The latest map of the survey is shown in Fig. 4. All the recorder samples from this wide survey are being returned to the Edinburgh Laboratory, where the staff is being augmented by additional members, some of whom are coming from the United States to get training in the methods which have been standard practice in Edinburgh since the survey was resumed after the War. I would like to stress the international nature of this work; there are 25 ships equipped to tow recorders from Denmark, the Faeroes, Germany, Holland, Iceland, Norway and Great Britain.

It is just thirty years since the present type of recorder was designed. It has only been improved in minor details because any major changes would have destroyed the basis of standardized sampling over a long period. However, there is a limitation to the method which we believe can be overcome in a new machine. The recorder on the survey is run at one standard depth: 10 metres below the surface. The need for sampling over a range of depth is accentuated by the fact that a number of plankton animals carry out a diurnal vertical migration; on this account the distribution of some species can only be considered from records taken at night, and for others the method is at present inapplicable. It is hoped in the future to modify the recorder so that it describes an undulating path as it is towed, rising and falling in every mile to cover the vertical range of the more important species; the technical difficulties, however, have not yet been fully solved, although we feel confident that they will be in time. Nevertheless, in spite of this limitation, it is clear, I think, from the results obtained that a survey run at a single depth can yield significant results. Over many years the average number of fish-eggs taken by the recorders in the southern North Sea give a graph which corresponds closely with that for the average yearly landings of trawl-caught fish in the same area; the two curves rise and fall together. It is, of course, what we should expect—more fish, more eggs—fewer fish, fewer eggs; yet to see the correlation come out of the survey gives confidence in its reliability. Again there has been a remarkable correspondence in the North Sea between the average quantities of the copepod *Calanus*, the principal food of the herring, and the annual variations in the growth of the three-year-old herring coming into the shoals as reported by the Lowestoft Fisheries Laboratory.

The object, in my lecture, was to discuss how we might make more of the beneficence of the sea than we are at present doing. Earlier (as explained in the introductory remarks) I had instanced a few ways in which we might be able to increase the yield of the fisheries; they would only be the small beginnings of a far greater use of these resources which must come when we have a fuller knowledge of this hidden part of the world. I have tried to show how we can only hope to gain a proper knowledge of it if we recognize that it is a constantly moving world and treat it as such. The biologists and the physical oceanographers are now ready to come together to give us one dynamic picture of its month-by-month changes. Only when this is done and the environment is studied as a changing physico-biological whole will man fully inherit the Earth by understanding its better half.

[1] *Bull. Mar. Ecol.*, 5, 65 (1961)

A MOLECULAR MODEL FOR BIOLOGICAL CONTRACTILITY: IMPLICATIONS IN CHROMOSOME STRUCTURE AND FUNCTION

By DR. ARTHUR COLE

Physics Department, University of Texas, M.D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston 25

JACOBSON[1] has suggested that the quasi-crystalline state of water may be a major factor in the 'structuring' of macromolecules. A helical molecule fits ideally into the water lattice if pitch-lengths and diameters are multiples of the water lattice dimension of 4·75 Å. The displacement of the water in the lattice exposes polar water groups which can bind to polar groups of the macromolecule and, therefore, stabilize the helix. Jacobson suggested that these principles seem to apply to the structure of the (double) helical deoxyribonucleic acid (DNA) molecule. Diffraction data[2,3] give a pitch-length of 33·6 Å for DNA, which is about equal to seven water-cell layers (33·2 Å). The pitch-length of 28·1 Å for DNA in the dehydrated *A* form is about equal to six water layers (28·4 Å). The diameter of 19·5 Å and the packing distance of 18·8 Å for parallel aligned molecules in less than 60 per cent relative humidity corresponds to about four water layers (19·0 Å). At 92 per cent relative humidity, the packing distance increases to about 23·5 Å (which corresponds to the incorporation of one water layer between molecules), while at higher humidities the molecules tend to dissolve out (separate) into the water.

Jacobson's[1] suggestion provides a basis for a possible contractile mechanism. If a hydrated molecular helix associates co-linearly with a fibrous molecule by ionic bonding, charged groups along the associated side will be neutralized and binding to water weakened. If one or more water layers per helical turn is lost from the affected side, a secondary coiling (Fig. 1) will result. The outer diameter, *D*, of the secondary coil will be:

$$D = \frac{2d}{1 - \frac{L - 4{\cdot}75\,n}{L}} \qquad (1)$$

where d is the diameter, L is the pitch-length of the primary helix, and n is the average number of water layers per turn displaced from the neutralized side (dimensions in Å).

This secondary coiling, which represents a distortion or change of the initial state, would be stabilized by the ionic binding energies and the fit of the larger helix into the water lattice structure. Further associations may be inhibited because of the necessity of bending the fibrous molecule to conform to the secondary coil configuration before extensive coupling can be achieved. Water bound between turns of the secondary coil would tend to set the pitch-length.

Advanced (tertiary or quaternary) coiling might be formed by successive associations with larger protein fibres. The formation and dissolution of such secondary and advanced coilings would be sensitive to agents such as monovalent and polyvalent ions, dehydrating agents, heat, etc., which affect both the associability and the water binding of molecules. Small ionic changes could lead to coiling ⇄ uncoiling transitions, if an initial concentration were close to a critical (equilibrium) value.

Co-linear associations also might be formed if an initial coupling between a relatively small monomer and a helical structure is followed by a linear polymerization of the small units along one side of the helix. Polymerization along the other side of the primary helix must be inhibited if the association is to lead to differential dehydration and advanced coiling.

The formation of a secondary coil can result in two kinds of contractile movement, depending on strand-length. Association of relatively short strands would produce a bending motion, while association of long strands would result in a large linear contraction of ten-fold to twenty-fold resulting from the multiple coiling (Fig. 2).

Implications in Chromosome Structure and Function

The helical structure of DNA[4] and the coiled mitotic chromosome[5] are generally recognized. Many suggestions for intermediate chromosome structure have been made; for example, the branched structure suggested by Taylor[6], the multi-stranded structure by Steffensen[7] and Kaufman and De[8], the side-chain structure by Schwartz[9,10], and the coiled coil structure of Inoué and Sato[11]. A structure presented here is based on a successive series of helical condensations of a linear thread composed of DNA segments covalently joined by short segments of either protein

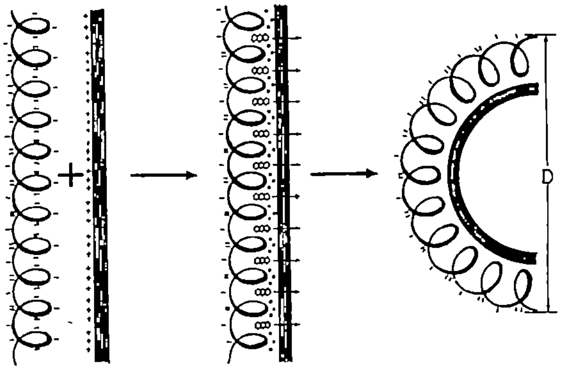


Fig. 1. Association of a hydrated helical molecule with a fibrous molecule. One-sided neutralization leads to a differential loss of water and secondary coiling

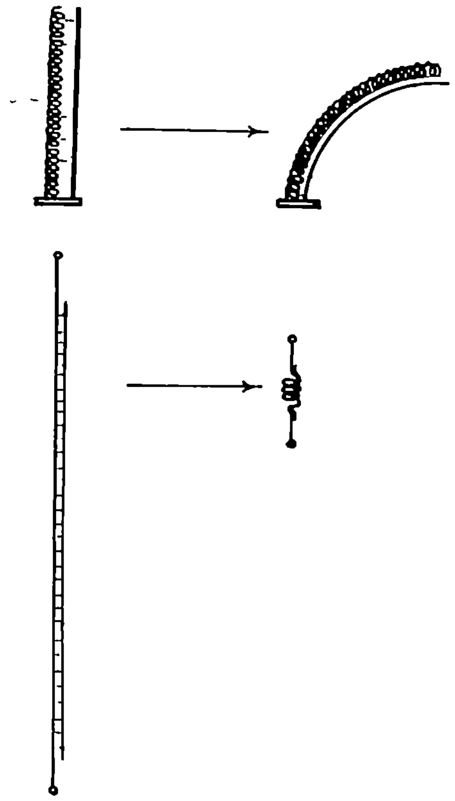


Fig. 2. Association of short strands leads to bending (top) Association of long strands produces large linear contractions because of multiple coiling

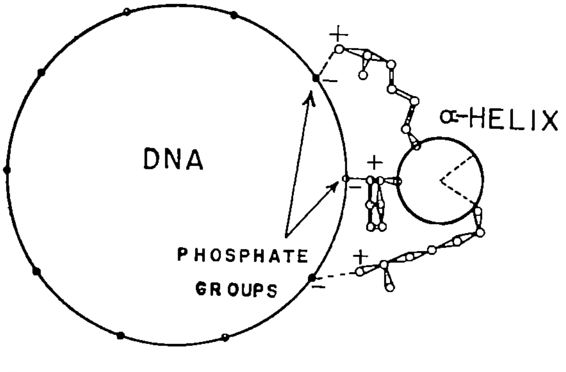


Fig. 3. Possible co-linear relationship between DNA and an α-helix. Arginine residues of the histone can couple to adjacent phosphate groups

or specialized DNA. The coilings are assumed to result from associations of DNA with histone protein.

Histones. Protein extracted from chromosomes by high ionic strength solutions generally consist of 2 or 3 (or 6) fractions[12-14]. For example, Phillips and Johns[13] show that a first, very small fraction is eluted from a carboxymethylcellulose column with 0·005 N hydrochloric acid; a second large fraction (containing about 2/3 of the protein), eluted by 0·01 N hydrochloric acid, is rich in lysine; and a third fraction, eluted by 0·02 N hydrochloric acid, is rich in arginine.

The histone mass is somewhat greater than or equal to the DNA mass[14] and probably couples to the DNA by ionic linkages to the phosphate groups[15]. The α-helix occurs in the protein of natural nucleoprotein[16] and synthetic nucleoprotein[17]. Stereochemical relations between histone and DNA have not been determined, although various co-helical relationships have been proposed[3,15,18,19].

It seems unlikely that the different histone fractions perform the same function, since they are of unequal amount and have different amino-acid contents, end groups, and elution (or associability) characteristics. It is suggested here that the arginine-rich fraction (defined here as histone *A*), equal to about 1/3 the DNA mass, is most closely coupled to DNA, and that the lysine rich fraction (defined as histone *B*) equal to about 2/3 the DNA mass, is less closely associated, while another fraction (histone *C*) is weakly associated.

Helical condensations. A co-linear relationship between an α-helical protein and DNA involves no initial distortion of the structures and provides for the coupling of basic protein residues to about 30 per cent of the DNA phosphate groups (Fig. 3). The protein mass corresponds to 31 per cent of the DNA mass. This association would produce a secondary coiling of 138 Å diameter (which also 'fits' into 29·0 water-layer units) if two water layers were differentially displaced from the DNA helix.

From work on the side-packing of DNA[2,3] it is likely that such a secondary coil could incorporate one or several water layers between turns because of the intermediate phosphate neutralization existing in this region. If a larger fibrous protein, histone *B*, were then to associate co-linearly with the secondary helix (fibril), and lead to the loss of one interturn water layer from the associated side, a 1400 Å diameter tertiary helix (chromanema) would result (Fig. 4A).

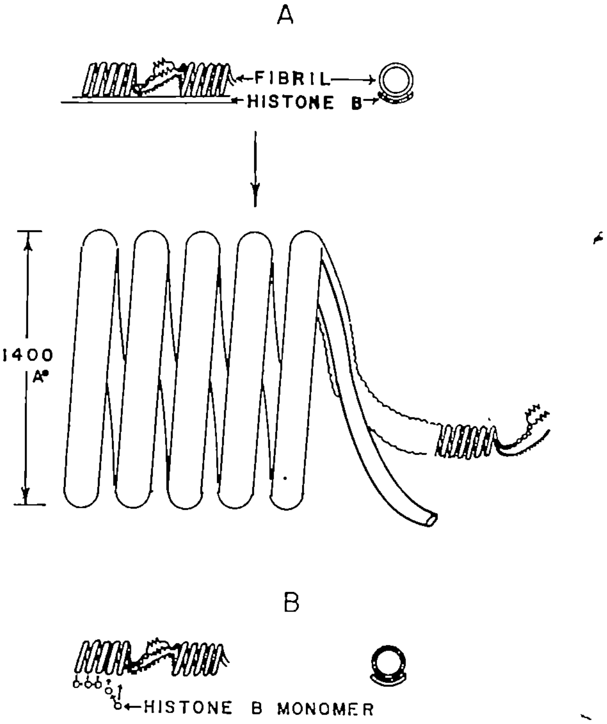


Fig. 4. A, associations of a DNA fibril with histone *B*, leading to a tertiary coiling (chromanema of 1400 Å diameter; B, alternative scheme for co-linear coupling, between a fibril and histone by means of a linear polymerization of histone monomers (40,000 mol. wt.) along one side of coil

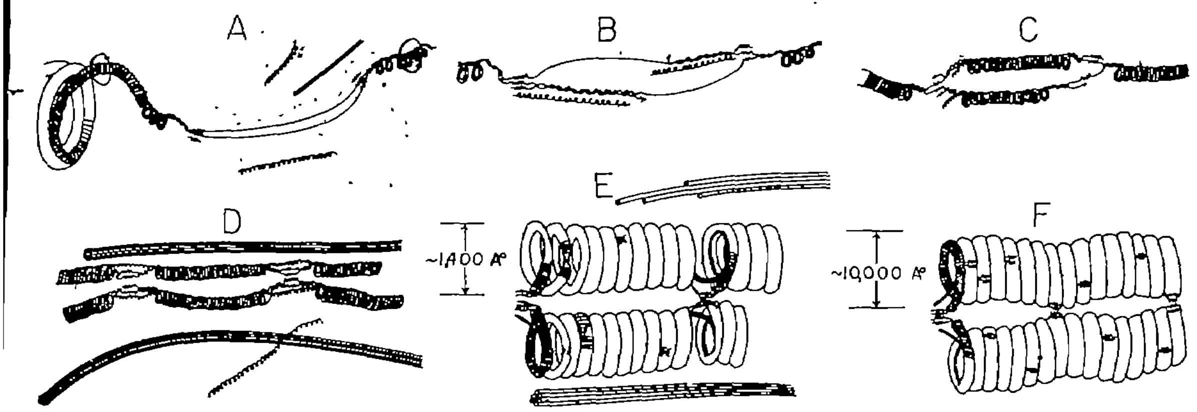


Fig 5. DNA replication scheme. Uncoiled segment (A) is replicated in opposite directions (B), and associates with histone *A* and coils into two fibrils (C) Adjacent segments are replicated (D). Association with histone *B* leads to chromanema (E). During mitosis, sister chromanema associate with histone *C* and condense into sister chromatids (F) (reduced scale)

It is estimated that in such a configuration, only about 15 per cent of the phosphate groups could be neutralized by a lysine-rich histone with a weight per length comparable with the fibril. Thus, the coupling of histone *B* would be considerably weaker than that of histone *A*.

An alternative possibility is that co-linear relationships are formed by a linear polymerization of histone monomers along one side of a helix. A histone monomer of 40,000 molecular weight[13] corresponds in size to a 20 × 200 Å rod (or a 50 Å sphere), which would conveniently fit along the fibril and equal about 50 per cent of the DNA mass (Fig. 4B).

The number of water layers bound between turns of the chromanema may be high because of the many charged phosphate groups in this region. If one interturn water layer is differentially removed following a co-linear association of histone *C* with the chromanema, the quaternary coil (chromatid) would be about 8μ in diameter. A more reasonable chromatid dimension of 1μ would require a differential removal of 11 water layers per turn. Histone *C* could form only weak associations with DNA because of the relatively smaller number of ionic bonds available when coupling large structures, and would exist as nucleoprotein only in the mitotic chromosome.

Although ambiguities arise in interpreting electron micrographs of chromosome structure, helical structures of the dimensions predicted for the secondary and tertiary coilings have been reported, and appear to be present in electron micrographs by Ris[5,20], Moses[21], Mirsky and Osawa[22], Kaufman and De[8], Yasuzumi and Ishida[23] and others.

Approximately 0·1 × 10^{-12} g of DNA is contained in a mammalian mitotic chromosome about 1μ × 5μ in size. A chromosome of this size formed by the suggested primary through quaternary coilings would contain 0·133 × 10^{-12} g of DNA and is, therefore, comparably condensed.

Replication. DNA replication probably involves an opening of the double helix into the two strands which then act as templates for the synthesis of new complementary strands[24]. Synthesis probably occurs linearly in one polarity along each DNA strand. Since complementary DNA strands have opposite polarity, synthesis must progress in opposite directions along complementary strands.

DNA synthesis occurs in interphase and lasts an appreciable fraction of the growth-cycle, that is, 7–10 h in mammalian cells[25,26]. Work on radiation-induced chromosome damage[27,28] indicates that two 'sub-units' of the chromosome tend to separate at the beginning of the DNA synthesis period. It may be that during the DNA synthesis period, a local change in ionic concentration in the region of the chromosome leads to a DNA dissociation with histone *B*, a weaker association with histone *A*, and a weakening of the base pair hydrogen bonding in the DNA molecule.

A general replication scheme is outlined in Fig. 5. Synthesis is initiated at specialized (link) regions of an uncoiled intermediate segment (Fig. 5A), and proceeds in opposite directions along the two strands (Fig. 5B). The new DNA associates with histone *A* and coils into two fibrils (Fig. 5C). Adjacent segments are similarly replicated (Fig. 5D) and then associate with histone *B* and coil into a chromanemal winding (Fig. 5E). During mitosis, the sister chromanema associate with histone *C* and condense into sister chromatids (Fig. 5F).

It is assumed that the short links (possibly protein), unlike helical DNA, do not form advanced coilings and maintain loose (ionic) attachments between sister chromosomes throughout the entire replication process. Although many points of attachment may be broken during the condensation sequence some attachments will remain. Thus, the model provides for chromosomal homology at every biological level, from the level of DNA complementary pairing to that of the mitotic chromosome.

Replication requires unwinding the DNA helix and, therefore, rotation of the chromosome. It is estimated that the relational rotation-rate of a mammalian chromosome replicating in this way would be about 100 turns per sec. Similar estimates for diverse materials such as *T*2 phage, *Escherichia coli* cells, yeast cells, and *Paramecia* yield a comparable rotation-rate; that all these materials synthesize DNA at a rate in the order of 10^{-14} g/h/chromosome.

This large mechanical activity represents a substantial objection to the model. It is known, however, that nuclei of living cells are engaged in much random motion. The stirring action could enhance the distribution of substrates.

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CHEMISTRY OF MUSCLE CONTRACTION

ADENOSINE TRIPHOSPHATE AND PHOSPHORYLCREATINE AS ENERGY SUPPLIES FOR SINGLE CONTRACTIONS OF WORKING MUSCLE

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CONTRACTING muscle is one of the most fascinating 'machines' in Nature, but, despite an enormous effort, it has so far not been possible to find the immediate source of energy which allows this mechano-chemical system to turn chemical free energy into external work. Following the work of Fletcher and Hopkins[1] in 1907, Parnas and Wagner[2] in 1914, Meyerhof[3] in 1920, it was generally accepted that the energy for muscle contraction came from the breakdown of glycogen to lactic acid. However, in 1930, Lundsgaard[4] showed that iodoacetate-treated muscles could contract many times without producing lactic acid. During these contractions, phosphorylcreatine (PCr) was found to break down and thus appeared to be the source of energy. Lohmann[5], in 1934, found that the co-factor for the breakdown of PCr in dialysed cell-free muscle extracts was adenosine diphosphate (ADP). Thus, it was generally accepted that the dephosphorylation of adenosine triphosphate (ATP) to produce ADP and inorganic phosphate was the initial reaction for the provision of energy for contraction in spite of the fact that the content of ATP remained unchanged except in extreme fatigue. This view was supported by experiments with isolated enzymes, muscle protein solutions, fibrils made from reconstituted actomyosin, and glycerinated muscle models which strongly suggest that ATP is in fact the immediate source of energy for muscle contraction[6].

However, in 1954, Fleckenstein, Janke, Davies and Krebs[7] published experiments showing that frog rectus abdominis muscles could contract and do work at 0° C without measurable net change of energetically equivalent quantities of ATP, ADP or PCr (that is, about 0·5 µmole/g at 50 per cent efficiency), while Mommaerts[8] reached similar conclusions on the basis of experiments with turtle leg muscles at 0° C in which measurements were made of ADP, adenosine monophosphate (AMP), creatine, creatinine and pyruvate during contraction and relaxation. Since then, many other phosphorus compounds have been tested and found not to undergo sufficient net changes during contraction. These include carnosine di- and mono-phosphates[9], all the inosine, guanosine and cytosine phosphates, phosphoenolpyruvate, all the phosphoglyceric acids[9,10] the phosphorus in the total acid-insoluble residue of muscle measured either as inorganic phosphate, phosphatido-peptide phosphorus, phospholipid phosphorus, ribonucleic or deoxyribonucleic acids[11]. Other relevant experiments[12] have been reviewed by Davies and Cain[13].

Since we had found that inorganic phosphate was certainly liberated during a single muscle contraction (Table 1), and no other compound could be observed to account for it, we re-investigated the possibility of a breakdown of PCr using gentler methods of extraction and more precise methods of analysis. These experiments showed that PCr in fact liberates creatine and inorganic phosphate during a single contraction[9,10,13,14] (Table 1). This finding has now been confirmed by Fleckenstein[15] and Mommaerts[16]. In normal rectus abdominis muscles of the female frog the inorganic phosphate and creatine content are sufficiently low (that is, 2·5 and 5·0 µmole/g) that changes can be observed during contraction, but the concentration of PCr is so high it is not possible to observe the expected changes with sufficient certainty. However, if the concentration of PCr is first lowered from about 12 to about 2·5 µmole/g by treatment with 2,4-dinitrophenol (DNP), (under conditions where the muscle can still do several complete contraction cycles) then the expected change in PCr can

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* Predoctoral Fellow of the U.S. National Institutes of Health.

Table 1. CHANGES IN INORGANIC PHOSPHATE (Pi) AND FREE CREATINE (Cr) IN NORMAL FROG RECTUS ABDOMINIS MUSCLES FOLLOWING ELECTRICAL STIMULATION AT 0° C

Type of exp.	Pairs of muscles	ΔPi (µmole/g muscle)	ΔCr (µmole/g muscle)	Average external work (g-cm/g muscle)	Length of stimulation (sec) (12 supramaximal pulses/sec)
Control-vs-activation	18	+0·08 ± 0·11	+0·11 ± 0·14	0–10	<0 2
Control-vs-shortening	9	−0·08 ± 0 20	+0 02 ± 0 21	0	0·5 − 1·0
Control-vs-contraction	21	+0·64 ± 0·14	+0·88 ± 0·20	120	1·0 − 2 5
Control-vs-contraction + relaxation + contraction	10	+1·20 ± 0·17	+1·22 ± 0·30	210	4·0 − 6·0
Control-vs-control	12	0·09 ± 0·10	0·29 ± 0 26	0	0 0
Contraction-vs-contraction + relaxation	7	+0·08 ± 0·14	— —	120*	1·0 − 2·5

* Both muscles of the pairs did the same amount of work.

The paired muscles from female *Rana pipiens* were dissected free, separated, and incubated in physiological bicarbonate saline solution at 25° C for 1–2 h. They were cooled to 0° C for 5 min and used as follows: In the activation experiments the muscles at rest length were stimulated and then frozen in liquid 'Freon' ($CF_2Cl_2 + CF_3Cl$) at −172° C as shortening began but before much external work had been done. In the shortening experiments the muscles were stimulated to shorten to one-half of their rest length under no external load and then frozen. Control–control values are for experiments in which neither member of the paired muscles was stimulated. Changes in Pi brought about by relaxation were studied by freezing one muscle at the height of 'after-loaded' contraction and permitting its pair to contract and then relax under load before being frozen. Muscles were extracted in aqueous methanol at −35° C for 7 days and the Pi and Cr measured by the methods of Wahler and Wollenberger[17], and Eggleton, Elsden and Gough[18]. Here and elsewhere, except when noted, all assays were done in duplicate. Results ± standard error of means.

Table 2. EFFECT OF ELECTRICAL STIMULATION IN HYPERTONIC SOLUTION AT 0° C ON THE PHOSPHORYLCREATINE (PCr) LEVEL OF FROG RECTUS ABDOMINIS MUSCLES

Pairs of muscles	No. of pulses (at 12/sec)	ΔPCr/pulse (µmole/g muscle)
4	12	0·00 ± 0·04
5	24	+0·01 ± 0 01
5	36	0 00 ± 0·01

These muscles were treated with 0·25 mM DNP at 20° C for 25 min and then for 20 min at 0° C in DNP-bicarbonate saline made hypertonic with 440 mM sucrose. Other conditions were as in Fig. 1. The work performed by these muscles was less than 3 g-cm/g muscle

Table 3. ASSOCIATION OF PHOSPHORYLCREATINE (PCr) BREAKDOWN WITH EXTERNAL WORK BUT NOT WITH DEGREE OF SHORTENING

Pairs of muscles	Per cent shortening $\frac{l_0 - l_s}{l_0} \times 100$*	ΔPCr (µmole/g muscle)	External work (g-cm/g muscle)	Stimulation time (sec) (12 pulses/sec)
Constant shortening—varying external work				
10	19·6 ± 2·24	−0·28 ± 0 15	21·7 ± 1·9	0·6 ± 0·1
10	20·2 ± 2 82	−0·36 ± 0·16	30·8 ± 2·3	1·2 ± 0·1
10	20·5 ± 2·27	−0·47 ± 0 26	46·6 ± 2·3	1·6 ± 0·2
8	21·1 ± 1·28	−0·58 ± 0 18	55·2 ± 0 9	2 0 ± 0·4
5	19·7 ± 3 13	−0·77 ± 0·24	64 2 ± 6·0	2 6 ± 0·5
Varying shortening—approx. constant external work				
11	10·7 ± 0·81	−0·44 ± 0·22	36 3 ± 4·7	1·9 ± 0·4
11	18·5 ± 0·75	−0·44 ± 0 16	37·3 ± 4·6	1·0 ± 0·2
11	23·4 ± 0·31	−0·48 ± 0·21	39 3 ± 5·1	1·2 ± 0·2
9	29·5 ± 1 08	−0·54 ± 0·11	50·8 ± 4·3	2 0 ± 0 4

*l_0 is rest length; l_s is length after shortening.

By varying the load, the muscles were made to do either different quantities of work while shortening the same amount, or the same amount of work while shortening to different lengths. The amounts of shortening and external work performed were determined from kymograph tracings. The muscles were treated as described in Fig. 1.

be observed to occur during a single contraction. The experimental techniques have been described previously[9,10,14].

The results are shown in Tables 1–3 and Fig. 1. It is clear that muscles made to do varying amounts of work broke down varying amounts of PCr to inorganic phosphate and creatine with a linear relationship between the amount of work and the amount of breakdown (Table 1, Fig. 1). Relaxation was not associated with any measurable change (Table 1). It is of interest that the observed breakdown of PCr is associated very much more with work than with either activation or shortening. The breakdown on activating the muscle and rapidly freezing it before it was allowed to do any work was quite small (Table 1). This was confirmed by stimulating the muscle in hypertonic saline (Table 2), where muscles are still activated but do not contract[20]. Similar small changes occurred in muscles which shorten against zero external load (Table 1). Furthermore, muscles performing an approximately constant amount of work with large changes in the degree of shortening showed no significant change in PCr breakdown associated with the shortening (Table 3), whereas for constant shortening and varying work a very clear correlation with breakdown of PCr was found (Table 3). Our direct chemical measurements show that in this muscle the breakdown of PCr can be associated with the work done and is rather independent of the amount of shortening that occurs.

It is of interest that DNP-treated muscles, which have low PCr but high creatine and inorganic phosphate, work with a reduced stoichiochemical efficiency (work per mole) (Tables 1 and 4, Fig. 1) which might well be expected on thermodynamic grounds.

In a mechano-chemical system the relation between output of heat and work done depends on the entropy and free energy of the chemical reactions, the efficiency of energy transduction and the absolute temperature. As Wilkie[21] has pointed out, when work is done by ATP usage under standard conditions at about 38 per cent efficiency, the reaction is thermally neutral. This type of situation may account for Hill's observation[22] of the striking appearance of work without extra initial heat production (above shortening heat) during the rising phase of a contraction, the time when we observe a breakdown of PCr. Assuming that results on sartorius muscles apply to rectus abdominis, our findings also show that the Fenn effect is to be expected; that is, " . . . when work is

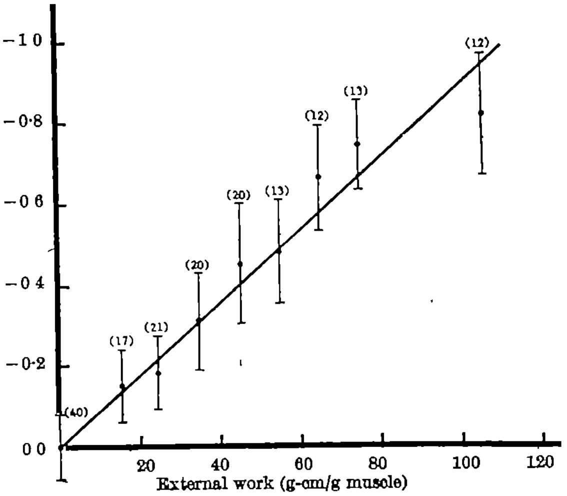

Fig. 1. Relationship of phosphorylcreatine breakdown to the amount of external work performed by 'after-loaded' frog rectus abdominis muscles contracting once or twice against a constant load to different degrees and for different times at 0° C. These muscles were isolated and incubated as described in Table 1, then treated with 0·25 mM DNP in bicarbonate saline solution for 40 min at 20° C and cooled to 0° C for 5 min. PCr was converted to creatinine by molybdate-catalysed acid hydrolysis and measured according to Barker and Ennor[19]. The figures in parentheses refer to the numbers of pairs of muscles used. Means ± *S.E.* Slope of line: 2,600 calories of external work/mole PCr

Table 4. PRODUCTION OF INORGANIC PHOSPHATE (Pi) WITHOUT CHANGE IN PHOSPHORYLCREATINE (PCr) AFTER THREE SMALL CONTRACTIONS AT 0° IN FROG RECTUS ABDOMINIS MUSCLES PRETREATED WITH DINITROPHENOL (DNP) PLUS FLUORODINITROBENZENE (FDNB)

Type of exp.	Pairs of muscles	External work (g-cm/g muscle)	ΔPCr (μmole/g muscle)	ΔPi (μmole/g muscle)
Control-vs-contractions	27	81 ± 5	− 0·10 ± 0 08	— —
Control-vs-contractions	12	79 ± 8	− 0·17 ± 0 08	+ 1·23 ± 0 48
Control-vs-control	4	0	0 17 ± 0·17	— —
Control-vs-contractions (DNP but no FDNB)	4	76 ± 8	− 1·02 ± 0 28	— —

The paired muscles were isolated and incubated as in Table 1. They were then placed in bicarbonate saline solution containing 0·25 mM DNP for 30 min at 20° C followed by 20 min at 0° C in saline containing both 0·25 mM DNP and 0·38 mM FDNB. These salines were gassed with 5 per cent CO_2 + 95 per cent N_2. Total time of stimulation 4–6 sec.

Table 5. BREAKDOWN OF ADENOSINE TRIPHOSPHATE (ATP) TO FORM ADENOSINE DIPHOSPHATE (ADP) AND ADENOSINE MONOPHOSPHATE (AMP) DURING CONTRACTION OF FROG RECTUS ABDOMINIS MUSCLES AFTER TREATMENT WITH FLUORODINITROBENZENE (FDNB)

	ATP (μmoles/g muscle)	ADP (μmoles/g muscle)	AMP (μmoles/g muscle)
Single contraction			
Control	1·25	0 64	0·10
After 1 contraction	0 81	0·90	0·24
Change ± *S.E.* for 9 pairs	− 0·44 ± 0·046	+ 0·26 ± 0 023	+ 0·14 ± 0·027
Double contraction			
Control	1·24	0·61	0·07
After 2 contractions	0·59	0·88	0·41
Change ± *S.E.* for 3 pairs	− 0·65 ± 0 045	+ 0·27 ± 0 051	+ 0·34 ± 0 037

External work ≃ 100 g-cm/g muscle/contraction.

The muscles were isolated and incubated as described in Table 1 then treated with 0·38 mM FDNB for 40 min at 0° C. ATP, ADP and AMP were measured fluorometrically according to a modification of the method described by Estabrook and Maitra[30]. AMP results are based on single analyses. In some experiments, the ATP contents of control and contracted muscles were also measured by the use of the specific firefly luminescence technique with a photomultiplier coupled to a recorder[31].

done by the muscle, an extra supply of energy is liberated . . . which is roughly proportional to the work . . . "[23]. Thus there will be an extra heat output after the shortening while, *inter alia*, the PCr used by working muscle is being re-synthesized. However, the existence of shortening heat (apart from work heat) has recently been questioned[24] so the Fenn[23,25] effect might be directly related to the breakdown of PCr rather than to its re-synthesis.

Lundsgaard[4] showed, many years ago, that the energy for muscular work in a series of contractions could be directly related to the splitting of PCr, and this has recently been confirmed for large series of twitches[24,26]. However, it was not possible to determine whether the breakdown occurred during contraction or relaxation or whether the energy was delivered at the beginning or throughout the contraction cycle, whereas our experiments show that this chemical change occurs while the work is being done by the muscle. It is clear, and has been pointed out by Hill[27] and others, that the splitting of PCr is not proof of the breakdown of ATP. However, this breakdown should be observable if creatine phosphoryltransferase could be inhibited *in situ* and thus allow a direct demonstration of the primary role of ATP in muscular contraction. Kuby and Mahowald[28] had found that crystalline creatine phosphoryltransferase is completely inhibited by 1-fluoro-2,4-dinitrobenzene (FDNB), and we have now used this inhibitor to find the changes in ATP during single contractions which have been sought so often during the past thirty years[29]. When isolated muscles were pre-treated with FDNB, the number of normal contractions which could be performed was reduced from more than 30 to about 3, yet even when they would no longer contract, they still contained the normal quantities of PCr found in untreated muscle.

Table 4 shows that after treatment with DNP + FDNB, inorganic phosphate increased on contraction although PCr remained unchanged. Table 5 gives results with FDNB alone which show that ATP was split, but that the net amount of ADP produced was much less than the net ATP breakdown which itself was less than the inorganic phosphate production observed in the earlier experiments given in Table 1.

In these earlier experiments with normal muscles, the Lohmann reaction[5] was apparently the over-all energy-supplying mechanism; that is:

$$\text{ATP} \xrightarrow{\text{actomyosin ATPase}} \text{ADP} + \text{inorganic phosphate} + \text{work, and}$$

$$\text{PCr} + \text{ADP} \xrightarrow{\text{creatine phosphoryltransferase}} \text{ATP} + \text{creatine}$$

Thus, the changes in inorganic phosphate and creatine should be identical as is shown in Table 1. The apparent discrepancy shown in Table 5 can be resolved by consideration of the action of myokinase. This enzyme catalyses the reaction:

$$2\,\text{ADP} \xrightarrow{\text{myokinase}} \text{ATP} + \text{AMP}$$

Thus, the combined action of actomyosin ATPase and myokinase leads to the generalized equation given in Table 6. This generalized equation fits the experimental results remarkably well for both conditions tested. These findings, together with those we have obtained previously, demonstrate clearly and directly that the energy is delivered from a breakdown of ATP. They also make it clear that, in normal working muscle, creatine phosphoryltransferase makes available the energy stored in phosphorylcreatine, but that in extreme conditions myokinase can also be observed to operate in muscle.

Myokinase activity is important because by cyclic regeneration of ATP from ADP free energy is in effect released from the breakdown of both the labile phosphates of some of the ATP. The enzyme also maintains low levels of ADP, which is an inhibitor of myosin ATPase[32], and it keeps the ratio of ATP to ADP high. On thermodynamic grounds, this is advantageous for free energy liberation from ATP splitting.

Our results fit the sliding-filament theory[33]. If the tension-generating system depends on the formation

Table 6. STOICHIOMETRY OF THE CHEMICAL REACTIONS WHICH OCCUR DURING CONTRACTION AT 0° C OF FROG RECTUS ABDOMINIS MUSCLES TREATED WITH FLUORODINITROBENZENE

Generalized equation for the combined action of actomyosin ATPase and myokinase

$$n\ \text{ATP} \longrightarrow (n-m)\ \text{ADP} + m\ \text{AMP} + (n+m)\ \text{Pi}.$$

One contraction of frog rectus abdominis (All quantities in μmoles/g muscle)

$$(0\ 44 \pm 0{\cdot}046)\ \text{ATP} \longrightarrow (0{\cdot}26 \pm 0\ 023)\ \text{ADP} + (0{\cdot}14 \pm 0{\cdot}027)\ \text{AMP} + (0{\cdot}64 \pm 0\ 14)\ \text{Pi}^{*}$$

On the basis of the figures for ATP and ADP, the values calculated from the generalized equation are 0 18 AMP and 0 62 Pi.

Two contractions of frog rectus abdominis (All quantities in μmoles/g muscle)

$$(0{\cdot}65 \pm 0{\cdot}045)\ \text{ATP} \longrightarrow (0{\cdot}27 \pm 0{\cdot}051)\ \text{ADP} + (0{\cdot}34 \pm 0{\cdot}037)\ \text{AMP} + (1{\cdot}20 \pm 0{\cdot}17)\ \text{Pi}^{*}$$

The calculated values are 0·38 AMP and 1·03 Pi.

* Since we have not so far measured changes in ATP, ADP, AMP, PCr, Cr, and Pi all on the same muscle pairs, the changes in Pi are taken from the results on normal muscle given in Table 1.

of cross-links between the side branches of the myosin and the actin, then fewer links would be formed during rapid unloaded contractions and more would be formed during slow, working contractions. Thus different amounts of energy would be required. The demonstration that myokinase is operating during the actual contraction means that the same ATP can be used several times. Such a re-use of a small amount of ATP is consistent with the view that the side-branches must form bonds, develop tension, and break the bonds many times during a single contraction by a mechanism involving ATP splitting; it is inconsistent with the view that a given amount of ATP binds *on to the* muscle protein and causes a complete contraction by an entropic electrostatic mechanism[34]. In this case, the ATP would remain bound during the whole contraction and would only be released at the end as ADP + inorganic phosphate. Thus, neither ATP splitting nor myokinase action would occur during the contraction.

Our results strongly support the view that the shortening of muscle is due to the summation of effects caused by a small number of bonds which are formed and broken repeatedly. It follows that an ATP-dependent series of micro-entropic contractions of the myosin side pieces after they had linked asymmetrically with the actin might well account for the overall process of muscle contraction by the sliding filament mechanism.

In summary, these results show that, with frog rectus abdominis muscles when work is done, ATP is used during single contractions, and that this ATP is normally rapidly reconstituted by the action of creatine phosphoryltransferase and myokinase while the contraction is taking place. There is a linear relationship between the external work done and the cleavage of high-energy phosphate compounds, but very little energy from this source is required for activation and shortening *per se* in these muscles.

We thank Miss Dzintra Klaupiks, Messrs. W. J. Brunton and W. A. Eaton for assistance during part of this work.

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NEWS and VIEWS

Controller of Rubber Research in Malaya: Sir Geoffrey Fletcher Clay, K.C.M.G., O.B.E.

Sir Geoffrey Fletcher Clay has recently retired from the post of controller of rubber research in Malaya, a position which he has held since 1958. Sir Geoffrey, a graduate of the Universities of Edinburgh and Cambridge, is well known as an agriculturalist. After the First World War he joined the Colonial Agricultural Service, spending many years in Uganda, where, in 1939, he was appointed director of agriculture. From 1942 until 1944 he was vice-chairman of the South African Production and Supply Council and from 1944 until 1946 development adviser for Northern Rhodesia and Nyasaland. For ten years, until 1956, he was agricultural adviser to the Secretary of State for the Colonies. Among his many other activities, Sir Geoffrey has been a member of the United Kingdom delegation to the conference organized by the United Nations on "Conservation of Resources" held at Lake Success in 1949. In 1952 he led the mission sent by the United Nations to Korea to prepare a five-year plan for the rehabilitation of Korean agriculture, forestry and fisheries. Sir Geoffrey has worked with the Food and Agriculture Organization on the problems of the reafforestation of the Mediterranean area, and has been closely associated with the Scientific Advisory Committee of the Empire Cotton Growing Association.

Dr. L. C. Bateman

Dr. Leslie Clifford Bateman has been appointed to succeed Sir Geoffrey Clay as controller of rubber research in Malaya. Dr. Bateman took up his position on August 1 and is now based in Kuala Lumpur. As controller he is responsible for the overall direction of the research and development programme for natural rubber carried out by the Rubber Research Institute of Malaya, the Natural Rubber Producers' Research Association and the Natural Rubber Bureau. Dr. Bateman was educated at Bishopshalt School, Uxbridge, and University College, London, where he gained honours in chemistry in 1935 and a Ph.D. in 1938, when he also won the Ramsey Memorial Prize for best student of the year. His postgraduate career included service as research assistant under Sir Christopher Ingold and Prof. E. D. Hughes and a senior studentship granted by the Department of Scientific and Industrial Research, held at Oriel College, Oxford, under Sir Robert Robinson. He received a D.Sc. at the University of London in 1955. He joined the then British Rubber Producers' Research Association as a physical chemist in 1941, and was promoted superintendent of chemical research in 1951, becoming acting director in 1953, and finally director of research in April 1954. Dr. Bateman has served the interests of the Natural Rubber Producers of Malaya for more than twenty years, and has been particularly successful in gathering around him a keen, enthusiastic and highly qualified staff at the Natural Rubber Producers' Research Association Laboratories at Welwyn Garden City, Herts. Dr. L. Mullins, at present deputy director of research at the Natural Rubber Producers' Research Association, will act as director during Dr. Bateman's absence, which will be for three years in the first instance.

Council for Scientific and Industrial Research

THREE new members of the Council for Scientific and Industrial Research have recently been appointed by the Minister for Science: Mr. W. J. Carron, president of the Amalgamated Engineering Union; Dr. S. C. Curran, principal of the Royal College of Science and Technology, Glasgow; and Dr. C. Sykes, deputy chairman and managing director of Thos Firth and John Brown, Ltd., Sheffield. They succeed the following, who have completed their term of office: Mr. L. T. Wright, general secretary of the Amalgamated Weavers Association; Dr. C. J. Smithells, past chairman of Magnesium Elektron, Ltd.; and Prof. B. Bleaney, professor of experimental philosophy in the University of Oxford, who has resigned from the Council on receiving a year's appointment at the University of Pittsburg.

The present constitution of the Council is: Sir Harold Roxbee Cox (chairman), Mr. L. H. Bedford, Mr. W. J. Carron, Prof. C. F. Carter, Dr. J. W. Cook, Mr. Frank Cousins, Dr. S. C. Curran, Mr. G. B. R. Feilden, Prof. E. R. H. Jones, Vice-Admiral Sir Frank Mason, Sir Harry Melville, Prof. O A. Saunders, Dr. C. Sykes and Mr. H. C. Tett.

Royal Society Visiting Professorships

PROF. R. DULBECCO, professor of virology, Division of Biology, California Institute of Technology, Pasadena, California, has been appointed Royal Society visiting professor for the academic year 1963–64. He is expected to take up the appointment in October 1963 and to work in the Departments of Genetics and of Virology at the University of Glasgow. Prof. Dulbecco is a leading virologist and has been the person chiefly responsible for the introduction of the quantitative analytical approach in the study of animal viruses. His interests lie in the three fields of virology, molecular biology and genetics.

The Royal Society and the Leverhulme Trust have established a scheme for the appointment of two visiting professors to India each year. Each visiting professor will visit a university or research institution in India for a period of about four months. It is hoped that in this way a contribution to higher education in India can be made. In implementing the scheme a committee in India will nominate the universities or other institutions to be visited; appointments will be made by the Council of the Royal Society, the finance and administrative arrangements being provided by the Leverhulme Trust Fund. The first appointments will be made so that the visiting professors can be in India during October 1963–February 1964.

The International Centre for Advanced Mediterranean Agronomic Studies

ON October 3, the Bari (Italy) Institute of the International Centre for Advanced Mediterranean Agronomic Studies, which was established under the ægis of the Organization for European Co-operation and Development and the Council of Europe, was officially opened. An inaugural lecture, on "International Co-operation and Economic Development", was given by the Italian Minister, Prof. Giuseppe Medici. The new buildings of the Bari Mediterranean Agronomic Institute, which have been built by the Italian Government, were officially opened on the same occasion. Forty students from countries in the Mediterranean area (Cyprus, France, Greece, Israel, Italy, Jordan, Malta, Morocco, Portugal, Spain, Syria, Turkey, Yugoslavia) are to attend the courses this year. Ten of them hold scholarships awarded by the Organization for European Co-operation and Development to nationals of member countries in the process of development. The others have received scholarships from the French and Italian Governments.

Cardio-respiratory Disease Research Project

THE London School of Hygiene and Tropical Medicine is to undertake a long-term research project on personal and environmental factors in cardio-respiratory diseases, including lung cancer, chronic bronchitis and coronary heart disease. The project has the support of the Tobacco Manufacturers' Standing Committee, which is to contribute up to £50,000 a year for ten years. This will also allow for a permanent endowment for the development of epidemiological studies at the School. The research project will have three main aims: (1) to investigate the various environmental factors, such as social, domestic and atmospheric conditions, affecting the onset and evolution of the commoner cardio-respiratory diseases; (2) to identify those physical and psychological characteristics, personal habits and those features of previous medical history which indicate a special predisposition to these diseases; (3) to study the interactions between these several factors, whether personal or external, in the production of cardio-respiratory disease.

It is planned to carry out surveys of the habits, health history and living conditions of different samples of the British population and to conduct physical examinations in smaller groups of individuals co-operating in this work. The later medical history of those taking part will then be carefully studied over a period of years. The whole project may take as long as ten years to complete, but some provisional results can be expected after four or five years. The honorary director of the project will be Prof. D. D. Reid, professor of epidemiology and director of the Department of Medical Statistics and Epidemiology at the London School of Hygiene and Tropical Medicine. Prof. Peter Armitage, professor of medical statistics at the School, will collaborate in this work, and the staff will be partly drawn from existing members of the Department and partly recruited specially for the project.

Films to Teach Science

SCHOOL science departments have, in the past, been hampered by the lack of specialized films suitable for use within the General Certificate of Education syllabus. To coincide with the beginning of the school year and the planning of new science courses, Sound-Services Film Library announces that it has been given distribution rights for the science teaching films of the Physical Science Study Committee produced by Educational Services, Inc. There are forty-eight titles in the 16-mm. film series, each film featuring well-known physicists who discuss and carry out experiments in a wide range of subjects. Apart from their use at the General Certificate of Education level, many will be of value to first-year university and technical college students. An illustrated brochure giving a synopsis of each film and an order form with details of hire and purchase charges can be obtained, free of charge, from Sound-Services, Ltd., Wilton Crescent, London, S.W.19.

Consolidating Fragile Iron Objects

THE conservation of objects, in which the iron has been completely converted to oxide, or in which only a small part of the metal remains as a slender core, has long been a problem to archæologists. In the September issue of the *Museums Journal* (62, No. 2; 1962) Messrs. R. M. Organ and P. Shorer describe an improved method for the consolidation of fragile iron objects. After a survey of the present methods, the authors advocate the use of a microcrystalline wax known commercially as 'Cosmolloid', whereby it is possible to consolidate an unstable object without prior stabilization and elimination of the ferric chloride. The grade 80 *H* is stated to be the most suitable for the purpose and the practical details are fully described in the paper quoted.

Nuclear Energy Research in Yugoslavia

THE first volume of papers from the Boris Kidrich Institute of Nuclear Sciences at Vincha appeared in July 1952, and until August 1953 the papers were published in the journal *Recueil de travaux de l'Institut de recherches sur la structure de la matière*. The title was then changed to the *Bulletin of the Institute of Nuclear Sciences 'Boris Kidrich', Belgrade*, and the *Bulletin* appeared annually with one volume per annum, except for 1953 and 1961, when two volumes per annum were published. During 1961 the number of papers submitted exceeded the possibility of the continuance of a standard volume per year, and accordingly with the commencement of Volume 13, 1962, the *Bulletin* is to be issued quarterly. The first issue (13, No. 1; April 1962) contains six articles, including a description of attempts made to measure the radiation of the *RA* reactor at Vincha by chemical dosimeters, and details of the '2*kC*' cobalt-60 radiation unit of the Boris Kidrich Institute at Vincha with its components—source, container, driving mechanism, radiation room, remote-control table, etc.

The Leopoldina German Academy of Science

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Mathematics*, Prof. B. N. Delaunay (Moscow), Prof. Karl Marguerre (Darmstadt), Prof. Hans Reichardt (Berlin), Prof. I. M. Vinogradov (Leningrad); *Physics*, Prof. Hans Falkenhagen (Rostock); *Chemistry*, Prof. Oskar Glemser (Göttingen), Prof. Ulrich Hofmann (Heidelberg), Prof. František Šorm (Prague), Prof. Friedrich Wessely (Vienna), Prof. Georg Wittig (Heidelberg), Prof. S. Ju. Junusov (Taschkent); *Physical Chemistry*, Prof. Klaus Schäfer (Heidelberg); *Botany*, Prof. Paul Collander (Helsinki), Prof. Harry Godwin (Cambridge), Prof. Roger Heim (Paris), Prof. Bruno Huber (Munich), Prof. Poul Larsen (Bergen), Prof. Wilhelm Menke (Cologne), Prof. Heinrich Walter (Stuttgart-Hohenheim), Prof. Frits W. Went (St. Louis); *General Biology*, Prof. Ivan Málek (Prague), Prof. A. I. Tolmatchev (Leningrad).

Geology of the South-West of England

IN January 1956 a conference was held at the University of Exeter of geologists and geomorphologists working in the south-west of England. It was discovered that there was a real need for contact between the scattered workers who were pioneering new lines of attack on the little-known geology of this difficult region. A great deal of progress has been made in the past six years to which the subsequent conferences held in 1958, 1960, 1961 and 1962 have made a useful contribution. At the last conference a decision was taken to set up a properly constituted society to hold regular annual conferences each January and to publish the abstracts of the conference proceedings. Abstracts of the proceedings of the second, third and fourth conferences had been published by the kind offices of the Royal Geological Society of Cornwall. The new society is named the Ussher Society in honour of W. A. E. Ussher (1849–1920), an officer of the Geological Survey of Great Britain who made fundamental contributions to several aspects of the geology and geomorphology of the region.

The first conference of the Ussher Society as such will be held at the University of Exeter during January 2–4, 1963. The secretary is Mr. M. Stone, of the Department of Geology, University of Exeter.

The Night Sky in November

FULL moon occurs on Nov. 11d. 22h. 04m. U.T. and new moon on Nov. 27d. 06h. 30m. The following conjunctions with the Moon take place: Nov. 4d. 19h., Saturn 0·6° S.; Nov. 6d. 18h., Jupiter 1° N.; Nov. 18d. 09h., Mars 0·7° N. Mercury and Venus are too close to the Sun for easy observation. Mars rises at 22h. 20m., 21h. 55m. and 21h. 25m. on Nov. 1, 15 and 30, respectively. It is at a distance of 106 million miles on November 15, and its stellar magnitude is +0·5. Conditions are becoming more favourable for observation. Jupiter sets at 0h. 40m., 23h. 50m. and 22h. 50m. at the beginning, middle and end of the month, respectively. It is in Aquarius, its stellar magnitude is −2·1 and its distance from the Earth in the middle of the month is 436 million miles. Saturn is in Capricornus, setting at 22h. 00m., 21h. 10m. and 20h. 15m. on Nov. 1, 15 and 30, respectively. Its stellar magnitude is +0·9 and its distance from the Earth on November 15 is 947 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Nov. 6d. 17h. 38·8m., 50 Aqr. (*D*); Nov. 13d. 19h. 08·9m., 104 Tau. (*R*); Nov. 16d. 02h. 53·3m., 56 Gem. (*R*); Nov. 16d. 05h. 46·2m., 61 Gem. (*R*); Nov. 20d. 02h. 41·4m., 53 Leo. (*R*). There is also an occultation of Saturn visible at Greenwich: Nov. 4d. 19h. 23·4m. (*D*); Nov. 4d. 19h. 42·7m. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The Taurid meteors are active during the first two weeks of November. Conditions for observation are favourable; the radiant is near R.A. 3h. 36m., Dec. +14°. The Leonids are active during November 15–17, but conditions for observation are unfavourable.

Royal College of Science and Technology, Glasgow

The Royal College of Science and Technology, Glasgow, in consultation with the University Grants Committee, has appointed an Academic Advisory Committee. In the period of development ahead of the College, as outlined in the Government statement of May 1962, and pending the report of the Robbins Committee, the Advisory Committee will make available to the College its experience in developments both in Scotland and England. The terms of reference of the Advisory Committee will cover matters of general academic policy. The following are members of the Committee: Prof. E. L. Hirst, professor of organic chemistry, University of Edinburgh (*chairman*); Dr. Charles Wilson, vice-chancellor and principal of the University of Glasgow and lately vice-chancellor of the University of Leicester; J. O. Blair Cunynghame, deputy chairman of the Royal Bank of Scotland; Sir Lindor Brown, Waynflete professor of physiology, University of Oxford; Prof. J. Diamond, Beyer professor of mechanical engineering, University of Manchester; Prof. E. H. Phelps Brown, professor of economics of labour, London School of Economics; Prof. M. W. Thring, Newton 'Drew professor of fuel technology and chemical engineering, University of Sheffield; Dr. S. C. Curran, principal of the Royal College of Science and Technology, Glasgow.

John Wiley and Interscience Publications: Dr. F. Betz, jun.

Dr. Frederick Betz, jun., has been appointed to the editorial staff of John Wiley and Sons, Inc., New York, as editor in charge of the earth sciences and geography publications. He will be responsible for developing texts and reference books for the Wiley and Interscience imprints in the fields of geology, mineralogy, geophysics, meteorology, oceanography and geography. Since obtaining his Ph.D. from Princeton University in 1938, Dr. Betz has had varied experiences. He was associated for seventeen years with the U.S. Geological Survey, in the United States and in Europe, and also has served with the U.S. Bureau of Mines, the Newfoundland Geological Survey, the Department of Geology of Lehigh University, and the Geological Society of America.

Macmillan and Co., Ltd.: Mr. B. J. Walby

Mr. Basil J. Walby has joined the editorial staff of Macmillan and Co., Ltd., London, to succeed Mr. D. Grisewood, who has been appointed lecturer in mathematics in the Royal University of Malta. Mr. Walby will be responsible for scientific and technical books for secondary schools, technical colleges, universities, etc., published by Macmillan and Co. He read mathematics and chemistry at King's College, London, and then spent two years as senior science instructor in the College of the Rhine Army. For four years after he was assistant editor of *The Analyst* and of *Analytical Abstracts*, and then carried out research in industrial mathematics with particular reference to computer applications at the Distillers' Co., Ltd. More recently, Mr. Walby has been acting as a management consultant in operational research with SIGMA.

Announcements

Sir Geoffrey Taylor, formerly Yarrow research professor of the Royal Society of London, has been awarded the Franklin Medal of the Franklin Institute, Philadelphia, "for his manifold contributions to the knowledge of natural sciences".

Lord Hailsham, Minister for Science, is to open the new £2 million National Lending Library for Science and Technology at Boston Spa, Yorkshire, on November 5. The library collects all the world's literature of advanced science, technology and certain aspects of medicine, and promotes its use among industrial, agricultural and commercial organizations, universities and colleges, research institutes and hospitals throughout Britain. It has already amassed the largest scientific loan collection—at least 350,000 volumes—in Western Europe.

The second Western National Meeting of the American Geophysical Union will be held at Stanford University, California, during December 27–29. Further information can be obtained from the American Geophysical Union, 1515 Massachusetts Avenue, N.W., Washington 5, D.C.

The third Summer Research Institute of the Australian Mathematical Society will be held at the Australian National University, Canberra, during January 8–February 15, 1963, under the directorship of Prof. B. H. Neumann, of the Australian National University. Further information can be obtained from Dr. G. E. Wall, Department of Mathematics, University of Sydney.

The fifteenth technical exhibition of the Oil and Colour Chemists' Association will be held in the Old and New Halls of the Royal Horticultural Society, London, during March 11–14. Further information can be obtained from the general secretary, R. H. Hamblin, Oil and Colour Chemists' Association, Wax Chandlers' Hall, Gresham Street, London, E.C.2.

A meeting on "Questioned Document Examination", in conjunction with the third international meeting on "Forensic Immunology, Medicine, Pathology and Toxicology", will be held at the School of Pharmacy, London, during April 16–24. Topics under discussion will include: writing materials: paper, instruments, inks; laboratory examinations: chemical, photographical, optical; recent advances and problems in questioned document examinations; the role of the questioned document examiner as an expert witness. Further information can be obtained from the Secretariat (Q.D.), Third International Meeting in Forensic Immunology, Medicine, Pathology and Toxicology, 28 Portland Place, London, W.1.

Erratum. In the fourth paragraph of the article by Dr. A. Compton entitled "Effect of Feeding X-ray Irradiated Coliform Bacilli to Tumour-bearing Mice", which appeared on p. 1273 of the September 29 issue of *Nature*, the last sentence should read: "It may be viewed . . . graft tumours are known to grow as a rule not so well in older, larger animals[4]".

RESEARCH INTO MANAGEMENT

By Dr. STANLEY HYMAN

Department of Management and Production, Kingston College of Technology, Surrey

THE process of management operates wherever a group of people is working to achieve some purpose. Although most studies of management have been made within the context of private manufacturing business, it is a universal problem with considerable power to influence individual welfare and happiness. It is unfortunate, therefore, that there have been so few attempts to find out more about it through scientific research techniques.

Most of the work that has been done has been concerned with particular management 'tools', like work study, or with sociological aspects of management, such as the social class of managers or the impact of technical change on industrial relations. Controlled investigations of what managers do and think are extremely rare. It is obviously very difficult to investigate managers at work, if only because the presence of the investigator is likely to disrupt their normal behaviour. Self-analysis has been tried, but leaves one inevitably unhappy about the chances of having been told the full story—even when the informant wishes to hide nothing.

In an inquiry started last October, it seemed wise to begin on a different basis. To find out something about management, the obvious beginning was to talk to managers about their work. While this would not answer the methodological problems, it seemed a good thing to start with the simplest and most obvious source of knowledge that was immediately available. A questionnaire was prepared for completion at interviews, covering all the major aspects of the firm—its size and activities, form of ownership, numbers employed, products manufactured, numbers of customers, advertising expenditure, trade union relationships, research and development policy, membership of trade associations, and attitudes towards and knowledge of Kingston College of Technology. Respondents were also to be asked about the problems of their work which caused them the greatest concern.

It was appreciated that there were snags in this type of work and that the first batch of interviews could be no more than a pilot survey. When they were completed the whole project would have to be reviewed. We wrote to forty firms who were local members of the British Institute of Management or represented on the College's Management Advisory Committee. We explained briefly what we had in mind and asked for appointments. Thirteen did not reply and were not written to again. Seven others were interested, but for various reasons interviews did not take place. The other twenty were interviewed by three members of the staff of the Management Department—Mr. S. H. J. Freemantle, Mr. J. L. G. Mogford and myself. Usually two of us went to an interview, but sometimes there was only one interviewer, and in two cases there were three. Opinions differed as to the advantage of multiple interviewers—they may hinder ease of discussion.

It was realized that the firms visited were not necessarily representative of management in general —their special memberships testified to an unusual interest and perhaps serious attitude towards management. However, it was thought that this 'defect' would not matter in the pilot survey. The firms represented a wide range of industries and sizes (measured by numbers employed). Apart from one retail organization, they were all manufacturing firms.

The main results of the pilot survey were:

(1) The lengthy questionnaire was found to be largely useless for our purpose. It tended to regulate the conversation, even though we did not press the sequence of discussion to conform to the questions. After a few visits, we abandonded the questions and merely began by saying: "We would like to increase our knowledge of management, and it seemed wise to begin by talking to managers. Would you care to tell us what your major problems are?". Although some respondents were suspicious of our intentions at first, in no case was resistance found when we had explained our aims. In fact, the outstanding finding of these interviews is the eagerness of managers—and these were all general managers or directors—to talk freely about their jobs, their firms and their responsibilities. They gave us facts and figures and seemed unconcerned with secrecy although we had in fact promised to observe it. There was almost a psychotherapeutic need for them to talk in confidence to responsible outsiders, although from a psychological point of view most of these initial interviews remained superficial.

(2) Most of the factories visited were part of larger organizations. We were surprised to find how many, particularly among those whom we thought were independent. In some cases, we did not probe the precise ownership. Some of the firms had been bought up in 'take-overs', some had asked to be taken over, some were merely subsidiaries resulting from 'natural' growth. Apart from perhaps six independents, the operating unit that we visited was 'protected' by a larger headquarters, usually some distance away, that was said to allow very large amounts of discretion to the local manager, exept in matters of major policy such as capital investment.

We decided on the following programme for the major part of our research, tentatively planned to last five years.:

(1) A detailed survey of the industry and commerce in the area served by the College, in order partly to define the 'universe' we were dealing with and to construct a representative sample. The members of the sample would be visited and eventually we should be able to consider making valid generalizations about management, at least in our area. In these aims we would seek the aid of geographers and statisticians from other departments of the College. We would continue to make whatever visits we thought interesting until the 'universe' and sample were established, as that might take considerable time.

(2) We would seek further interviews with those of our initial survey who seemed especially valuable to us and who might be willing to give us perhaps another six hours of time to enable us to get a deeper and wider knowledge of their work, personalities and organizations.

(3) Two problems mentioned most frequently in the initial interviews were concerned with (*a*) the manager's lack of time to deal with all his duties properly, and (*b*) the apparent impossibility of finding competent subordinates to whom could be delegated a field of responsibilities with complete confidence. It seemed that these two problems were closely related to one another, but that some managers would have been sorry if a solution had been found. Managers are short of time partly because they cannot find subordinates. Hence the manager becomes increasingly indispensable and has no time to find and train subordinates. Hence he ceases seriously to ensure proper delegation. Hence the firm is dangerously dependent on his health, ability, adaptability and expectancy of working life.

(4) We formed a tentative hypothesis that a great deal of management problems were social in origin—coming from the society outside the firm—and that possibly a great number of benefits also came from these external sources. We thought that these external sources of help might be the substitute in the small firm for benevolent head offices in the large organizations. We propose, therefore, to investigate the role of local government, local offices of ministries, trade associations, the banks, and consultants, in helping or hindering managers in their work.

(5) We propose to make comparative studies of management outside the business firm. A beginning has been made with a hospital, and it is hoped to investigate the management problems of the professional practice, initially among architects.

THE UNDERGROUND LABORATORY OF THE NATIONAL CENTRE FOR SCIENTIFIC RESEARCH, FRANCE

FRANCE is one of the countries which has made a considerable contribution to the development of spelæology and biospelæology. An underground laboratory with modern equipment was developed after the Second World War, through the efforts of Profs. Jeannel and Fage and with the financial assistance of the National Centre for Scientific Research. A decree, dated February 11, 1948, specified that it should be built at Moulis, a small village in the Pyrenees, five kilometres west of Saint Girons. It took seven years to build, and was opened on June 26, 1954. The underground laboratory consists of a cave laboratory, which is equipped with low-voltage current and compressed air, and has a storage tank to collect the water of the underground stream running through it. The supply of water under pressure is ensured by a system of pumps and a hanging reservoir. The surface laboratory is some 300 yards from the cave, and includes laboratories, a library, bedrooms, a living room and a kitchen; the scientists are thus entirely independent. The original building has had an extra storey added to it and was considerably enlarged during 1960–61.

The laboratory was designed by zoologists and equipped for biological research. The cave laboratory is arranged so that cavernicolous animals can be raised there under optimum conditions, and investigations carried out *in situ*. It would be of great interest to carry out physical spelæology studies together with the biological investigations, as, for example, the study of the creation of underground cavities, the flow of underground streams and rivers, the composition of air and water in caves, underground meteorology, the creation and destruction of crystals and the composition of the clay found in the caves. A fuller knowledge of the physical media would, moreover, provide the biospelæologist with data of the highest value. For such work, however, specially equipped laboratories would have to be built and staffed with physicists and chemists. This will be a task for future generations.

Biospelæological research over the past fifteen years has dealt with three closely related problems: (1) the development and life-cycle of cavernicolous animals (worms, crustaceans, arachnids, insects, molluscs, fish and amphibians); (2) their metabolism; (3) their nutrition. The last-named has led to the birth of a new science, 'spelæobacteriology'.

One of the most serious gaps in biospelæological knowledge concerned the initial stages of cavernicolous life, about which little was known. Not only was survival of the animals made possible in the cave laboratory, but also almost all the species raised there also reproduced. The results obtained have been surprising and have justified the enterprise*.

Cavernicolous coleoptera can be divided into two main groups: Bathysciinae, which are omnivorous insects related to the Silphidae, and Trechinae, which are carnivorous insects related to the Carabaeid family. The way in which they have developed was unknown until recently. This problem has been resolved in the course of the past ten years by Mme. Glaçon-Deleurance's observations. There are several types of development of cavernicolous Bathysciinae, the degree of specialization of which is correlative to the 'cavernicolarity' of the insect. The peculiar cycle of the most cavernicolous of the Bathysciinae, such as *Speonomus longicornus*, was at first thought to be a deviation due to captivity. The initial results were soon confirmed, however, and there is now no doubt that the cycle observed in the laboratory is the same as that occurring under normal conditions.

In the most specialized species of Bathysciinae, the female lays only one egg at a time; this egg is extremely large and completely filled with vitellus. It hatches into a larva which has a short larval stage, (from a few hours to a few days). This explains why such larvæ had never been found in the caves, and were unknown until Mme. Glaçon-Deleurance carried out her observations. The larva rapidly builds itself a clay case in which it pupates. In the course of its brief larval life, the insect does not feed, grow or moult; it therefore has only one larval stage, whereas coleoptera normally moult twice and thus have three stages. The peculiar growth-cycle of this highly specialized species is related to the normal cycle by a series of intermediate species. Mme. Glaçon-Deleurance also resolved the problem of the development of Trechinae and, particularly, of

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* *French Science News*, No. 1, January–March, 1962. (Paris: Ministère des Affaires Étrangères—Direction Générale des Affaires Culturelles et Techniques, 1962.)

Aphaenops, which is the most remarkable cavernicolous animal of the Pyrenean caves.

The comparative physiology of cavernicolous creatures and their epigeous counterparts was studied by Mme. Derouet-Dresco, who had worked with Prof. Fontaine. She has carried out research work on both aquatic crustaceans and spiders. In a remarkable thesis presented last year, M. R. Ginet mentioned that the growth of *Niphargus* is much slower than that of *Gammarus*, but that its life-span is far longer. After a comparison between the respiration-rates of epigeous spiders and the cavernicolous species, Mme. Derouet-Dresco showed that metabolism of the cavernicolous animals is less active than that of their closest biological relatives above ground, and can be compared with the metabolism of an ageing creature. This explains their extremely slow development and also their long life.

Biospelæologists have long suspected that the clay in the caves played an important part in the life of cavernicolous animals, either in their nutrition or in their reproduction and development. Research on the nutritional implication started when Rene Ginet carried out breeding tests with *Niphargus* in 1956. He showed that the young *Niphargus* continues to develop, for a certain length of time, in tanks containing clay but with no actual food of any sort, whereas when given food but no clay, all the young *Niphargus* die during their second moult. A research programme was therefore drawn up to investigate the properties of the clays.

Victor Caumartin, the co-ordinator of the research team, used an electronic microscope to discover a wide variety of bacterial flora in this underground clay, as well as certain types of porous rock, such as dolomite. One of his discoveries was a hitherto unknown ferrobacterium to which he gave the name of *Perabacterium spelai*. This bacterium is anærobic in its natural medium, and is completely autotrophic. It fixes the nitrogen of the air and gets its carbon from 'ferric carbonate'. The energy necessary for its metabolism is supplied by the decomposition of ferric carbonate, which frees ferrous oxide, and this in turn is transformed by oxidation into ferric oxide. When the supply of ferrous salts in the clay is exhausted the bacteria disappear and the clay becomes a strictly inorganic substance.

This remarkable bacterial species is the only one which has been studied in any detail, and its discovery sheds a new light on the clay found in these underground cavities. It is not only an inorganic mixture, but also a living medium which produces organic substances and can serve as food for cavernicolous creatures. The bacteria, and also the fungi, can synthesize the vitamins and growth substances which are normally supplied to animals by grass. This discovery shows the possible existence of a purely endogenous cycle within the depths of the Earth, to which no exogenous contribution is made. The closed cycle could be similar to that observed in the great marine trenches. Ginet and Miss Gounot give an initial, although tentative, insight into the interdependent nutrition cycles of the subterranean world.

The basic cycle is dependent on autotrophic bacteria and on ferro-bacteria in particular. A second, as yet incompletely known cycle is probably dependent on heterotrophic and saprophytic bacteria, and on various protista, amœbæ and flagellate zoospores. Limivora, such as Nematoda and Oligochæta, feed on this 'living clay' as do young *Niphargus* and *Proteus*. The Carnivora (adult *Niphargus* and *Planaria*) feed on Limivora. Finally, *Proteus*, which can be considered as a supercarnivore, feeds on *Niphargus* and *Troglocaris*. These cycles have as yet been observed only with aquatic animals, but it is probable that similar nutrition cycles govern the life of terrestrial cavernicolous animals.

THE BRITISH GELATINE AND GLUE RESEARCH ASSOCIATION

THE twenty-second meeting of the Research Panel of the British Gelatine and Glue Research Association was held on November 8, 1961, with the director of research, Dr. D. A. Sutton, in the chair.

Dr. F. S. Steven (University of St. Andrews) presented the first paper on his work with Dr. G. R. Tristram, entitled "The Presence of Non-Protein-Nitrogen associated with Soluble Calf Skin Collagen". It has been found that soluble collagen extracted by the acetic acid method and precipitated by sodium chloride contains a significant amount of non-protein nitrogen even after five successive dissolutions and precipitations. This 2 per cent of non-protein nitrogen material is left in solution when the collagen is adjusted to pH 11 and acetone is added, or it can be obtained by other procedures such as a combination of dialysis at pH 3·5 and acetone precipitation of the dialysate. It cannot be dialysed from the (precipitated) collagen at pH 7 nor does the addition of acetone free it at this pH. It would appear, therefore, that the non-protein nitrogen material is physically bound to the protein and it was shown by paper chromatography to contain eleven ninhydrin-positive components of which nine were amino-acids. Similar experiments were carried out with alkali-processed gelatines. The discussion centred round the possible bearing of these findings on the Courts and Stainsby multi-chain model for gelatin, since a relatively small percentage of amino-acids would cause a disproportionate drop in the found average chain weight as compared with the weight average molecular weight. Dr. Sutton pointed out that the authors' results were consistent with the supposition that only about 1 per cent of the non-protein-nitrogen material was amino-acid so that the bulk of it was presumably peptides, which would have a smaller effect on the ratio of chain weight to molecular weight.

The second paper, by Mr. N. T. Crosby and Dr. G. Stainsby (University of Leeds), "The Stability of Solutions of Solubilized Collagens", reviewed relationships between collagens extracted from skin in the cold by acid buffers and the soluble eucollagens which become extractable after skin has been soaked in cold alkali. The differences between the two groups are mainly quantitative, for example, soluble eucollagen preparations lie within a wider intrinsic viscosity-range and have slightly lower denaturation temperatures. Certain qualitative differences do exist, however, such as the absence of the 640 Å periodicity in reconstituted eucollagen fibrils and their lowered isoionic point arising from the loss of side-chain amide groups during the treatment with alkali. The accumulated data on eucollagens were discussed in the light of the most recent literature on the fine structure of soluble collagens. The subsequent discussion served to re-emphasize our present lack of knowledge on the number and nature of the inter-helix covalent links in insoluble collagens.

The final paper "Glueflok" by Dr. J. Wootton and Mr. W. B. Langston (British Gelatine and Glue Research Association), described experiments in which colloid-milled aqueous suspensions of cross-linked (insoluble) gelatines and animal glues were assessed as flocculating agents for a variety of suspensions, including quartz, colliery wash waters and paper fibres. Such suspensions were described by M. B. Goren and the name 'Glueflok' has now been proposed for them. The advantages of 'Glueflok' over soluble gelatine or glue are: (*a*) the formation of a much larger and more stable floc, (*b*) a three to ten-fold increase in flocculating efficiency, (*c*) independence of flocculating efficiency on *p*H over a wide range of *p*H and, (*d*) absence of resuspension when an excess is added. 'Glueflok' was also compared with the synthetic polyamide flocculating agents and efforts directed towards preparing it in the form of a dried powder were described. D. A. Sutton

THE NATIONAL CHEMICAL LABORATORY

THE report of the National Chemical Laboratory, 1961*, comprises the reports of the Steering Committee and of the director, Dr. J. S. Anderson, together with lists of sub-committees of the Steering Committee, staff in post at December 31, 1961, publications and patents in 1961, and of standard samples of organic compounds. The brief report of the Steering Committee, signed by the chairman, Dr. C. E. N. Bawn, records that after considering during the year the development of the Laboratory's research programme over the next five years, the Committee has planned for some growth of research, and in particular for continued development of the work in chemical thermodynamics over a period of years. The Committee agreed that in the field of corrosion of metals, the Laboratory's function should be to discover the fundamental processes underlying the complexity of corrosion phenomena, and work on the basic chemical and electro-chemical aspects of corrosion is to be strengthened accordingly, while the advisory work of the Group is to be continued. A more concentrated research attack in the inorganic-hydrometallurgical field is now planned for the Extraction of Metals Group, but, in consultation with the National Physical Laboratory Executive Committee, it has been agreed to dissolve the joint Pure Metals Research Sub-Committee. The Steering Committee is seriously concerned at the limitations on these developments which are imposed by the space at present available and a new site to enable additional accommodation to be provided is a matter of urgency.

The Director's report notes steady progress in implementing the Laboratory's revised research programme. During the year research on uranium and beryllium extraction, carried out on behalf of the Atomic Energy Authority, was continued, and now represents rather more than half the work of the Extraction of Metals Group, which has now been able to tackle some important fundamental problems that arise generally in hydrometallurgical work and in the chemistry of mineral processing; together with the Mineral Processing Group of the Warren Spring Laboratory, the Group constitutes a very powerful research team. The Inorganic Chemistry Group has discontinued work on the extreme purification of semi-conductor materials, and that on the separation of the rare earth elements is nearing completion. The work of finding significant technological outlets for materials that have become readily accessible is taking the Group into the field of solid-state science. As part of this development an X-ray Diffraction Unit has been set up. In chemical thermodynamics systematic measurements of the free energies and physicochemical properties of organic compounds, including heats of combustion, heat capacities at low temperatures, vapour compressibilities, vapour pressures and latent heats of vaporization and critical properties, is being carried out, but a comparable programme on inorganic materials is limited by lack of space. The Radioisotope Applications Unit continued to work in the Wantage Research Laboratory of the Atomic Energy Authority and, although short of staff, was able to initiate studies of the milling of coal to produce pulverized fuel for power stations, of the back streaming of oil from diffusion pumps using neutron-irradiated silicone oil, and of the uniformity of sizing of cotton yarn prior to weaving. Staff in post at the end of the year included 60 scientific officers and 7 research fellows; 111 experimental officers; 31 scientific assistants; 2 works technical officers; and 20 clerical and 43 industrial staff; the total authorized complement was 293 compared with 264 at the end of 1960.

In the Director's Research Unit several interesting features have emerged from an examination by field emission microscopy of the behaviour of small amounts of copper deposited by controlled evaporation on to a tungsten field emitter tip, and apparatus has been set up for the study of processes at the metal–gas interface with the field ion microscope. The New Materials Group has continued its thorough study of systems based on the $>BN<$ unit as a possible analogue of the $>C=C<$ unit in extended π-band systems, and the chemistry of aluminium nitrogen systems is being explored. The present objectives of work on organic semiconductors are examination of the possibility of synthesizing useful polymeric organic semiconductors and the study of the electrical properties of organic polymers in charge-transfer association with molecular species of high electron affinity. Progress is reported in the development of electron-exchanging polymers and the polymeric regenerable oxidants; this study has been extended to a more thorough polarographic investigation of the functional groups concerned while some progress is also reported in the study of high molecular organic semiconductors.

Basic research started during the year in the Extraction of Metals Group has been inspired either by difficulties or lack of knowledge encountered during applied hydrometallurgical investigations, or by ideas which could lead to major changes in hydrometallurgical processes. Under the general heading of the chemistry of leach liquors, the solution chemistry of silica is being investigated, and an investigation of the kinetics and mechanism of solvent extraction has been started. The extraction of beryllium from flotation concentrates continues to be the principal problem sponsored by the Atomic Energy Authority, and in the programme undertaken for the Authority, progress has continued in developing a contracting device whereby solvent extraction can be carried out directly on pulps or slurries without prior liquid/solid separation, and in the understanding of anion-exchange resins prepared from polyvinyl chloride, capable of absorbing uranium, but not silica, from acid solution. In the Inorganic Group a section has been set up to study the solid-state chemistry of rare earth compounds, and work has been commenced on comparative studies of chemical and catalytic activity, phase relations and physical properties of oxides and chalcogenides. Work on materials of interest as optical masers has involved exploratory studies of radically new kinds of matrix for excited transition metal ions, investigations of extremely pure alumina crystals and the role of impurities.

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* Department of Scientific and Industrial Research. Report of the National Chemical Laboratory 1961: Report of the Steering Committee and Report of the Director. Pp. iii+75+4 plates. (London: H.M.S.O., 1962.) 5s. 6d. net.

The Corrosion of Metals Group reports that relatively simple techniques have yielded new information on the conditions in which the presence of different anions in solution inhibits corrosion, and relationships established for mild steel have been shown to apply also for zinc. Some indication has been obtained of the relative corrosion rates of several metals at high temperatures and pressures and measurements of corrosion rates and electrochemical studies continue to furnish information on the influence of different strains of bacteria on corrosion processes, while nutritional studies are being used to extend our knowledge of the biochemistry of the bacteria. Data that should lead to improved methods of estimating the relative aggressiveness of both soils and waters are being acquired. Attention has been directed to the sintering of evaporated metal films with the view of extending the fundamental work on oxidation, and in this connexion an investigation of the interaction of hydrogen with pure iron has yielded results of general interest.

ATOMIC ENERGY RESEARCH IN AUSTRALIA

THE ninth annual report of the Australian Atomic Energy Commission* covers the year ended June 30, 1961, in which capital expenditure was reduced by £174,458 to £1,229,542. With the completion of major buildings at the Research Establishment expenditure on construction fell substantially. There was an increase of £60,254, however, in the amount required for research plant and for equipment needed as the new buildings came into use and net current expenditure on research rose by £256,232 with the growth of staff and research activity.

The seven main sections of the report deal respectively with the development of the uranium industry in Australia during 1944–61; the search for uranium; uranium mining; raw materials; research; nuclear power; and general activities. The financial accounts and details of research contracts, postgraduate research studentships and the names of the staff of the Commission are given in appendixes to the report.

Production of uranium oxide at Rum Jungle was the highest for three years. Output of copper also increased. An important new ore-body was established at Rum Jungle Creek South, ore reserves being placed at about 600,000 tons of ore of an average grade of approximately 6·0 lb. uranium oxide per ton. There is no copper in this deposit. The three companies producing uranium oxide for sale under contract to the United Kingdom Atomic Energy Authority continued to operate successfully. Rewards totalling £14,000 were paid for three uranium discoveries. Reward payments for new discoveries have now been discontinued.

The Commission's Research Establishment at Lucas Heights is organized into a number of research sections, and an Operations Division which is responsible for the operation of the research reactors *Hifar* and *Moata* and for the engineering and administrative services. Research continued on the high-temperature gas-cooled reactor system of the thermal breeder type (*HTGCR*) based on a beryllium moderator in the form of a metal or oxide, and operating on a uranium–thorium fuel-cycle. This type of reactor is considered to offer excellent prospects for economical nuclear power in Australia in the decade 1970–80. By June 1961 the Establishment had commissioned most of the major equipment and buildings essential to the research programme. The reactor *Hifar* completed a full year of routine operation in September, when it was overhauled and afterwards run at a maximum power-level of 10 MW. The new low-power (10 kW) graphite and water-moderated reactor *Moata* became critical on April 10. It provides a neutron source for reactor physics experiments.

The effect of neutron irradiation on the physical and mechanical properties of beryllium metal is being investigated by irradiation at high temperatures and at *Hifar* reactor temperatures, followed by post-irradiation annealing. The main damage was revealed to be the formation within the metal of new atoms of the gases helium and tritium, leading to swelling and distortion of the metal. The effects of strain heat, temperature and heat-treatment on the mechanical properties of beryllium are also being studied. Other fuel materials being investigated include beryllium oxide; a cermet, consisting of a dispersion of mixed uranium and thorium oxide in beryllium; and graphite fuel mixtures. Methods are being developed for the recovery of valuable materials from irradiated *HTGCR* fuels. The Commission's chemical processing research effort has been concentrated on the problems of fuels containing beryllium, and on beryllium recovery and purification, and results have been most encouraging. A time-of-flight system is under construction for the measurement of the neutron energy spectrum from reactor cores and is under test on *Hifar*. A theoretical group has extended earlier simpler calculations of the uranium-233–

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* Commonwealth of Australia: Australian Atomic Energy Commission. Ninth Annual Report for the year ended 30th June, 1961. Pp 84. (Coogee, N S.W. Australian Atomic Energy Commission, 1962.)

thorium–beryllium system to cover some aspects of burn-up and reactivity, but the full development of work on practical reactor behaviour must await better computing facilities.

Greater awareness in Australia of the potential of radioisotope applications is reported, and an increase is forecast in their uses in all fields, covering a wide range of techniques. However, in Australia radioisotope methods are still being used to a lesser extent than in other industrialized countries. Eighteen investigations were undertaken for outside organizations. In ten of these, radioisotopes produced in *Hifar* were used. Extensive assistance was given to the Hunter Valley Research Foundation in a study of silt movement in Newcastle Harbour. Other effluent tracer investigations were carried out for industrial organizations in New South Wales and Tasmania. A service was set up for labelling organic compounds with radioactive hydrogen (tritium). The long-term production of cobalt-60 for teletherapy use continued. At June 30, 1961, cobalt being irradiated had reached an estimated activity of 22,500 curies, of which approximately 80 per cent was at a sufficient strength for teletherapy use.

The main project of the small physics research group is the development, in conjunction with the Radiophysics Division of the Commonwealth Scientific and Industrial Research Organization, of a technique using radioisotopes to trace fine particulate matter in the atmosphere—namely, what happens to silver iodide when released at the base of a cloud. The Technical Physics Section evolved techniques for making solid-state α-particle detectors, using semiconductor materials, and research is being conducted into their performance and properties. Shortage of staff and laboratory space prevented much expansion of biological research; but some investigations have been made. In the section of the annual report dealing with health and safety it is reported that the quantity and variety of radioactive and toxic material stored and handled at the Research Establishment increased greatly during the year. Nevertheless, no person received a significant exposure to radiation or beryllium.

The Australian Institute of Nuclear Science and Engineering, with its headquarters at Lucas Heights, is an organization established jointly by the Commission and the Australian universities, and a portion of the annual report is devoted to its activities. The Establishment collaborated closely with the Institute in more than thirty projects, details of which are given in the report. An Institute Fellow was appointed to work at the Research Establishment on improving existing methods and developing new methods of using radioactive techniques in geological age determinations. The Commission awarded a number of undergraduate scholarships each year during 1954–57 as part of its plan to overcome a shortage of graduates in geology, geophysics, metallurgy and chemical engineering. A total of 28 students was awarded scholarships; the majority have since graduated and ten students were still undergoing training during 1960. Fifty-four separate research contracts have been awarded by the Commission to universities and hospitals in Australia, of a total value of £269,000.

S. WEINTROUB

UNIVERSITY ORGANIZATION IN INDIA

THE Proceedings of the Third Conference of the Vice-Chancellors of Indian Universities*, convened by the University Grants Commission and the Ministry of Education at New Delhi during October 28–29, 1961, include a notable inaugural address by Dr. K. L. Shrimali, Minister of Education, which indicates a striking contrast in the attitude of the Indian Government to universities compared with that of the South African Government. Dr. Shrimali was deeply concerned over recent events in Aligarh Muslim University and was fully aware that they represented a major failure of education, but it was for the universities themselves to correct this prejudice, intolerance and bigotry. They were not concerned solely with the training of minds but also with the education of emotions, cultivation of good taste and sense of decency and the development of character. Dr. Shrimali thought that the tutorial system could provide the answer to most problems, but, as free corporations, universities had the authority to make what changes they considered necessary to discharge their responsibilities to society, and a high sense of responsibility on the part of the universities was the essential condition for the preservation of their autonomy. Dr. Shrimali recognized that both Government and universities were liable to err, and it was their duty to correct each other, but the only way in a free society was to reach agreement through discussion. The whole temper of Dr. Shrimali's address was in line with Sir Eric Ashby's recent Chancellor's Lecture to the University of the Witwatersrand, and he urged that, more than money, to improve the quality of education are needed dedicated teachers who would stimulate thinking among the students, give them a vision of true greatness and inspire faith to dedicate themselves to the realization of great ideals.

The Proceedings also incorporate the address of Dr. D. S. Kothari, chairman of the University Grants Commission, on education and the universities, discussing the expansion of Indian universities. In this Dr. Kothari deals with the acute problem of student wastage and measures to reduce this and some proposals of the report on general education, such as refresher courses and seminars for a selected number of college and university teachers, the establishment of centres of advanced study on an all-India basis and promotion of academic mobility (for which purpose the universities must be bilingual, using English and the regional language). Establishment of about 12 new universities is envisaged during the Third Five Year Plan, issued in June 1961, to provide the anticipated additional 100,000 places required to keep pace with the expansion of the population. The Proceedings also include the recommendations of the Committee on Standards of Education, the Committee on Instruments of Education (dealing with entrance qualifications and tests for admission, training of teachers, quality and availability of university text-books, common calendar of terms, examination dates and publication of results and uniformity of standards) and the Committee on 'Emotional Integration', which considered the preparation of text-books, the role of student unions and the resolution of religious conflicts. It was recommended that all universities which make available a certain minimum number of places for students from other parts of India and those bearing denominational names should discard the denominational component of their title. The Committee on Standards of Education recommended an increase in the number of technical schools, polytechnics, trade schools, etc., and improvement of the student–teacher ratio to at least 1 : 7 or 1 : 10. English was considered the only appropriate medium of instruction in postgraduate education at present, and the Committee recommended that the University Grants Commission should play a more active part in the development of all higher education. Research centres should be built up around outstanding personalities.

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* Government of India. University Grants Commission and Ministry of Education. Proceedings of the Vice-Chancellors' Conference, 1961. Pp. v+58. (New Delhi: University Grants Commission and Ministry of Education, 1962.)

THE CHALLENGE OF HUNGER

THE greatest challenge that faces mankind to-day and in the coming decades is the problem of feeding the growing population of the world. While the world population is increasing at an unprecedented rate, food production in the areas of the world where most people do not have enough to eat is lagging. Thus, to-day, mankind as a whole is not being adequately fed, and there is a population increase of such dimensions that by the end of this century—possibly earlier—the number of mouths to be fed will be more than double.

For several years the Food and Agriculture Organization of the United Nations has pointed out the dangers inherent in this situation. It has stressed that food production as a whole, but especially in the underdeveloped and developing countries, is not increasing fast enough. The rate of population-growth in the developing countries is more than 2 per cent, while the volume of agricultural production is increasing at slightly less than 3 per cent per annum —a margin that is clearly precarious. Any major set-back in agriculture in any part of the world, owing to climatic or other natural factors, could quickly wipe out the gains which have been so painfully won.

Statistics set out by Dr. Binai Ranjan Sen, the director-general of the Food and Agriculture Organization, reveal the realism of this warning. Estimates of world agricultural production in 1960–61 (excluding mainland China) show an increase of no more than 1 per cent over production in the previous season—which is less than the estimated rate of population growth of 1·6 per cent. If mainland China is included in the world agricultural production figures, the total production of 1960–61 would probably be less than that of 1959–60 instead of a 1 per cent increase. Set-backs due to bad weather in several regions of the world indicate that the 1961–62 production may again be small.

Since the Second World War there has been a great expansion in the production of food and agricultural products, but by far the largest part of this has taken place in Europe, in North America and Oceania. All these are countries where standards of living are high and the people are well fed and adequately nourished. In the food-deficient regions of Africa, Asia and Latin America, the increases have so far barely kept pace with the population increase. Thus, although the Far East Region, which is the worst nourished and most populous area in the world, has had a good harvest for three successive years and, excluding mainland China, the 1960–61 food production per head has only brought consumption levels up to those of 1939.

In other words, improvements in diets which could have been expected as a result of increases in food production have, in fact, been largely nullified by population increase. In Latin America, for example, *per capita* food production continues to lag behind pre-war levels. In the Near East, however, *per capita* production is now about 10 per cent greater than the low levels prevailing in the pre-war period. In Africa, a rather stationary situation prevailed with some ups and downs. These regional figures reveal wide differences among individual countries.

A statistical survey of the incidence of hunger and malnutrition was recently made by Dr. P. V. Sukhatme, director of the Statistics Division of the Food and Agriculture Organization, based on the international reference scale for calorie requirements and on the variation in energy expenditure among men of the reference age group. Applying the Food and Agriculture Organization calorie standards as a basis of comparison for the quantitative aspects of the diet, the study showed that calorie supplies in Europe, North America and Oceania are of the order of more than 3,100 calories *per capita* per day and exceed the requirements by about 20 per cent. In the Near East, Africa and Latin America, they are about equal to the requirements, amounting to some 2,400. In the Far East, the supplies are of the order of only 2,050 compared with a calorie requirement of some 2,300. The supply thus falls short of the requirement by 11 per cent.

This gap is particularly striking when compared with the total calorie supplies of other regions. Thus, the shortage in the Far East is of a quantity large enough to feed the entire population of the Near East. Even for other regions where the overall picture looks balanced, there are wide variations within the region. In the Near East, for example, there is apparently enough food in a group of countries where one-half of the population lives: the other half lives in a relatively poorer area. The calorie supplies of this area are only 2,100–2,200 *per capita* per day. It is therefore justifiable to conclude that many millions of people in the world still go hungry at least for a part of their lives.

Thus, the recent study conducted by the Food and Agriculture Organization* has shown that the gap of 11 per cent between the calorie supplies and the calorie requirements in the Far East is shared by one-fifth to one-fourth of the population of the region. Since this region comprises half the total world population, it is clear that a conservative estimate of the proportion of undernourished in the world is between 10 and 15 per cent. In actual numbers this means that between 300 and 500 million go hungry for part of their lives, even in normal times.

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* *UNESCO Courier*, July–August (1962).

ELECTRON TRANSFER AND CARCINOGENESIS

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THE idea that electron transfer may be involved in chemical carcinogenesis was first proposed by us[1,2]. We postulated that the interaction of carcinogenic aromatic hydrocarbons with cellular receivers, which according to our general theory must occur through the *K* region of the hydrocarbons, involves the spreading out of the π electrons of the hydrocarbon into the bonds which unite the hydrocarbon to the cell. These bonds would then become partially double, the whole electronic cloud of the hydrocarbons assuming an approximately quinonoid configuration. The advantage of this hypothesis resides in the fact that it enables an interpretation of the metabolic transformations undergone by these carcinogens. Experimental results obtained by Bhargava and Heidelberger[3] seem to confirm the quinonoid nature of the bonds between the hydrocarbon and the tissue.

In our theory the existence of such an electron transfer between the carcinogen and the cell is dependent on certain electronic characteristics of the *K* and *L* regions of the carcinogens which constitute the primary conditions for the manifestation of carcinogenic activity. It may be added that within the series of carcinogenic molecules satisfying the primary conditions related to the *K* and *L* regions (aromatic hydrocarbons or methylbenzanthracenes) restricted correlations have been observed between carcinogenicity and the energy of the electronic transition to the lowest excited state or the ionization potential of the carcinogens[2,4].

Recently, a theory has been proposed which tries to link carcinogenesis with the possibility of an electron transfer in a more fundamental and specific way. Quantum mechanical calculations[5] having indicated the possibility of the existence of energy bands in proteins, Mason[6-9] has postulated that carcinogenic activity depends directly on the existence of an electron transfer from the highest filled band of the protein to one of the empty levels of the hydrocarbons. He postulated that in order for such a transfer to occur, an unfilled level of the hydrocarbon must match in energy with the highest filled level of the protein. Assuming that the highest filled molecular orbital of the hydrocarbon is at the same height as the bottom filled band of the protein, he arrives at the conclusion that in order to be carcinogenic an aromatic hydrocarbon must possess a transition energy in the range of 1·10–1·18, in units of β (the resonance integral of the usual Hückel approximation of the molecular orbital method).

It must be stressed in the first place that the basic physical hypothesis of Mason (the existence of a charge transfer from a protein to the hydrocarbon) is at present entirely arbitrary. Moreover, even if the principle of such a hypothesis is accepted, strong objections may be raised[10-14] to the model proposed by Mason. It has, in particular, been observed that according to the general theory of charge transfer complexes, the matching of energy-levels is not necessary for the formation of such complexes, which depend rather on the ionization potential of the donor and the electron affinity of the acceptor. It has also been observed that the adjustment of the energy-levels of the hydrocarbon and the protein should be made with respect to the free electron as energy zero and not by equating the energy of the lower filled band of the protein with the highest filled level of the hydrocarbon. Such equating leads to the erroneous prediction that the ionization potentials of aromatic hydrocarbons of the dimension of the carcinogens should be of the order of 11–12 eV, while most probably they are of the order of 7 eV [14]. Moreover, recent refined calculations on the possible energy bands in proteins[15-17] lead to results appreciably different from the older ones used by Mason.

In fact, stripped of its arbitrary and superfluous assumptions, Mason's proposition amounts simply to the existence of a correlation between carcinogenicity and the presence in a molecule of a transition energy between a filled and an empty orbital in the range $1{\cdot}10\beta$–$1{\cdot}18\beta$. The proposition is supposed to be valid whatever are the orbitals involved in the transition. Stated in this rigorous way the correlation scarcely has any physical significance. We wish to show, moreover, that the correlation is far from being as satisfactory as claimed by Mason.

Mason investigated 23 cata-condensed aromatics comprising 6 carcinogens and 17 inactive compounds. His criterion allows him to distinguish between the active and the inactive molecules, with two exceptions. When overlap is included in the calculations (in which case the 'active' range of the transition energies should be, following Mason, 1·248–1·348, in units of γ, the resonance integral with overlap) the exceptions are more numerous. To justify this result, Mason states[7] that the calculations without overlap have been shown to be somewhat better than the calculations with overlap in other investigations. This statement, however, is unfounded. Usually, the introduction of overlap does not change the general validity of the results. In problems involving the energies of the molecular orbitals and the transition energies of aromatic hydrocarbons, as is the case here, it definitely represents a refinement because it suppresses the degeneracy of the transitions.

In order to check the validity of Mason's proposed correlation we have extended the investigations to a series of other cata-condensed aromatic hydrocarbons, which have been investigated for carcinogenic activity. The compounds studied included the weakly carcinogenic 1,2,5,6-dibenzphenanthrene and 1,2,3,4-dibenzphenanthrene, the strongly - carcinogenic 1,2,3,4-dibenzpyrene, the very potent carcinogen 3,4,9,10-dibenzpyrene and fifteen inactive compounds. The results are indicated in Table 1, where it can be seen that Mason's correlation breaks down completely in these compounds. Thus, when the calculations are carried out without overlap, three carcinogens (including the extremely potent 3,4,9,10-dibenzpyrene) out of four have no transition energy in the 'active' range and, following Mason's criterion, should not be carcinogenic. On the con-

Table 1. TRANSITION ENERGIES IN AROMATIC HYDROCARBONS IN THE LIMITS OF MASON'S 'ACTIVE' RANGE

Compound	Transition energy Without overlap (in β units)	Transition energy With overlap (in γ units)	Carcinogenic activity
1,2,3,4-Dibenzphenanthrene	—	1·320, 1·334	+
1,2,5,6-Dibenzphenanthrene	1·100, 1·152	—	+
1,2,3,4-Dibenzpyrene	—	—	+ + +
3,4,9,10-Dibenzpyrene	—	1·319	+ + + +
1,2,6,7-Dibenzpyrene	1·110	1·296, 1·312	—
2′,3′-Naphtho-3,4-pyrene	—	1·327, 1·331	—
1,2,7,8-Dibenznaphthacene	—	1·252	—
1,2,9,10-Dibenznaphthacene	1·161	1,285, 1·297 1·329, 1·331	—
1,2,3,4-Dibenznaphthacene	1·102	1·276, 1·322	—
2,3,7,8-Dibenzphenanthrene	1·109	1·310	—
2,3,5,6-Dibenzphenanthrene	—	1·321	—
1,2-Benzpyrene	—	1·317	—
2,3,8,9-Dipenzperylene	1·115	1,263, 1·303 1·341	—
1,2-Benznaphthacene	1·172	1·267	—
2′,1′-Anthra-1,2-anthracene	1·135, 1·140	1·254, 1·299 1·314, 1·322	—
1′-2′-Anthra-1,2-anthracene	1·152	1·270, 1·294 1·318, 1·320	—
4,5,10,11-Di(1′,2′-naphtho)-crysene	1·178	1·253, 1·295 1·309, 1·316 1·343, 1·345	—
3,4,9,10-Di(2′,3′-naphtho)-pyrene	—	1,295, 1·313	—
Naphthodianthrene	1·177	1·276	—

trary, ten of the fifteen inactive hydrocarbons have a transition energy in the 'active' range and, following the same criterion, should therefore all be active. The disagreement between Mason's theory and experiment is even more striking when the calculations with overlap are taken into account: two carcinogenic hydrocarbons have a transition energy in the 'active' range, but this is also the case for all the non-carcinogenic compounds. A number of inactive compounds even have a few (up to six) transition energies in the limits of Mason's 'active' range. The only molecules which do not possess a transition energy in Mason's 'active' range are two carcinogens.

Mason has also included in his correlation a number of molecules other than the cata-condensed aromatics. There is no need to discuss this extension of Mason's theory here because it is obvious that it is very easy to find a great number of molecules of these other types which do not obey the correlation.

One of the foreseeable results of the attractiveness and at the same time arbitrariness of Mason's proposition was to give rise to a series of similar and just as unfounded propositions. Mason postulated electron-transfer from a protein to the carcinogen; Birks[12,18] suggests electron transfer in the reverse direction, from the carcinogen to the protein, and Ladik *et al.*[19,20] claim that the process may involve the nucleic acids rather than the proteins. At present none of these propositions has any acceptable basis. It may be said quite generally that the fact that, in the limited series of the carcinogenic hydrocarbons, restricted correlations may be established with some electronic characteristics such as the ionization potential or the transition energy to the first excited state cannot be of primary significance when a great number of non-carcinogenic hydrocarbons have the same characteristics. We may recall that the *K–L* regions theory of carcinogenesis, on the contrary, accounts satisfactorily for the activity or inactivity of practically all the aromatic hydrocarbons. The conditions imposed on the *K* and *L* regions seem thus to be the primary necessities for the existence of carcinogenic activity. On the other hand, the hypothesis that a 'local' electron transfer may be involved in carcinogenesis has found some experimental confirmation in the recent work of Szent-Gyorgyi *et al.*[21] on the formation of charge transfer-complexes between carcinogens and iodine. The involvement of such a transfer in carcinogenesis is, however, subject to the fulfilment of the primary conditions for the interaction between the carcinogen and the cellular receiver, based on the characteristics of the *K* and *L* regions.

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CHEMICAL HETEROGENEITY OF HÆMOGLOBIN O

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WE wish to report on two samples of hæmoglobin O (Hb–O), characterized by identical electrophoretic and chromatographic properties. Hb–O has first been described in Indonesia by Lie-Injo Luan Eng and Sadono[1]. Ramot *et al.*[2] have since reported the occurrence of Hb–O in an Arab family, in which hæmoglobin S (Hb–S) was also observed. In this family the Mendelian inheritance of the gene responsible for Hb–O was also shown.

Two samples of blood containing Hb–O have been examined: sample I came from an Indonesian woman seen in London and one may presume that the Hb–O

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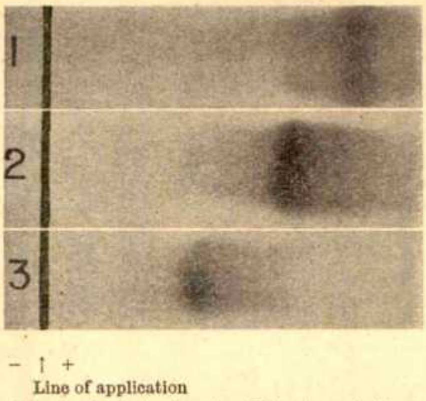

Fig. 1. Starch-gel electrophoresis of the hæmoglobins isolated from sample II. 1, Hb-F; 2, Hb-S; 3, Hb-O$_{\text{Ar}}$

of this individual may be identical with the type described by Lie-Injo Luan Eng and Sadono[1]. Sample II came from a member of the Arab family described by Ramot *et al.*[2], namely the sister of the propositus. We will refer to the Hb–O in samples I and II as Hb–O Indonesia and Hb–O Arab (Hb–O$_{\text{Indon}}$ and Hb–O$_{\text{Ar}}$) respectively.

The two samples were subjected to preparative column chromatography on carboxymethylcellulose according to the method of Huisman and Meyering[3]. The identity and the purity of the hæmoglobins eluted from the columns were determined by starch-gel electrophoresis according to Poulik's method[4] after concentration on small columns of carboxymethylcellulose. Two hæmoglobins were isolated from sample I; they were shown by starch-gel electrophoresis to be hæmoglobin A (Hb–A) and Hb–O$_{\text{Indon}}$. Three hæmoglobins were isolated from sample II: fœtal hæmoglobin (Hb–F), Hb–S and Hb–O$_{\text{Ar}}$. Hb–F was eluted as the first and Hb–O as the last peak. The starch-gel electrophoresis of the hæmoglobins isolated from sample II is shown in Fig. 1.

No Hb–A was found in sample II. Ramot *et al.*[2] had found Hb–A as well as the abnormal adult hæmoglobins Hb–O and Hb–S in the propositus and his sister. When this newly obtained sample was now found to contain no Hb–A, the propositus was re-examined and again no Hb–A was found on this occasion. A careful search for evidence of a previous blood transfusion had been made by Ramot *et al.*[2] before they accepted the finding of three adult hæmoglobins A, O and S as representing a genuine blood dyscrasia, and a transfusion had also been strenuously denied by the parents of the children. However, in view of the present observations, it must be assumed nevertheless that the children had been transfused at some time when the nomadic family had been receiving medical care before they were seen in Israel and that the third adult hæmoglobin – A was an acquired and not an inherited character.

The hæmoglobins isolated were heat-denatured and digested with trypsin and the tryptic digests were analysed by 'fingerprinting' according to Ingram[5], using the different chromatographic solvent described by Baglioni[6]. The pattern of the tryptic peptides of Hb–O$_{\text{Indon}}$ was found to be identical to that obtained from Hb–A. The absence of any detectable difference in the pattern of the soluble tryptic peptides suggested that a chemical difference could possibly be found among the insoluble peptides obtained by tryptic digestion, which are removed by centrifugation prior to fingerprinting. It has been shown by Hunt and Ingram[7] that after tryptic digestion about one-third of the hæmoglobin molecule (the so-called 'core') is insoluble at neutral *p*H.

The cores of Hb–A and of Hb–O$_{\text{Indon}}$ were isolated, digested with chymotrypsin and fingerprinted. The pattern of peptides obtained from these two cases were found to differ. In the Hb–A core fingerprint two spots give a strong positive reaction for histidine when tested with the Pauly reagent; these two spots were found to be missing in the Hb–O$_{\text{Indon}}$ core fingerprint. One histidine-positive spot was observed in the Hb–O$_{\text{Indon}}$ core fingerprint which was not present in the Hb–A core fingerprint; this spot is indicated by an arrow in the Hb–O$_{\text{Indon}}$ core fingerprint shown in Fig. 2.

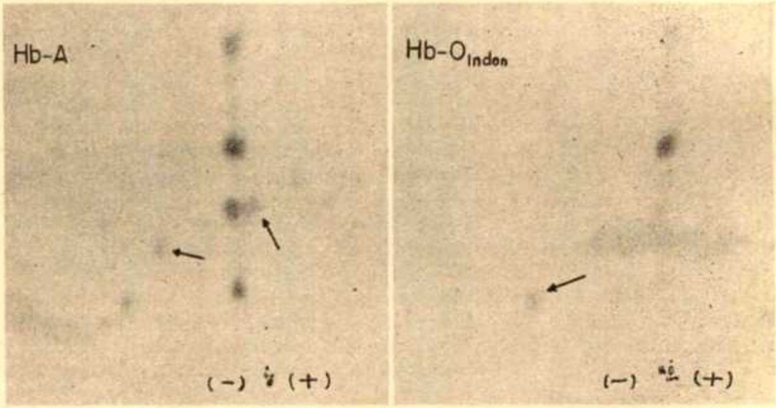

Fig. 2. Fingerprints of the chymotryptic digests of the cores of Hb-A and of Hb-O$_{\text{Indon}}$. The arrows in the Hb-A core fingerprint indicate the two peptides missing in Hb-O$_{\text{Indon}}$ core fingerprint. The arrow in Hb-O$_{\text{Indon}}$ core fingerprint indicates the extra peptide, which gives a positive reaction for histidine

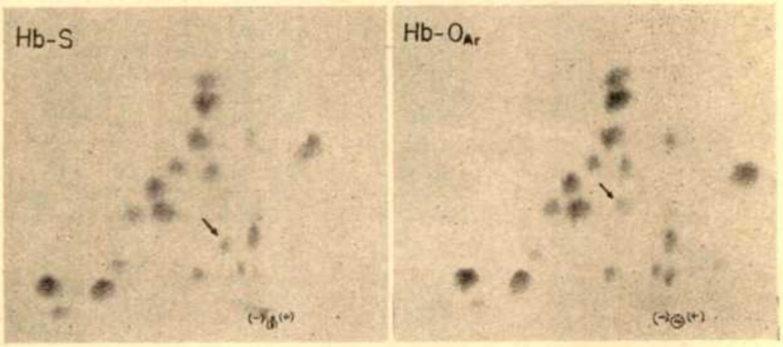

Fig. 3. Fingerprints of the tryptic digests of Hb-S and of Hb-O$_{\text{Ar}}$ isolated from sample II. Arrows indicate the abnormal peptides characteristic of these two hæmoglobins

The core was also prepared from isolated α- and β-chains of Hb–A, donated by Dr. Michael Naughton, Division of Biochemistry, Massachusetts Institute of Technology. They were digested with chymotrypsin and fingerprinted[7,8]. It was shown that the two peptides missing in the Hb–O$_{\text{Indon}}$ core fingerprint correspond to two peptides obtained from the α-chain core; no peptides occupying the same position in the peptide map have been observed in the fingerprint of the β-chain core. It thus seems likely that a chemical difference exists between Hb–A and Hb–O$_{\text{Indon}}$ in that part of the amino-acid sequence of the α-chain corresponding to the core, that is, from residue 100–139.

The hæmoglobins isolated from the Arab child were also fingerprinted. The peptide patterns of Hb–F and Hb–S were found to correspond to the pattern described by Hunt[9] and Ingram[5] for these hæmoglobins. In the Hb–O$_{\text{Ar}}$ fingerprint one peptide was seen to have moved from the band containing uncharged peptides to a new position nearer the cathode. This peptide gave a positive reaction for tyrosine and was identified as peptide 5. In Fig. 3 are shown the fingerprints of Hb–S and Hb–O$_{\text{Ar}}$ obtained from sample II; the peptides altered in Hb–S and Hb–O are indicated by arrows.

A peptide pattern somewhat similar to that observed in Hb–O$_{\text{Ar}}$ has been described by Benzer, Ingram and Lehmann[10] for one type of Hb–D, designated first as Dγ and later as D$_{\beta\ \text{Punjab}}$. Bowman and Ingram[11] have examined an unrelated sample of Hb–D showing the same peptide pattern and have localized the peptide alteration in the β-chain of this hæmoglobin. In a recent investigation by Baglioni[12] several samples of Hb–D were shown to be identical to this type of hæmoglobin D. In this hæmoglobin the glutamic acid residue in position 121 of the β-chain of Hb–A is substituted by a glutamine residue.

The peptide altered in the Hb–O$_{\text{Ar}}$ has been prepared by high-voltage electrophoresis and paper chromatography, following the same procedure which was used to isolate the peptide in Hb–D$_{\beta\ \text{Punjab}}$. The amino-acid composition of the peptide altered in Hb–O$_{\text{Ar}}$ designated peptide O$_{\text{Ar}}$–5, of D$_{\beta\ \text{Punjab}}$–5, and of A–5 are shown in Table 1; the analysis of peptides A–5 and D$_{\beta\ \text{Punjab}}$–5 have been reported previously[12].

Peptide O$_{\text{Ar}}$–5 shows the presence of two glutamic acid residues instead of the three found in peptide A–5. The *N*-terminal amino-acid of peptide O$_{\text{Ar}}$–5 has been determined by Sanger's method (1955); dinitrophenyl phenylalanine was identified by two-dimensional paper chromatography according to Frankel-Conrat *et al.*[13]. The results of the *N*-terminal amino-acid determination and of the sequence studies on peptides A–5 and D$_{\beta\ \text{Punjab}}$–5 previously reported are shown in Table 2.

The results of the amino-acid analysis of peptide O$_{\text{Ar}}$–5 and of the *N*-terminal amino-acid determination suggest that the residue corresponding to the *N*-terminal glutamic acid of A–5 is missing in O$_{\text{Ar}}$–5. This finding can only be accounted for by a substitution of an arginine or a lysine residue in Hb–O$_{\text{Ar}}$ for the glutamic acid occupying position 121 in Hb–A. The peptide bond between the arginine or lysine and the phenylalanine in position 122 could be split by trypsin, and phenylalanine, which is the second residue in peptide A–5, becomes the *N*-terminal of peptide O$_{\text{Ar}}$–5. No free arginine has been observed in the fingerprint of Hb–O$_{\text{Ar}}$; one would expect free arginine to be released from a . . . Lys. Arg. Phe . . . sequence. Most likely the glutamic acid in position 121 is substituted by a lysine residue in Hb–O$_{\text{Ar}}$. This substitution accounts for the difference in electrophoretic mobility between Hb–A and Hb–O$_{\text{Ar}}$; Hb–O$_{\text{Ar}}$ migrates on paper or starch electrophoresis close to Hb–C and Hb–E, in which as Hunt and Ingram[14] have shown a lysine residue replaces a glutamic acid residue. It is noteworthy that two different substitutions have been found to involve residue 121, in Hb–O$_{\text{Ar}}$ and in Hb D$_{\beta\ \text{Punjab}}$. A similar situation has been previously observed for Hb–S and Hb–C; a valine in Hb–S and a lysine in Hb–C substitutes for a glutamic acid in position 6 of the β-chain of Hb–A[14].

Table 1. AMINO-ACID ANALYSIS OF PEPTIDE A-5, D$_{\beta\ \text{Punjab}}$–5, AND O$_{\text{Ar}}$–5

	A-5	D-5	O$_{\text{Ar}}$–5
Threonine	0·95	0·89	0·94
Alanine	2·16	2·16	1·85
Glutamic acid	2·84	2·88	1·87
Proline	1·97	2·14	2·14
Valine	1·15	0·93	1·27
Tyrosine	0·71	0·64	(0·13)
Phenylalanine	1·01	1·14	0·91
Amount of peptides (μM)	0·26	0·07	0·24

The molar ratio of the amino-acids is given, and the peptides are defined as βTp XIII according to the recommendations for the nomenclature of hæmoglobins by Gerald and Ingram (ref. 18).

The two samples of Hb–O examined carry chemical alterations in different parts of the hæmoglobin molecule: Hb$_{\text{Indon}}$ in the α-chain and Hb–O$_{\text{Ar}}$ in the β-chain. Chemical heterogeneity of hæmoglobins, which behave identically on chromatography and electrophoresis, has previously been shown by Benzer *et al.*[10]. Three samples of Hb–D obtained from unrelated individuals living in different countries were found to carry chemical changes in different parts of the molecule. Three samples of Hb–G have also been shown to carry different chemical changes[15–17]. According to Gerald and Ingram's[18] recommendations for the nomenclature of hæmoglobins, Hb–O is defined as $\alpha_2^A\ \beta_2{}^{121\text{Lys}}$.

While this work was in progress yet a third example of Hb–O was found by Lehmann and Vella[19], this time in the Sudan. They studied this sample by hybridization methods and found it to be a β-chain abnormal hæmoglobin. They direct attention to a possible anthropological significance of hæmoglobin O,

Table 2. SEQUENCES OF PEPTIDES A-5, D$_{\beta\ \text{Punjab}}$–5 AND O$_{\text{Ar}}$–5

Residue No. in the β-chain of Hb-A	121 —	122 —	123 —	124 —	125 —	126 —	127 —	128 —	129 —	130 —	131 —	132
A-5	Glu	Phe	Thr	Pro	Pro	Val	NH_2 | Glu	Ala	Ala	Tyr	NH_2 | Glu	Lys
D$_{\beta\ \text{Punjab}}$–5	NH_2 | Glu	(Phe	Thr	Pro	Pro	Val	NH_2 | Glu	Ala)	Ala	Tyr	NH_2 | Glu	Lys
O$_{\text{Ar}}$–5		Phe	(Thr	Pro	Pro	Val	NH_2 | Glu	Ala	Ala	Tyr	NH_2 | Glu)	Lys

The sequences of residues in brackets have not been determined.

one abnormal in the α-chain found in an Indonesian and presumably the same Hb–O as that seen by Lie-Injo Luan Eng and Sadono[1] in that part of Asia, and the other Hb–O abnormal in the β-chain, and seemingly associated with Arabs.

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CHROMOSOMAL REARRANGEMENTS AND THE EVOLUTION OF HAPTOGLOBIN GENES

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THE three common types of haptoglobin (Hp 1–1, 2–1 and 2–2)[1–3] have recently been divided into a total of six types (Hp 1F–1F, 1F–1S, 1S–1S, 2–1F, 2–1S and 2–2)[4] on the basis of electrophoretic differences in their component polypeptide chains dissociated by treatment with a thiol in the presence of urea. Family studies[5] indicate that these types are the expression of the combinations of three alleles, Hp^{1F}, Hp^{1S} and Hp^{2}, each of which gives rise to the production of a corresponding polypeptide chain (hp 1Fα, hp 1Sα and hp 2α). All haptoglobins contain an additional polypeptide chain, the β-chain, which appears to be unaffected by the genotype at the *Hp* locus.

Structure of haptoglobin α-polypeptide chains. The results of our investigations[6] of the differences between the three common haptoglobin α-polypeptide chains may be summarized in part as follows: Amino-acid analyses of hp 1Fα and hp 1Sα suggest the replacement of a single lysine residue in hp 1 Fα by an acidic amino-acid (or its amide) in hp 1Sα. In agreement with these analyses we find that the peptides obtained from hp 1Fα and hp 1Sα after chymotryptic digestion are indistinguishable by finger-printing[7] except for the replacement of one peptide (F) in hp 1Fα by another peptide (S) in hp 1Sα. The effect of *p*H on the mobilities of the α-chains and the native haptoglobins is also consistent with the substitution of a basic amino-acid in hp 1Fα by an amidated acidic amino-acid in hp 1Sα. On the other hand, although the amino-acid composition of hp 2α is very similar to the compositions of hp 1Fα and hp 1Sα, the peptides obtained from hp 2α differ from those obtained from hp 1Fα and hp 1Sα (Fig. 1) more extensively than can be accounted for by amino-acid substitutions. Thus, all peptides present in digests of hp 1Fα and hp 1Sα are found in digests of hp 2α together with an extra peptide (*J*); two peptides (*N* and *C*), which are common to all three α-chains, are relatively deficient in hp 2α. The amino-terminal amino-acid of peptide *N* and of the whole α-chains is valine, and the carboxy-terminal amino-acid of peptide *C* and of the whole of α-chains is glutamine, which suggests that these peptides originate from the ends of the α-chains. Amino-acid

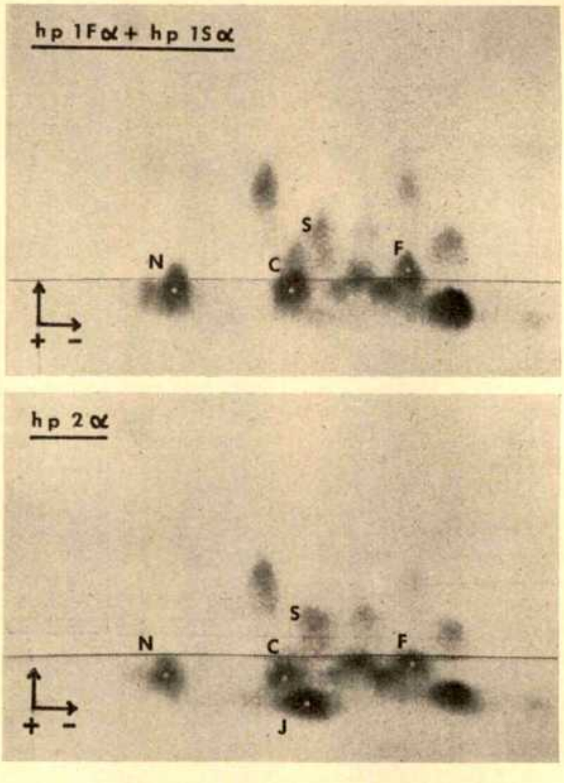

Fig. 1. Peptides from α-chymotrypsin digests of hp 1Fα + hp 1Sα, and hp 2α. The peptides labelled *F* and *S*, characteristic of hp 1Fα and hp 1Sα, both occur in hp 2α. Peptides *N* and *C* are relatively deficient in hp 2α; *J* is additional

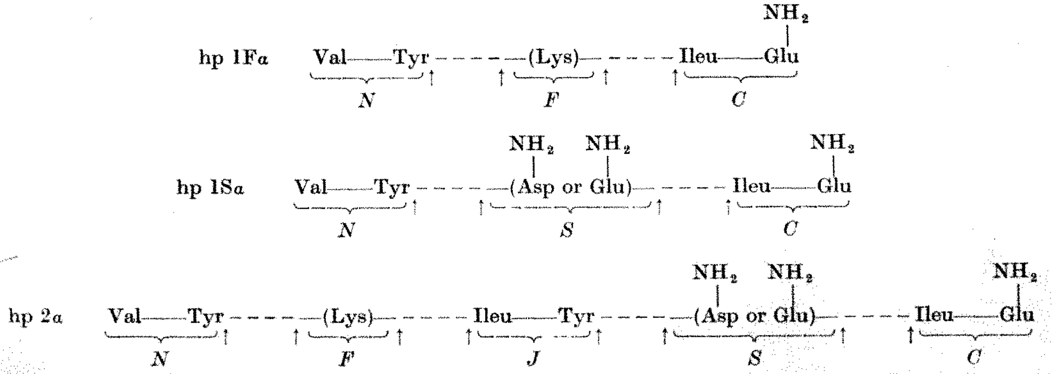
hp 1Fα
Val——Tyr - - - - - -(Lys)- - - - - Ileu——Glu
NH$_2$
N
F
C
hp 1Sα
Val——Tyr - - - - - -(Asp or Glu)- - - - - Ileu——Glu
NH$_2$ NH$_2$ NH$_2$
N
S
C
hp 2α
Val——Tyr - - - - -(Lys)- - - - - Ileu——Tyr - - - - -(Asp or Glu)- - - - - Ileu——Glu
NH$_2$ NH$_2$ NH$_2$
N
F
J
S
C

Fig. 2. Outlines of the proposed structures for the three common haptoglobin α-polypeptide chains showing their characteristic features. The order of peptides F and S in hp 2α is arbitrary and could be reversed. The arrows indicate points of attack of chymotrypsin. Amino-acids at the ends of the chymotryptic peptides have been determined by the dinitrofluorobenzene and carboxypeptidase methods; amino-acids in parentheses are present in the corresponding peptides but their positions are not known

analyses of the peptides *N*, *C* and *J* and examination of the peptides obtained from them by further enzymatic digestion with subtilisin or trypsin, suggest that *J* is slightly smaller than the peptide which would be obtained by joining *C* and *N* with a peptide bond. Peptide *J* has the same amino-terminal amino-acid (isoleucine) as *C*, and the same carboxy-terminal amino-acid (tyrosine) as *N*. Furthermore, almost all our preparations (see following) of the polypeptide hp 2α show both the peptides (*F* and *S*) characteristic of hp 1Fα and hp 1Sα. These finding are understandable if almost the whole of the amino-acid sequence of the hp 1α polypeptides occurs twice in a single molecule of hp 2α, and lead us to suggest the structures for the three haptoglobin α-polypeptides shown in outline in Fig. 2.

The general correctness of the proposed structures has now received confirmation from measurements of the molecular weights of the α-chains, and of the yields from them of *N*-terminal amino-acid (valine) and of C-terminal amino-acid (glutamine), as shown in Table 1.

Table 1

	Molecular weight*	*N*-valine† (per mg)	*C*-glutamine‡ (per mg)
hp 1Fα	8,860 ± 400	0·034 μM	0·063 μM
hp 1Sα		0·035 μM	
hp 2α	17,300 ± 1,400	0·021 μM	0·032 μM

* By Archibald method.
† By dinitrofluorobenzene method, uncorrected for losses during hydrolysis and chromatography.
‡ By carboxypeptidase method, uncorrected.

Evolution of haptoglobin genes. The substitution of one amino-acid residue in hp 1Fα by a different residue in hp 1Sα indicates that the genes Hp^{1F} and Hp^{1S} may differ only in a single base pair, as has been suggested for the genes controlling the hæmoglobin variants *A*, *S*, *C*, etc.[8]. On the other hand, the structure of hp 2α, in which most of the amino-acid sequence of the hp 1α polypeptides is repeated in a single molecule, indicates that the gene Hp^2 did not evolve from Hp^{1F} or Hp^{1S} by mutations involving small changes in base composition. The simplest hypothesis we can suggest (outlined in ref. 6) is that the gene Hp^2 was evolved from Hp^{1F} and Hp^{1S} by non-homologous crossing-over occurring in a heterozygous individual Hp^{1F}/Hp^{1S}. The suggested mechanism is illustrated diagrammatically in Fig. 3, and is similar to the type of process proposed by Serebrovsky[9] to account for chromosomal rearrangements in general. The net result of this non-homologous crossing-over is the production of one chromosome containing a small duplication and one with a corresponding deletion.

Chromosomal rearrangements and gene evolution. Duplication of existing genes as an important factor in evolution has been discussed previously by several workers (for example, Bridges[10], Lewis[11], and Ingram and Stretton[12]), but the consequences at the molecular level of *partial* gene duplications do not appear to have been foreseen. Our studies with the haptoglobin system indicate that partial gene duplications can lead to the production of a continuous polypeptide which 'bridges' the beginning of the duplication. We use the term 'gene' here, and in what follows, to mean that portion of the DNA coded for a single polypeptide chain, such as an α-chain of haptoglobin. No assumptions as to the length or nature of the DNA between such genes are

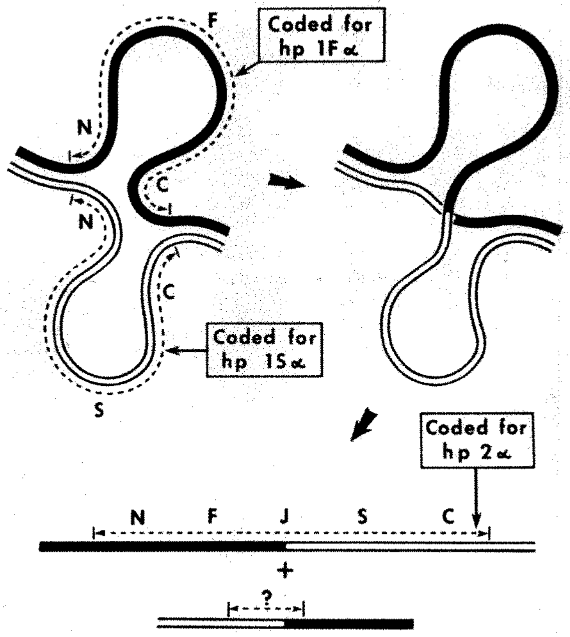

Fig. 3. Diagrammatic representation of the possible evolution of the gene Hp^2 from Hp^{1F} and Hp^{1S} by non-homologous crossing-over in a heterozygote. The parental chromatids involved are distinguished by double and heavy lines. The regions coded for the α-polypeptides are indicated by dotted lines, and the letters locate the approximate regions corresponding to the chymotryptic peptides *N*, *F*, *S*, *C* and *J* referred to in the text

made, although we assume that the beginnings and/or ends of genes are specified in some way, and we recognize that the relative frequency but not the possibility of occurrence of some of the events to be discussed may be influenced by the presence and the amount of any DNA not coded for amino-acid sequences.

Partial gene duplications may or may not be completely functional so far as protein synthesis is concerned, depending on the particular point in the chromosome where the duplication begins, and on the nature of the base pair code specifying amino-acids and the ends of polypeptide chains. On theoretical grounds we would expect coding restrictions to limit the number of duplications of random length leading to the production of a polypeptide bridging the beginning of the duplication. For example, less than one in three random duplications would make 'sense' at the beginning of the duplicated region if each amino-acid is coded by one particular triplet of base pairs. On the other hand, if an amino-acid can be specified in more than one way the requirements for continuity of the corresponding polypeptide may be met more frequently at the beginning of the duplication, but may fail to be met at some other point (see Crick *et al.*[13] for further discussion of this problem). If integral numbers of amino-acid coding units are involved, the requirements for continuity may be met still more frequently and the whole of the duplication may make 'sense'. Although partly functional and non-functional duplications are thus conceivable, our experiments indicate that completely functional partial gene duplications occur.

Once it is realized that a new gene able to code for an uninterrupted sequence of amino-acids can be formed from pre-existing genes by chromosomal rearrangements, the possible importance of this process for the evolution of new proteins becomes apparent. Three of the many conceivable examples of such evolutionary events, related to duplications which could have been formed by non-homologous crossing-over, are indicated diagrammatically in Fig. 4.

A duplication, *I*, within the boundaries of a single gene, leads to a repeated sequence of amino-acids in the corresponding polypeptide (subject to the involvement of an integral number of amino-acid coding units). Unexpected properties can result from such repetitions, as, for example, the polymerization tendencies of the polypeptide hp 2α. Although the repeated sequence in hp 2α is extensive, there appears to be no *a priori* reason why shorter ones could not exist. The consequences of the involvement of non-integral numbers of amino-acid coding units in a partial gene duplication will, as indicated here, depend on the nature of the code. For example, the new gene might give rise only to that part of the (pre-existing) polypeptide sequence which is coded by the DNA preceding the duplicated region, or the pre-existing sequence may be followed, as a result of the shift in reading of the DNA code (cf. Crick *et al.*[13]), by a completely new polypeptide sequence extending over part or almost all of the duplication. Any partial gene duplications which are non-functional in polypeptide synthesis, or give rise to phenotypically inactive products, may behave like amorphic mutations. However, unlike amorphic mutations due to deletions, they will revert to the normal gene occasionally, as a result of unequal but homologous crossing-over within the locus in homozygotes (see following), or as a result of unequal but homologous sister strand or intrachromatid crossing-over in heterozygotes[14].

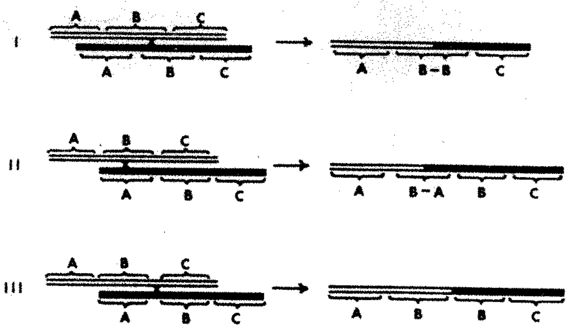

Fig. 4. Diagrammatic representation of the possible formation (by non-homologous crossing-over) and the consequences of duplications of several types. The brackets indicate DNA coded for polypeptide sequences. I, An intragenic duplication of the type suggested as involved in the evolution of Hp^2 from Hp^{1F} and Hp^{1S}; II, A duplication involving parts of two genes, leading to the formation of a new gene *B–A* without loss of the pre-existing genes *A* and *B*; III, a duplication of the same extent as II, but formed by crossing-over between genes. The result is a classical gene duplication, such as may have been involved in the evolution of the hæmoglobin polypeptide chains

A duplication involving parts of two genes, *II*, can lead to the formation of a new gene coded for a polypeptide sequence combining parts of the sequences of two pre-existing proteins if integral numbers of coding units are involved. Such a polypeptide might combine in one molecule properties formerly present in separate molecules. For example, in the case of enzymes it could have amino-acid sequences enabling it to combine with two substrate groups where its 'progenitors' could each combine with only one of them. (If non-integral numbers of coding units are involved the situation is similar to that discussed here.) Yet the evolution of such radically new proteins, by a single genetic event, need not be accompanied by loss of any genes for pre-existing proteins (Fig. 4). The alternate chromosome (not shown in the figure) formed during the non-homologous crossing-over leading to duplication *II* also has a new gene (*A–B*) which could code for a sequence of amino-acids with the portions corresponding to the genes *A* and *B* in the order A–B, in contrast to the order B–A for the duplication. This alternate chromosome, however, lacks the genes *A* and *B*, and so may have selective disadvantages.

Non-homologous crossing-over occurring between genes can lead to complete duplication of an existing gene (*III*).

Many types of chromosomal rearrangements, in addition to duplications, have been detected by genetic and cytological studies; for example, deletions, inversions, shifts, transpositions, etc. None of these would appear on *a priori* grounds to be confined to integral numbers of genes. We can therefore expect that protein variants will eventually be found corresponding to them, although until the base pair-amino-acid code is more completely known it is difficult to predict what proportion of these arrangements will fulfil the requirements for continuity in the corresponding polypeptides. The frequency of occurrence of cytologically detectable chromosomal rearrangements in naturally occurring populations of *Drosophila* and other species certainly indicates that re-arrangements are common evolutionary events.

Other proteins, in addition to the haptoglobins, have structures which suggest that chromosomal rearrangements were involved in their evolution. Thus, the porcine pituitary hormones α-melanotropin (αMSH) and adrenocorticotrophin (ACTH) both contain the same sequence of 13 amino-acids[15]. αMSH has an acyl group on the *N*-terminal amino-group and the terminal carboxyl-group is amidated. On the other hand, ACTH has a free *N*-terminal amino-group and 26 additional amino-acid residues on the *C*-terminal end. βMSH lacks the *N*- and *C*-terminal amino-acids of αMSH and has short, completely dissimilar sequences on both ends of the common sequence. Two amino-acid residues in the common sequence are interchanged in position. These structures are strongly suggestive of evolution involving chromosomal rearrangements at some stage. Many proteolytic enzymes (for example, trypsin and chymotrypsin) have portions of their sequences in common, but in general their structures differ greatly[16]. They could also have evolved by chromosomal rearrangements.

Consequences of unequal but homologous crossing-over at the Hp locus. Several consequences of the intragenic duplication probably involved in the gene Hp^2 can be predicted by considering other systems involving duplications. The 'Bar' allele in *Drosophila* is associated with a cytologically demonstrable duplication[17,18]. Homozygotes for 'Bar' are relatively frequently involved in unequal but homologous crossing-over[19], as a result of the displaced synapsis possible when there is a duplication in the genome. (Unequal but homologous crossing-over must be distinguished from non-homologous crossing-over, although both involve displaced synapsis. The latter, as indicated in Fig. 3, involves crossing-over between chromosomal regions which are not equivalent in their gene structure; it is consequently a rare event, and the subsequent action of the genes involved will be dependent on the requirements for continuity discussed here. Unequal but homologous crossing-over, on the other hand, involves crossing-over between chromosomal regions which are of equivalent structure but which are not in the same relative positions on the two chromosomes. Unequal but homologous crossing-over will not usually change the 'sense' of the DNA involved, and its frequency will be determined only by the frequency of displaced synapsis and by the map length of the corresponding duplicated or displaced chromosomal segment.) This unequal crossing-over in *Drosophila* gives rise to the formation of "double 'Bar' ", associated with a cytological triplication, or to reversion to 'wild-type' which lacks the original 'Bar' duplication. Homozygous individuals of the genotype Hp^2/Hp^2 are in many ways analogous to homozygotes for the 'Bar' allele. Unequal crossing-over would therefore be expected to occur occasionally in such individuals, either with the production of a triplicated haptoglobin gene (instead of the usual duplicated Hp^2 gene) or with reversion to one of the original 'wild-type' genes Hp^{1F} or Hp^{1S} (see Fig. 5 I). In the absence of information about the nature of the gene Hp^2 these events would be interpreted as mutations and the *Hp* locus would be described as having a high mutation rate.

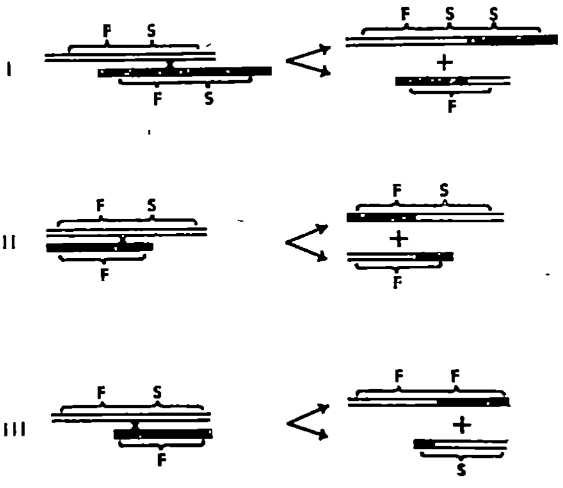

Fig. 5. Diagrammatic representation of the consequences of: I, unequal but homologous crossing-over occurring during displaced synapsis in a Hp^2/Hp^2 homozygote, leading to the possible evolution of a haptoglobin triplication or to reversion of Hp^2 to Hp^{1F} or Hp^{1S}; II and III, crossing-over within the locus in a heterozygous individual (Hp^2/Hp^{1F}) during the two possible synaptic configurations. This crossing-over will always be unequal and can lead to the production of four types of Hp^2 gene, and to the formation of a gamete containing the gene Hp^{1S} in an individual of genotype Hp^2/Hp^{1F}

A rare and unusual type of haptoglobin (the Johnson type), discovered by Dr. E. R. Giblett in a Negro woman and her daughter in Seattle, is associated with a rare gene, Hp^{2J}, in heterozygous combination with the common Hp^{1S} allele. Preliminary tests, involving comparison of the mobilities of the α-chains in starch gels at different starch concentrations[20], indicate that the corresponding polypeptide *hp* 2Jα, is considerably larger than *hp* 2α. This suggests that the gene Hp^{2J} could be the triplication formed by unequal but homologous crossing-over in a homozygote Hp^2/Hp^2 during displaced synapsis. This suggestion receives some confirmation from the recent discovery of the Johnson type in other widely separated racial groups (Dr. E. R. Giblett has found it in a Canadian Mennonite and two Hawaiian Chinese, Dr. K. Henningsen in a Dane, Dr. J. Hirschfeld in a Swede, Dr. R. L. Kirk in an Australian Dutch, Dr. J. Lundevall and Mrs. E. A. Fleischer in three Norwegians, Dr. B. Romat in a Kurdish Jew). It seems highly improbable that the gene Hp^{2J} in the Seattle Negroes and these other genes have a common ancestor; yet they appear to be identical as judged by the phenotypes. The cause of their identity could be a common mechanism of formation rather than descent from a common ancestor. If the gene Hp^{2J} does prove to be an intragenic triplication then the Johnson type (genotype Hp^{2J}/Hp^{1S}) provides an excellent example and a possible explanation at the molecular level of the stable position effect, since the Johnson phenotype is readily distinguished from *Hp* 2–2, in which the same genetic material is distributed equally between the two chromosomes.

Other unusual serum samples very similar to but not identical with the Johnson type have also been found and sent to us for testing. Unfortunately, all had been shipped considerable distances and were not freshly collected, so that the observed small differences are difficult to evaluate. However, differences would be expected in terms of the triplication hypothesis, since there should be eight different forms of a triplicated haptoglobin gene (see later) and consequently sixteen heterozygous combinations with the genes Hp^{1F} and Hp^{1S}.

Synapsis in heterozygotes Hp^2/Hp^{1F} and Hp^2/Hp^{1S} can occur in two ways (Fig. 5 II and III), as a result of the duplicate nature of the gene Hp^2. Crossing-over within the locus (which will always be unequal) should therefore lead eventually to the occurrence in the gene pool of all the four possible types of Hp^2. Thus, if we assume that the original Hp^2 gene was FS, then intragenic crossing-over in heterozygotes will lead to the formation of Hp^2 genes which are FF, SS, and SF. The relative chances of finding these types is difficult to predict, since we have insufficient knowledge about the frequency of the corresponding unequal crossing-over, and of the selective forces involved, to be able to estimate the equilibrium frequencies of the different genes. However, we may already have had some samples of hp 2α from individuals who are $Hp^{2(FF)}/Hp^{2(FF)}$ or $Hp^{2(FS)}/Hp^{2(FF)}$, as three out of fifteen single donor hp 2α preparations showed complete, or almost complete absence of the peptide S characteristic of hp 1Sα (Fig. 1), but clearly showed the corresponding F peptide. In any event, we predict that four types of Hp^2 gene are possible, and are undertaking a search for them.

In summary, we conclude that the haptoglobin system provides examples of the effect on the structure of a single polypeptide chain of both a 'point' mutation and chromosomal rearrangements. We suggest that proteins with radically changed properties can be formed as a consequence of the single genetic event of a chromosonal rearrangement involving non-integral numbers of genes. Chromosomal rearrangements of this type appear to provide a mechanism for achieving more rapid and extensive changes in protein structure during evolution than are possible by point mutations, even when preceded by gene duplication. New genes formed by chromosomal rearrangements need not be accompanied by loss to the organism of any pre-existing genes.

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A THEORY OF S GENE STRUCTURE

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BECAUSE of technical limitations, comparatively few compound loci in higher plants are amenable to detailed analysis. Exceptional in this respect is the *S* gene controlling self-incompatibility. Some understanding of this complex gene at the phenotypic level has been derived from its behaviour in autopolyploids; in spontaneous and induced mutants; and in interspecific crosses. Thus different *S* alleles in diploid pollen may diversely show independent action, dominance, or competitive interaction[1,2]; the last, which is expressed after selfing, often results in self-compatibility. Spontaneous and irradiation-induced mutants involving the *S* alleles in *Oenothera organensis*[3], *Prunus avium*[4], *Trifolium pratense* and *T. repens*[5,6] have shown that the *S* gene consists of at least two independently mutable units, one governing the specificity of the pollen, the other that of the style; a change or loss of specificity in one or the other leads to self-compatibility. Brewbaker and Natrajan[7], however, found self-compatible plants in *Petunia inflata* which were not due to mutations of the *S* allele. These plants, which arose in the selfed generation following X-irradiation of the pollen mother cells, were found to carry a supernumerary *S*-bearing centric chromosome fragment, and self-compatibility was due to competitive interaction between the two different *S* alleles carried by pollen bearing the fragment. The *S* allele mutation hypothesis was therefore challenged, but its general validity was firmly re-established by Lewis[8].

Recent advances in the analysis of the *S* gene complex have been made through the examination of interspecific crosses. From a large number of crosses between several genera and families, Lewis and Crowe[9] and Crowe[10] showed that there were two kinds of self-compatible species: Sc species, which have an allele S_f that is similar to the experimentally induced pollen-part mutant; and SC species, which have a self-fertile allele not yet recovered in mutation studies. The two groups of species are distinguished by their interactions in crosses with self-incompatible (SI) species, thus:

SI × Sc—Compatible; Sc × SI—Compatible
SI × SC—Incompatible; SC × SI—Compatible

SI styles accept Sc pollen but reject SC pollen.

To explain this, Lewis and Crowe suggested a modified form of Straub's[11] hypothesis, that specificity molecules are attached to a growth substance and that a mutant allele which has lost its pollen specificity unit produces a 'bare' pollen growth substance. Such unprotected pollen growth substance is inactivated by a specific incompatibility substance in the style. The problem arose that if both Sc and SC species had lost their pollen specificity why was only the SC pollen rejected by SI styles. Experimental evidence with induced mutants, taken in conjunction with published evidence on self-fertility in certain species suggests[9] that Sc species had become self-compatible comparatively recently, whereas in SC species self-compatibility was long established. This observation, while explaining the evolutionary status of the two groups of species, does not account for the sharp differences in the behaviour of their two kinds of *S* alleles. A clue to the solution of this problem is provided by studies in *Nicotiana*.

N. alata (SI) and *N. langsdorffii* (Sc), as expected, are usually reciprocally compatible. However, Anderson and de Winton[12] found one strain of *N. alata* which rejected the pollen of *N. langsdorffii*. This behaviour was controlled by a self-incompatible *S* allele, named S_F, which when present in the style of *N. alata* rejected the pollen of *N. langsdorffii*. That this allele is not so rare as at first thought has been shown by me (unpublished results): 15 out of 34 plants of *N. alata* from three different seed sources were heterozygous for this allele.

The S_F allele in *N. alata* produces a certain specificity in the style which matches not only that of its own pollen but also that produced by the S_f allele in *N. langsdorffii*. It is obvious that a pollen specificity, as between S_F and S_f, that is common to two species, must be of a primary nature. It has been assumed[6,9] that S_f has lost its pollen specificity making *N. langsdorffii* self-fertile. The fact, however, that pollen carrying this allele is rejected by *N. alata* styles carrying S_F shows that a certain specificity remains in the S_f, an allele which occurs in species that have recently become self-fertile. This would suggest that the primary specificity still remains after the secondary (common) specificity has been lost, and that the primary specificity is physiologically interposed between the growth substance and the secondary specificity.

An allele which has lost secondary specificity in both pollen and style would still express primary specificity in both organs and would be self-incompatible. The S_F allele may be such an allele. Another allele which has lost secondary specificity in the pollen only would still express primary specificity in the pollen and secondary specificity in the style and would therefore be self-compatible The allele S_f of Sc species may be such an allele. Pollen with the S_f allele would be rejected by a style containing the S_F allele because the primary specificity is expressed in both pollen and style.

On the above hypothesis, the SC species, in which self-compatibility was long-established, would have an allele, here called S_C, which has lost both the primary and the secondary specificity in the pollen and thus produces an unprotected pollen growth substance. Pollen with such an allele is, therefore, rejected on an SI style. Evidence suggesting the existence of a growth substance in the style has been found by me (unpublished results) in two Mexican *Solanum* species, *S. pinnatisectum* and *S. ehrenbergii*. Styles of certain strains of these species, supposedly homozygous for an allele, named S_{IC}, reject all SI pollen. This situation in the style corresponds to that presented by the S_C allele in the pollen; in the former the style is incompatible with all SI pollen, in the latter the SC pollen is incompatible with all SI styles. These reactions suggest that the S_{IC} allele is probably a stylar counterpart of the S_C allele, and produces an unprotected stylar growth substance which is inactivated by a specific substance in SI pollen.

An investigation of cross-compatibility relationships in 9 *Nicotiana* species by me (unpublished results) has shown that, of the self-compatible species, *N. glauca*, *N. longiflora*, *N. rustica*, *N. paniculata*, *N. sylvestris*, *N. glutinosa* and *N. tabacum* belong to the SC group and only *N. langsdorffii* to the Sc group; *N. alata* is an SI species.

Since it has been assumed that the SC species have an unprotected pollen growth substance, in order that these species be self-compatible their styles should lack specificity, either secondary or primary. But if the absence of these specificities alone left the stylar growth substance unprotected, they should be incompatible as females with Sc and SI pollen. However, this is not so; the styles of SC species are compatible with Sc and SI pollen, suggesting that their growth substance is still protected even when both specificities are lost. This would indicate that, contrary to the previous assumption, specific substances are not responsible for the protection of the growth substance; and leads to the assumption of a separate 'protective substance'. This component would be attached to the growth substance and would prevent its inactivation after both specificities are lost. The S_C allele, therefore, appears to have lost both specificities for pollen and for style, but has lost the protective substance only for pollen.

The apparent widespread occurrence of the S_C allele in flowering plants[9] suggests that this state of the *S* gene represents the minimum requirement of a dynamic species; for if the protective substance was lost in both pollen and style the species having such an allele would be completely incapable of exchanging genes with other species having any specificity and would thus be greatly restricted in its evolutionary development.

It is therefore tentatively suggested that the physiology of the *S* gene is controlled by 4 components: growth substance, protective substance, primary specificity and secondary specificity, each with corresponding pollen and stylar units attached in that order, the component for secondary specificity being added last. There appear to be two possibilities of mutations of *S* alleles: (1) those that could give rise to variants of the common S_I alleles through recombination, presumably on Fisher's model[13] and (2) those that give rise to new forms of *S* alleles, through loss mutations. The second group may involve the loss of one or more of the 3 components, protective substance, primary specificity and secondary specificity. The fourth component, growth substance, may or may not be a part of the *S* gene complex, since it is basic to all functional pollen. Considering that viable mutations of these units, occurring singly or in multiples, take place only sequentially starting from the secondary specificity, 16 kinds of *S* alleles are possible. Six of these alleles have been either directly identified through the study of spontaneous and induced mutants of *S* alleles or deduced from the results of interspecific hybridization; these are listed in Table 1 and shown diagrammatically in Fig. 1.

Generally, the available data on intra- and interspecific crosses can be explained satisfactorily on the basis of the above theory of the *S* gene structure. The reported exceptions usually involve wide intergeneric crosses[9] and rarely interspecific crosses[14]. They are mostly in the SI × SI combinations which, contrary to expectations, are incompatible. It is likely that in certain wide crosses factors other than

Table 1. Hypothetical Structure of Different *S* Alleles

Type of allele	Pollen / Style: Growth substance	Pollen / Style: Protective substance	Pollen / Style: Primary specificity	Pollen / Style: Secondary specificity	Species (References in the text)
S_I (normal)	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	All normal homomorphic self-incompatible species.
S_f	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{-}{+}$	Mutants in *Oenothera organensis*, *Trifolium pratense*, *T. repens*, *Prunus avium* and many wild self-compatible species.
S_{fc}	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{-}$	Mutants in *Trifolium pratense* and *Prunus avium*.
S_F	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{+}{+}$	$\frac{-}{-}$	Certain strains of *Nicotiana alata*.
S_{Ic}	$\frac{+}{+}$	$\frac{+}{-}$	$\frac{+}{-}$	$\frac{+}{-}$	Certain strains of *Solanum pinnatisectum* and *S. ehrenbergii*.
S_C	$\frac{+}{+}$	$\frac{-}{+}$	$\frac{-}{-}$	$\frac{-}{-}$	Many wild self-compatible species.

S-gene incompatibility, such as gross physico-chemical differences in the nature of styles, may be responsible for pollen-style inhibition. However, it is possible that, in association with enzymes which help in the pollen-tube penetration of the stigmatic cuticle[15,16], there is a general enzyme operating in distant crosses which inactivates all secondary specificity, thus allowing the expression of the primary specificity in both pollen and style and therefore causing incompatibility.

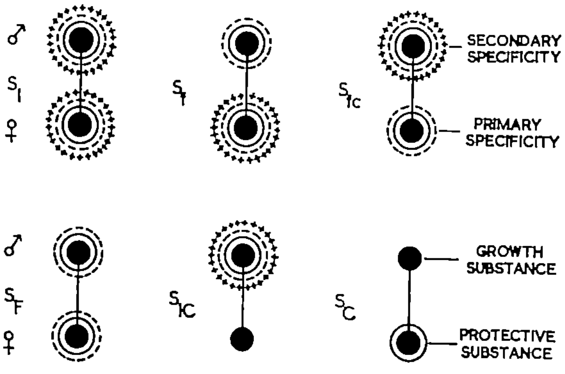

Fig. 1. Diagrammatic representation of the 6 kinds of *S* alleles described in Table 1

A detailed investigation of *S* alleles in intra- and inter-specific hybrids, and the attempt to produce them artificially, should help to elucidate the fine structure of the *S* gene.

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DEVELOPMENT OF THE THYMUS IN THE HUMAN FŒTUS AND ITS RELATION TO IMMUNOLOGICAL POTENTIAL

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RECENT work by Auerbach[1], Miller[2], and Kalmutz[3] has emphasized the importance of the thymus in the development of the lymphoid system and the dependent property of immunity. The possibility of exploiting fœtal tolerance to achieve tissue transplants in man may be limited by the duration of the initial period of immune inactivity.

Elsewhere[4] we have described the emergence of the small lymphocyte of the peripheral blood in man and contrasted the timing of this event with the rodents, rats and mice. A similar, and perhaps more significant, contrast is to be found in the growth of the thymus.

Thymic weights of 289 young and mid-term human fœtuses are recorded in Fig. 1. These are supplemented by figures from the few relevant publications[5-7] which are mostly concerned with older fœtuses. At first sight the rate of growth throughout fœtal life appears to be fairly regular, but when expressed in relation to the body-weight (Fig. 2) it is seen to level off at the beginning of the third trimester. A gradual decline follows which is already perceptible at birth and continues thereafter. The initial upgradient from 12 to 24 weeks can be deduced fairly accurately from the data we have collected. The later points, however, which define the acme of the curve and the shape of the decline are less certain. First, some of the figures on which they are based were published as mean thymic weights in groups of fœtuses within a particular weight range and with a mean crown-rump length, not an entirely satisfactory mode of presentation. Secondly, an opportunity for error arises from the possible delay in older fœtuses between delivery and death, during which time the thymus may partly involute as a result of stress; and a further objection is that stillbirths of premature infants and deaths within 24 h of delivery may be due to some disease which itself affects the thymus. Erythroblastosis fœtalis is an example[6], but such diseases form only a minority of the cases and will not unduly affect the mean values. Nor is the effect of delay likely to be serious, although there may be some adventitious flattening of the top of the curve.

Fig. 3 presents similar data relating to splenic weight. The curve here is somewhat different. The development of the spleen lags behind that of the thymus and the increase in relative weight continues up to the time of birth[5,6,8]. The spleen is, of course, a composite organ, more versatile in hæmopoiesis than the thymus, more prone also to engorgement with blood. The latter factor may partly account for the apparent discrepancy of observed values where our data overlap others and for the difference between the mean weights at birth as given by Potter[6] and by Coppoletta and Wolbach[8].

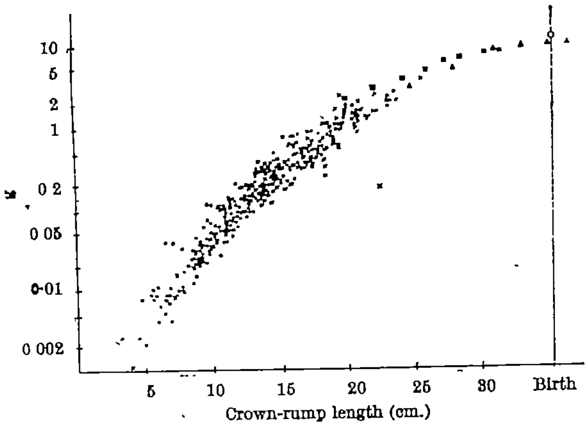

Fig. 1. Weight of fœtal thymuses in relation to crown-rump length. ●, Hysterotomy cases; ×, spontaneous abortions; ■, Silverman (ref. 5); △, Potter (ref. 6); ○, Russkoff (ref. 7)

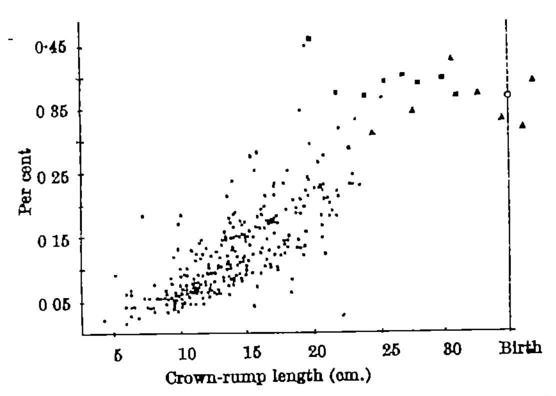

Fig. 2. Weight of fœtal thymuses as a proportion of body-weight. ●, Hysterotomies and spontaneous abortions; ■, Silverman (ref. 5), △, Potter (ref. 6), ○, Russkoff (ref. 7)

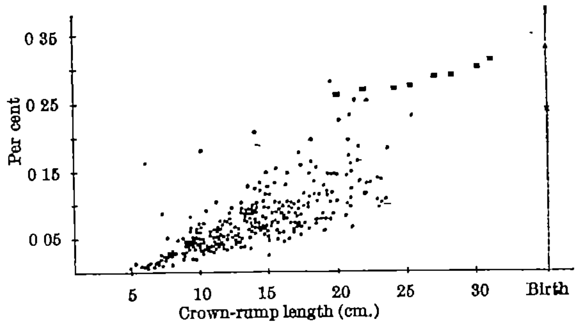

Fig. 3. Weight of fœtal spleens as a percentage of body-weight. ●, Hysterotomies and spontaneous abortions, ■, Silverman (ref. 5); △, Potter (ref. 6); ×, Coppoletta and Wolbach (ref. 8)

The three events in lymphoid development which are susceptible to quantitative analysis[5–10] are shown in Fig. 4. No firm conclusions should be drawn from the relationships; but the evidence is compatible with the hypothesis that cells from the thymus colonize the other lymphoid tissues of the body. It is also tempting to surmise that while the circulating lymphocytes of the fœtus are derived from the thymus post-natal lymphocytes may originate elsewhere also.

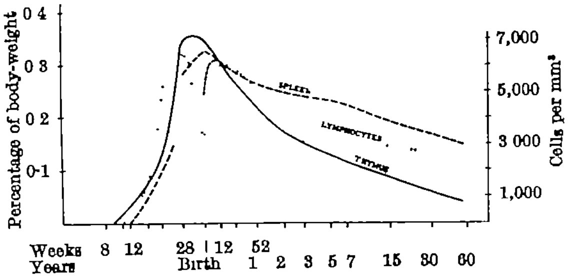

Fig. 4. Development of lymphoid system. Data from refs. 4–10

In Fig. 5 the development of the thymus has been compared in three species[10–13]. In the mouse the period of life in which tolerance to foreign antigens can be easily induced ceases within the first ten days of post-natal life[14], corresponding to the phase of most rapid thymus growth[13] and the attainment of its peak ratio. In the sheep, by contrast, immunological potential has been demonstrated in the second half of gestation[15,16], and the development of the thymus seems to be correspondingly advanced. If, as is possible, the two events are causally connected the phase of immune passivity in man may be restricted to early fœtal life. Silverstein[17] has deduced, from histological evidence in congenital syphilis, that six months may be the limit, but any such figure must almost certainly be qualified in the context of other antigens and variations in antigen-dosage. To this early capacity for immune reaction

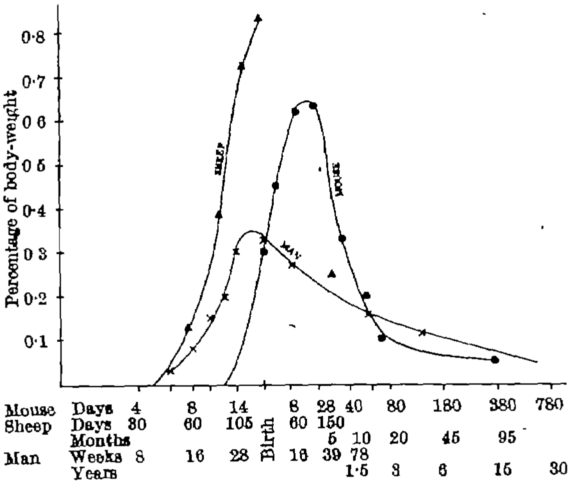

Fig. 5. Comparison of thymic developments in man (from Fig. 2 and ref. 10), mouse (refs. 1, 13) and sheep (refs. 11, 12 and our own unpublished data)

may be attributed the failure so far to achieve hæmopoietic transplants in the human fœtus, though the occurrence of natural blood-group chimæras in man in evidence that such transplants are theoretically possible.

We thank Dr. J. F. A. P. Miller for permission to use his data and for helpful criticism. This work was supported by a grant from the Medical Research Council.

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EFFECT OF BONE-MARROW ON REGENERATION OF LIVER OF X-IRRADIATED RATS

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ONE of the most important problems in experiments carried out in radiation biology is to find out how the ionizing radiation exercises its effects on the inhibition of cell-division. Regenerating liver seems to be a very good subject for experiments because, while dividing cells are very rare in intact liver, numerous cell-divisions can be noticed in liver which is regenerating. In the case of rat liver the intensity of cell-division can be easily followed by simple weighing, and this facilitates the examination.

The effect of X-irradiation on regenerating liver has been examined by several authors, however with conflicting results[1–15]. On the basis of the examinations it can be certainly stated that while normal liver is relatively resistant to irradiation, and regenerating hepatic tissue exposed to local irradiation behaves similarly (when irradiating the whole body), the degree of regeneration, the intake of phosphorus-32 by the liver under regeneration and its DNA synthesis decrease.

As is well known from the works of Jacobson[16,17] and Lorenz[18], protection of the spleen and bone-marrow, that is, injection of spleen and medulla suspension, diminishes the lethal effect of X-rays and has an effect on the regeneration of the bone-marrow. With regard to the effect of the bone-marrow on liver, Kretchmar and Congdon[19] noted an increase in the weight of the whole liver in mice exposed to X-irradiation and treated with bone-marrow suspensions. In the course of our present experiments we wished to clarify the effect of bone-marrow extracts on liver regeneration of normal and X-rayed rats.

Partial hepatectomy was carried out according to the method of Higgins and Anderson[20] on 173 male and female albino rats (130–320 g body-weight) bred at this Institute.

In 51 animals used for producing bone-marrow extracts, the weight of the liver lobules removed by partial hepatectomy and that of the remaining portions was measured, and we found that the part removed through partial hepatectomy represented 63·2 per cent of the whole liver, hence the residuum was 36·8 per cent. The percentage degree of regeneration has been established on the basis of Canzanelli's formula:

$$\frac{\text{Weight of the whole liver under regeneration} - \text{Weight of the residuum}}{\text{Weight of the two liver lobules removed by operation}} \times 100$$

while the hepatic tissues formed within one week were calculated in 100 g of body-weight.

Whole-body X-irradiation was effected 24 h after the operation. In each case a dose measured in 500 r. air was applied from a distance of 40 cm, with a 180 kV, 15 m.amp, 0·5-mm copper filter, where the air doses in r./m was 67·6.

In order to protect the region of the liver a lead plate covering the body from the xyphoid process to the upper contour of the pelvis was placed on the abdomen of the animal fixed on the operating table. Partial protection of the bone-marrow was effected by exposing only the region between the lower part of the sternum and the upper edge of the pelvis. We use the term 'partial protection of the bone-marrow' because we were unable to protect either

Table 1. EFFECT OF LIVER AND BONE-MARROW SHIELDING, AS WELL AS EFFECT OF NORMAL AND X-RAY-TREATED BONE-MARROW ON LIVER-REGENERATION OF WHOLE-BODY IRRADIATED ANIMALS

No. of exps.	Group	Mortality (per cent)	No. of animals	r./100 g	Per cent	Note	
1	Partially hepatectomy	10	27	2·27 ± 0·08	89·7	—	
2	Partially hepatectomy + X-ray	32	17	1·48 ± 0·05	59·6	$t_{42} = 13{\cdot}898$	$P \ll 0{\cdot}01$
3	Partially hepatectomy + X-ray + liver shielding	21	25	1·68 ± 0·05	68·7	$t_{40} = 2{\cdot}270$	$P < 0{\cdot}05$
4	Partially hepatectomy + X-ray + liver shielding + partially bone-marrow shielding	13	26	1·78 ± 0·06	77·7	$t_{41} = 4{\cdot}400$	$P < 0{\cdot}01$
5	Partially hepatectomy + X-ray + bone-marrow administration	12	14	2·13 ± 0·05	88·6	$t_{29} = 8{\cdot}170$	$P \ll 0{\cdot}01$
6	Partially hepatectomy + X-ray + irradiated bone-marrow administration	27	8	1·45 ± 0·09	57·2	$t_{23} = 0{\cdot}303$	$P > 0{\cdot}5$

Table 2. EFFECT OF ADMINISTRATION OF NORMAL AND IRRADIATED BONE-MARROW SUSPENSION ON LIVER REGENERATION OF NON-IRRADIATED ANIMALS

No. of exps.	Group	Mortality (per cent)	No of animals	r./100 g	Per cent	Note	
1	Partially hepatectomy	10	27	2·27 ± 0·08	89·7	—	
2	Partially hepatectomy + administration of bone-marrow suspension	8	11	2·82 ± 0·10	104·8	$t_{36} = 6{\cdot}170$	$P < 0{\cdot}01$
3	Partially hepatectomy + administration of irradiated bone-marrow suspension	11	15	1·51 ± 0·08	58·3	$t_{40} = 10{\cdot}250$	$P \ll 0{\cdot}01$

the lower thoracic and lumbar vertebræ or the lower ribs against irradiation.

The bone-marrow extract was obtained from the femur and tibia of one rat, and suspended in sodium chloride solution. This was injected into the femoral vein of one rat. The injected suspension contained $2{\cdot}1 \times 10^7$ cells on the average. The injection was carried out within 2 h after irradiation.

These experiments have again shown that treatment with bone-marrow extract decreases the number of deaths caused by irradiation.

Table 1 shows that under the effect of X-irradiation the loss in weight in the hepatic regenerate remains significant even if the liver is protected during irradiation of the whole body. At the same time when protecting the bone-marrow, that is, when the liver is subjected to X-rays, though the weight of the regenerate is smaller than that of the normal one, it surpasses significantly the weight of the liver regenerate exposed to the irradiation of the entire body. Injection of bone-marrow suspension being administered, regeneration of the liver continues with maximum intensity. The bone-marrow extract exposed to irradiation *in vitro* is ineffective. The results of our experiments are supported by mathematical calculations.

On the basis of the 117 appreciable experiments performed on rats it can be stated that the speed of hepatic regeneration decreases during irradiation and this effect can be explained not only by the direct influence exerted on the liver but also by the general reaction of the whole organism. An important component of this system, exerting a general reaction, is the bone-marrow.

The ineffectiveness of the X-ray-treated bone-marrow can be explained in two ways: (1) the supposed protecting material of the cell elements becomes inactive under the effect of irradiation; (2) under the effect of ionizing radiation toxic elements are produced which prevent the regeneration of the liver. The first possibility has already been shown by the protecting effect of bone-marrow suspension. The second possibility has been investigated through the effect of intact and irradiated bone-marrow on regeneration of liver in rats not exposed to X-rays. The results of these experiments are shown in Table 2.

It appears clearly that while regeneration of the liver of rats not exposed to irradiation is significantly advanced by normal bone-marrow suspension, the bone-marrow exposed to X-rays causes inhibition as if the whole body of the animal had been irradiated. Hence, when explaining the effect of bone-marrow, both possibilities (the protecting and the toxic effect) have to be taken into consideration.

In the course of our experiments we have noticed several phenomena little understood so far. One is that irradiation of the whole body hinders hepatic regeneration even if the region of the liver itself is protected against irradiation with lead plates. The other is that besides the protecting functions of the bone-marrow (prolongation of life and rapid regeneration of the lesions of bone-marrow and the alimentary canal) a further effect exercised on hepatic regeneration has been discovered, and at the same time also the toxic effect of the X-ray-treated bone-marrow has been noticed. It is also interesting that in the course of the research for the ætiology of pernicious anæmia, the effect of the liver on the bone-marrow has been recognized, and we have now found that both normal and irradiated bone-marrow also have a certain effect on the liver.

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A SEX-ASSOCIATED PROTEIN IN LIVER TISSUE OF THE RAT AND ITS RESPONSE TO ENDOCRINE MANIPULATION

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SEXUALLY associated protein differences in animal tissues have been recognized for some time. Reports of these differences have related chiefly to enzyme activities[1,2] or to certain yolk proteins[3]. A protein in the serum of pregnant and fœtal rats has been described[4] which persists for the first three weeks of post-natal life, and the suggestion has been made that this protein may be a growth-associated substance which occurs in either sex in the presence of rapidly growing tissues. In this article, further work on a protein of unknown function, normally present in high concentration in the liver of the male rat (but absent from female liver[5,6]) will be described, together with observations on the effects of endocrine manipulation.

A sub-Wistar strain maintained at this laboratory was used for correlations of the sex-associated protein with age. The remainder of the investigations were carried out with Sprague–Dawley rats 4–5 months of age. No protein differences were observed between the two strains.

The effects of endocrine disturbances were examined on 10 groups of animals (castrated and uncastrated of both sexes) using commercial preparations of testosterone propionate, testosterone phenylacetate, and œstradiol dipropionate (Ciba preparation of 'Perandren' and 'Ovocylin').

To assay for the sex-associated protein, rats were stunned and decapitated, and the liver perfused, homogenized, and the soluble phase prepared as previously described[7]. Low molecular weight materials were removed by passage through a 'Sephadex *G*–50' (Pharmacia, Uppsala, Sweden) column equilibrated with 0·01 M *tris* chloride buffer at *p*H 8·4. 15 ml. of the Sephadex column effluent of known optical density at 280 mμ were transferred to a 2 × 25 cm column of DEAE-cellulose and chromatographed at 6° C. The column had been previously packed with DEAE-cellulose in the hydroxide form under 15 lb. air pressure and equilibrated with 0·01 M *tris* chloride buffer at *p*H 8·4. Proteins were eluted from the column with the same buffer at a flow-rate of 1·5 ml./min. The column had a hold-up volume of 42 ml. and 59 theoretical plates. The effective distribution coefficient for the male protein was 0·09. The column eluant was analysed continuously and the data recorded automatically at 260 and 280 mμ (Fig. 1) using an automatic analytical system[8].

Since the area under the sex-associated protein peak was found to compare favourably with the results from Kjeldahl nitrogen determinations, all chromatographic data presented here are based on the spectrophotometric measurements.

Mobility and sedimentation measurements were made in Miller–Golder[9] buffers at an ionic strength of 0·1. Amino-acid analyses were conducted on 24-h acid and base hydrolysates using a Spinco amino-acid analyser.

The sex-associated protein rechromatographs as a single component. Preliminary immunochemical experiments, however, show at least two other components present in small amounts in the purified material. An $S_{20,w}$ of 2·6 has been found for the protein isolated by improved techniques as compared with the value of 3·13 previously reported. The material appears to be homogeneous in the ultracentrifuge. The mobilities observed at *p*H 7·48, 8·12, and 8·59 were $-1{\cdot}6$, $+1{\cdot}8$ and $+2{\cdot}1 \times 10^{-5}$ cm^2 V^{-1} sec^{-1}. In each instance only one peak was observed. The isoelectric point obtained by interpolation is *p*H 7·7. The dry protein contains 15·5 per cent nitrogen and approximately 1 per cent amino hexose. The amino-acid chromatogram contains a peak suggesting the presence of galactos-

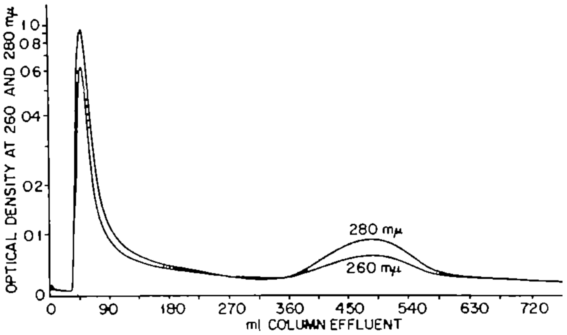

Fig. 1. Continuous-flow chromatogram of male rat liver soluble-phase proteins on DEAE-cellulose. Column loaded with 309 O.D. units of soluble phase dialysed against 0·01 M *tris* chloride buffer at *p*H 8·4 on 'Sephadex *G*-50' columns. The DEAE-column was eluted with the same buffer at a flow-rate of 1·5 ml./min. The component starting at 360 ml. is the sex-associated protein absent from the female

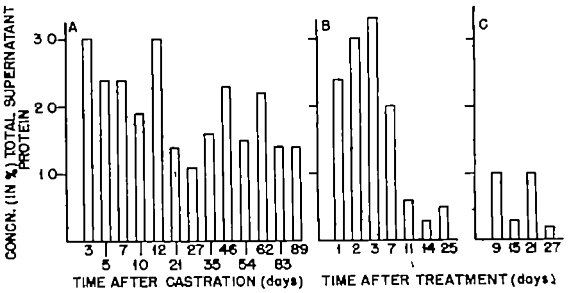

Fig. 2. Changes in the sex-associated protein component with respect to experimental treatment with œstradiol and with castration. One animal was used for each analysis. *A*, castrated males, *B*, castrated males treated with œstradiol, *C*, normal males treated with œstradiol

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† Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

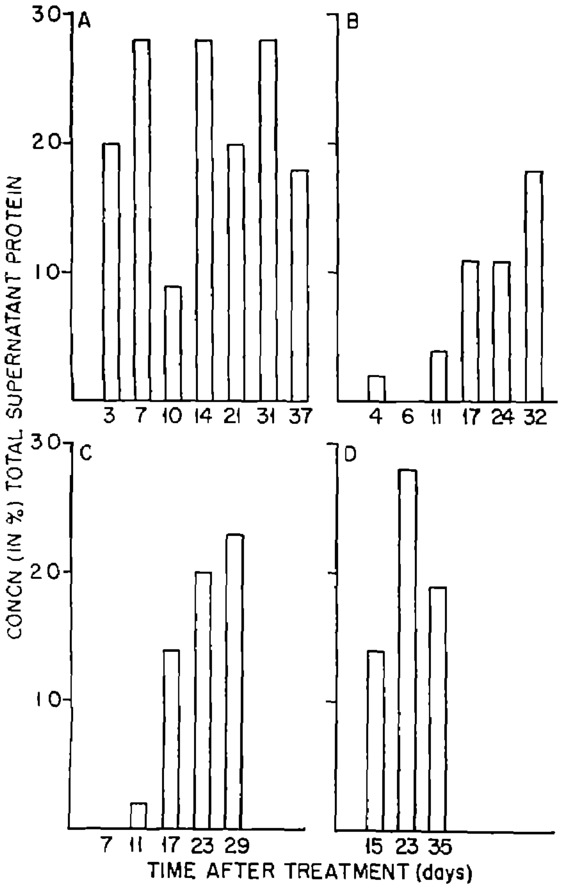

Fig. 3. Changes in the sex-associated protein component with respect to experimental treatment with testosterone. One animal was used for each analysis. *A*, male castrates treated with testosterone after being castrated 84 days. Time with reference to hormone treatment; B, females treated with testosterone, *C* spayed females treated with testosterone, *D*, normal males treated with testosterone

amine. Only a trace of cystine was observed which may have come from the minor contaminants suggested by immunochemical studies. The sex-associated protein has not been observed in male rat kidney, brain or testis.

The concentration of sex-associated protein observed in the soluble phase of the normal adult male liver has a mean value of 3·2 ± 0·3 per cent. This is a lower value than obtained previously[5], the difference being the result of improved chromatographic methods.

The effect of castration in the male was followed for 89 days (Fig. 2*A*). While considerable individual variation was seen, the concentration of the protein tended to be reduced to about 1·5 per cent Changes in the concentration of sex-associated protein in males given 2 mg œstradiol dipropionate intramuscularly immediately after castration were studied at intervals for 25 days (Fig. 2*B*). Little change was seen during the first 3 days. The concentration of sex-associated protein declined abruptly between the seventh and eleventh days, giving a total change much greater than was observed with castration alone.

To examine changes in the concentration of sex-associated protein as a result of female sex hormone treatment. normal male rats were given one intramuscular injection of 2 mg of œstradiol dipropionate (Fig. 2*C*). Observations were not started until the ninth day, at which time the concentration of sex-associated protein had already dropped to 1 per cent. Three subsequent determinations confirmed the pronounced reduction.

The effect of male sex hormone on males which had been castrated for 84 days was examined after injection of 10 mg each of testosterone propionate and testosterone phenylacetate. The results are shown in Fig. 3*A*. While the results of this experiment are inconclusive, there is some evidence for protein restoration. When female rats were given the same hormone treatment, sex-associated protein appeared and gradually increased in concentration during the 32-day observation period (Fig. 3*B*). It was of interest to see if the presence of ovarian tissue was important in the production of sex-associated protein in females treated with male sex hormone. The experiment was therefore repeated on spayed females (Fig. 3*C*). The results were similar to those observed in intact females. A somewhat higher concentration of sex-associated protein was eventually attained, although the onset of the response was slower. In control experiments on spayed females given no hormones, no evidence of sex-associated protein was observed.

To see whether excess male sex hormone increased the amount of sex-associated protein, normal males were given 15 mg of testosterone phenylacetate intramuscularly. The first observations (Fig. 3*D*) were made at 15 days and the last at 35 days. The only effect observed was a slight depression in the amount of the protein.

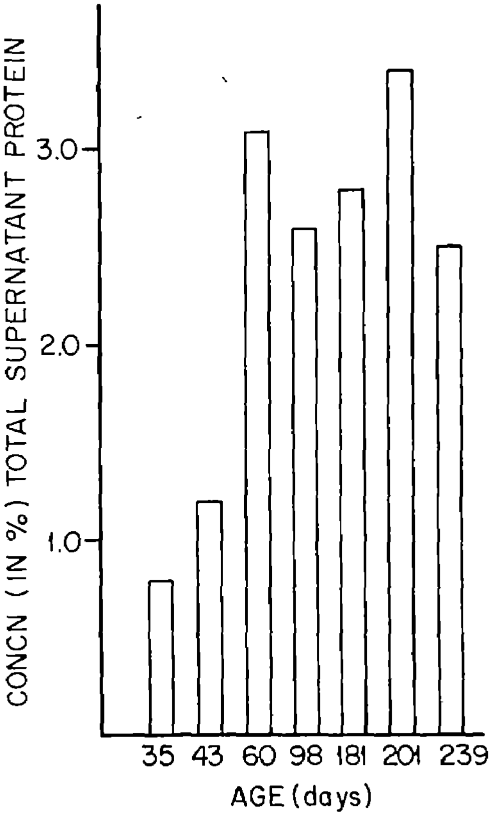

Fig. 4. Concentration of sex-associated protein component with respect to age. Pooled samples from 3–4 animals were used for each analysis at 98 days or less; one animal was used for each of the remaining analyses

The effect of age on the concentration of sex-associated protein was studied using livers of male *WR* (a sub-Wistar stock) rats. As shown in Fig. 4, animals 60 days of age and older gave similar values, whereas the composite samples tested at less than 60 days have lower concentrations. The protein is present in considerable amount at 35 days of age, however, and apparently increases rapidly in concentration between days 43 and 60.

These investigations demonstrate that the sex-associated protein of the male rat is susceptible to endocrine manipulation. The most striking instance is the female rat treated with testosterone. Here the previously absent protein appears and reaches an appreciable concentration. Results based on testosterone treatment of male and male castrates are less conclusive (but suggest that testosterone causes the production of the protein and that œstradiol has the opposite effect). Other androgens and œstrogens may be similarly effective but have not been tested.

The possibility exists that the hormones used may have exerted their effect through complex interactions with other endocrine systems. The presence of the protein in moderate concentration in the immature male rat is one such suggestion as is also the persistence of a considerable amount of the material in the castrated male. The treatment also of the long-term castrated male with testosterone appeared not to have restored the protein to levels found in the normal male. The inhibiting effect of large doses of testosterone in 2 of 3 intact males could also have involved indirect mechanisms, interactions with the pituitary gland or with the liver metabolism of adrenal hormones, for example. The pursuit of these and other interesting aspects involving the sex-associated protein are being deferred pending development of more precise and less time-consuming analytical techniques.

Evidence of a dose-dependent response of the sex-associated protein to hormone treatment has been shown in additional studies not reported here. Consequently, any attempt to obtain statistically valid results would have been complicated by the necessity of testing both the dose response and the animal variability in each group. The experiments described here were designed to show that there is a relation between male and female hormones and the existence of the sex-associated protein. The results show this unequivocally but do not explain the nature of the interactions demonstrated.

I thank Dr. Norman G. Anderson for advice.

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FATAL INFECTION OF NEW-BORN HAMSTERS BY AN ADENOVIRUS OF HUMAN ORIGIN

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LACK of pathogenicity for laboratory animals is generally accepted as one of the criteria for the characterization of adenoviruses of human origin[1-3]. However, symptomless infection of rabbits by adenovirus type 5 has been described by Pereira and Kelly[4] and evidence that several adenovirus types may cause inapparent infections in guinea pigs, cotton rats, hamsters and dogs has been presented[3-6]. Furthermore, Hull *et al.*[7] showed that intracerebral inoculation of certain types of human adenovirus into monkeys may give rise to inflammation of the choroid plexus. More recently, the pathogenicity of human adenoviruses for new-born pigs has been demonstrated by Betts *et al.*[8].

In the investigation reported here adenovirus type 5 is shown to cause fatal infections when inoculated subcutaneously into new-born hamsters.

The virus inoculum used in these experiments was obtained from HeLa cells infected with the prototype strain of adenovirus type 5 and was partially purified by treatment with a fluorocarbon compound[9] as described by Pereira and Valentine[10]. This virus material contained $10^{10\cdot5}$ TCD_{50}/ml., estimated by titration in HeLa cell cultures incubated for 4 weeks.

All of 35 one-day-old hamsters inoculated subcutaneously with 0·05 ml. of undiluted virus material died within a period of 31 days. No deaths were observed during the first 3 days following inoculation. The number of deaths after this period showed a sharp rise between 4 and 10 days, involving 23 (65·7 per cent) of the 35 animals under examination. Between the 11th and the 27th days, only 4 deaths were observed, and all the remaining animals died during the following 4 days.

Inoculation of a 10^{-3} dilution of the same virus material into 11 new-born hamsters resulted in 5 deaths, the first occurring on the 9th day and the remainder between the 19th and 27th days after inoculation. The survivors showed retarded development when compared with uninoculated litter mates (Fig. 1), but were still alive 4 months after the inoculation.

No post-mortem examinations could be carried out either because of cannibalism or because the animals could not be examined shortly after death. However, 3 animals from the group inoculated with undiluted virus material and not included in the percentages given here were killed and examined for histological changes and for the virus content of various tissues.

A part of each tissue collected from these animals was homogenized, centrifuged and the supernatants titrated in HeLa cell cultures. From the results shown in Table 1 it is seen that the virus was present in all tissues examined although the titres showed

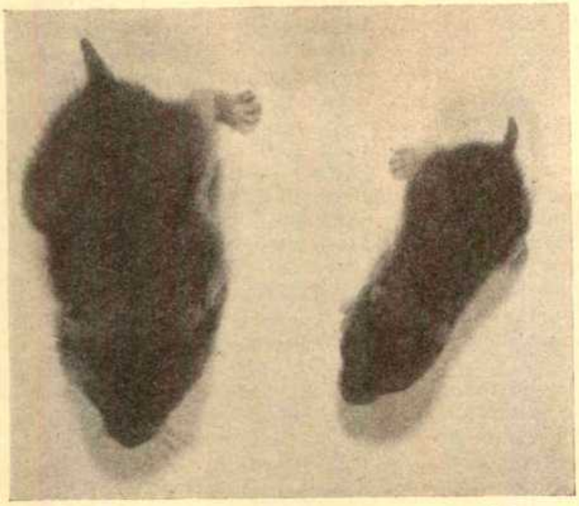

Fig. 1. 20-day-old hamsters. The animal on the right was inoculated one day after birth with 0·05 ml. of a 10^{-3} dilution of the virus material. Uninoculated litter mate on the left

considerable variation from tissue to tissue and from animal to animal.

Table 1

Hamster No.	Time (days) after inoculation	Virus content* Liver	Spleen	Kidney	Lung	Thymus
101	5	$10^{6\cdot5}$	$10^{5\cdot7}$	$10^{4\cdot3}$	$10^{5\cdot6}$	$10^{2\cdot8}$
102	6	$10^{5\cdot0}$	$10^{5\cdot6}$	$10^{2\cdot7}$	$10^{3\cdot0}$	$10^{1\cdot2}$
103	12	$10^{4\cdot6}$	$10^{3\cdot5}$	$10^{3\cdot7}$	$10^{4\cdot0}$	$10^{2\cdot3}$

* TCD_{50}/ml. of supernatant of 10 per cent tissue suspensions.

Histological examination revealed varying degrees of abnormalities, the most striking occurring in the liver tissue of hamster 101 (Fig. 2), which showed many parenchymatous cells with nuclear changes characteristic of adenovirus infection, that is, condensation of chromatin into a basophilic central mass surrounded by vesicular spaces. There were also cells with very shrunken pyknotic nuclei. However, actual necrosis of the liver was slight. Lesions seen in the remaining tissues consisted mainly of necrotic cells with pyknotic nuclei, but the extent of these alterations was extremely variable and their significance remains to be determined.

Although the present findings demonstrate the pathogenicity of adenovirus type 5 for new-born hamsters they do not allow a categorical statement to be made regarding virus multiplication in this host. The latter is, however, likely in view of the characteristic histopathological lesions correlated with the high virus content found in some of the tissues examined.

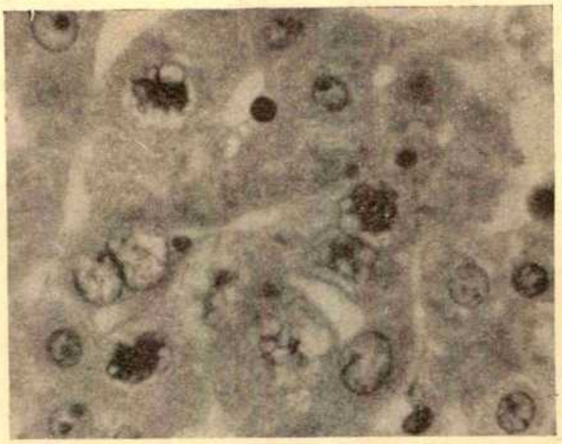

Fig. 2. Section of liver tissue from hamster 101. (Hæmatoxylin–eosin × c. 950)

The irregular results regarding the extent of lesions and virus content in different animals suggest that new-born hamsters may react in different ways to infection. This is also suggested by the apparently bimodal distribution of survival times, especially notable among the animals inoculated with the larger virus dose.

Note added in proof. Since this communication was submitted for publication it has been observed that human adenovirus types 12 (Trentin, J. J. Yabe, Y., and Taylor, C., *Science*, **137**, 835; 1962) and 18 (Huebner, R. J., personal communication) are capable of inducing malignant tumours in new-born hamsters.

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PATTERN AND MECHANISM IN THE REAGGREGATION OF SPONGES

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WHEN the various types of cell of an organism reaggregate from a mixture of cells they tend to sort out so that those of each tissue group together. This sorting out demonstrates a specificity which it has been usual to regard as being due to the specific adhesion of cells of like type. The concept of specific adhesion has greatly influenced theories about the nature of cell adhesion. However, as I have pointed out[1,2], since the various tissue masses in reaggregates are always positioned in definite relationships to one another the sorting out would result equally well from a mechanism which determined the general position of a given cell type in the reaggregate without the existence of any form of specific adhesion.

To enlarge on this point: it is not a sufficient explanation of the pattern of tissues found after sorting out to invoke a hypothesis of specific adhesion because, although this might sort out the cell types, it would not arrange the tissues in any given pattern. On the other hand, any mechanism controlling the position in the tissue pattern taken up by a given cell type would automatically sort out the cell types as well. For these reasons examination of the pattern found in reaggregates may be most informative about the nature of mechanisms of reaggregation. When mixtures of the cells of two species of sponges and other animals have been allowed to reaggregate, each cell type tends to form separate reaggregate bodies, as Wilson[3] first pointed out. Nevertheless de Laubenfels[4] stated that he had succeeded in obtaining interspecific mixtures of sponge cells in single reaggregate bodies, though unfortunately he gave few details of the experimental technique used. Sara[5] found that two closely related species of *Leucosolenia* do not show any sorting out in mixed reaggregates. Among vertebrates Moscona[6] has shown that mouse and chick cells do not sort out if they come from homologous organs, though mixtures of cells from two newt species do sort out[2]. In view of such exceptions, it seems that the mechanism for sorting out species is not developed universally.

In general, it has been assumed that the same type of mechanism is responsible for both the sorting out of tissue types and of different species. The experiments to be described here are directed to test whether the two types of sorting out are alike and whether specific adhesion or some other mechanism can account for sorting out and pattern formation in reaggregates. In reaggregation, phenomena other than adhesion play a considerable part. During the period when the cells are coming together they crawl over one another rather than on the surrounding substrate—a form of behaviour called 'contact promotion'[2]. Although movement is obviously related to adhesiveness it is not related in a linear manner, for extreme lack of adhesion will defeat movement owing to the resulting slip and likewise extreme adhesion will prevent movement owing to there being too much grip on the substrate for the cell to overcome. Later in reaggregation the cells become less mobile, and there is some evidence[1,2,7] that this happens at differing times for each cell type. Possibly the cells become 'contact inhibiting'[8] before they become immobile. Thus it seems that several types of cell behaviour are involved in the sorting-out process and that they involve movement as well as differences in adhesion. This argument suggests in itself that a specificity of adhesion is not a sufficient mechanism to account for the sorting out. Extensive cell movement is required for sorting out, and large differences in adhesiveness will be required to produce any marked specificity in the process. Yet these differences in adhesiveness will make some cell types less mobile so that their movement and thus the sorting out will be impeded.

For these reasons it seems that the sorting-out mechanism might depend on the control of the types of cell behaviour shown by cells. Evidence that this was so was found for the sorting-out of tissue types in amphibian reaggregates[1]. These results supported the following hypothesis: during the various stages of reaggregation the cells pass through a sequence in time of types of cell behaviour. Different cell types, which separate out in reaggregation, pass through this sequence at differing rates. There is thus a temporal specificity in the types of behaviour shown by each cell type. Any two cells which show the same types of behaviour at the same time associate together, but those which do not, become separated in the reaggregation process.

This hypothesis has been tested for sponge reaggregation by the following method, which, in addition, tests whether the species specificity and the tissue specificity are brought about by the same mechanism. Four species of sponge were used, *Halichondria panicea*, *Hymeniacidon perleve*, *Microciona sanguinea* and *Suberites* (*Ficulina*) *ficus*. Of these, *M. sanguinea* cells reaggregate most rapidly, taking about 3 h to form rounded bodies at 16°–17° C, *H. panicea* cells take about 8 h to reach the same stage at 16°–17° C and *S. ficus* and *H. perleve* cells reaggregate more slowly, taking some 15 h to reach this stage. Disaggregation was carried out by immersing fragments of the sponge bodies in 0·004 M ethylenediamine tetra-acetate (EDTA) in 0·55 M sodium chloride buffered with 0·004 M 2-amino-2-hydroxymethyl 1,3-propanediol–hydrochloric acid (*tris*-hydrochloric acid buffer), for 24 min. At the end of this period the fragments were pressed slightly and a dense suspension of cells formed. The cells were diluted with filtered (sterile) sea-water to give suspensions of 0·7–0·9 million cells per mm^3. For each reaggregate 5 ml. of this suspension was mixed with 10 ml. of sterile sea-water and the cells were then allowed to settle down on a coverslip. After 1 h the medium was replaced with more filtered sea-water. These cultures were grown for 20 h at 16°–17° C and then fixed and stained either as whole mounts or in section.

If a temporal specificity exists it would display itself by leading to the sorting out of those pairs of species which reaggregate at different rates. This in itself is little proof of the existence of such a mechanism: but if, when one of these species is started reaggregating and after an interval the cells of the second species are placed on top of it, so that they are initially 'sorted out', they become intermingled during the course of reaggregation; then this is strong evidence for the existence of a mechanism controlling sorting out by the timing of some process. In order to check intermingling, it is necessary to be able to identify the species type of cells. In live reaggregates the colour of masses of cells is a rough guide to cell type, but the degree of hæmatoxylin staining is a most satisfactory guide. By these means it is fairly easy to identify the main species type of a mass of cells. Control reaggregates of the four species each cultured alone were made. Mixed reaggregates were made from the following pairs of species: *M. sanguinea* and *Halichondria panicea*, *M. sanguinea* and *S. ficus*, *Hymeniacidon perleve* and *S. ficus*, and *Halichondria panicea* and *S. ficus*. These reaggregates were prepared with 0, 2, 4, 6, 8 and in some cases 10 h between the start of reaggregation of the first species and the start of the second, placed on top of the first.

When the reaggregate has been completed and sufficient time for any sorting out that might occur has passed (20 h) one or other of the following four types of pattern of separation of the various cell types in the mixed reaggregates can be recognized (see also Fig. 1).

(1) Complete separation of the reaggregates of each species. This was found when cells of *Microciona*, *Suberites* and *Halichondria* taken in pairs were started reaggregating simultaneously.

(2) Chain aggregates: though separation of the cells of each species occurs, reaggregates of each

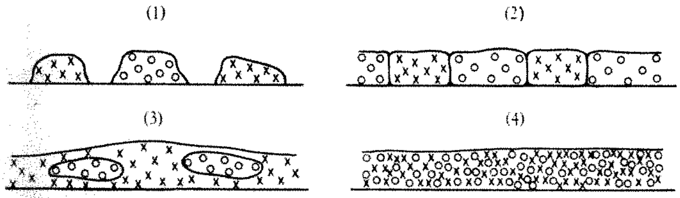

Fig. 1. The four types of reaggregate seen in idealized section. (1) Complete separation of 2 cell types; (2) chain aggregates: types separate in groups, but each group adheres to another; (3) concentric coating: one cell type invests the other; (4) intermingling. ○, Cells of one type; ×, cells of second type

species can adhere to those of another, frequently a 'segmented' chain of aggregates forms. This was produced when *Halichondria* cells were added to *Suberites* cells after the latter had reaggregated for 2 h, when *Microciona* cells were added to *Suberites* after 6 h reaggregation, when *Microciona* cells were added to *Halichondria* cells after 2 h and when *Hymeniacidon* cells were added to *Suberites* cells after the latter had reaggregated for 4 h.

(3) Concentric coating: one cell type forms a layer, coating a core of the other type. *Microciona* cells formed the coat when they were added to *Suberites* cells after the latter had reaggregated for 8 h; they also formed the coat when added to *Halichondria* cells which had been reaggregating for 4 h, and *Halichondria* cells formed the coat when added to *Suberites* cells after 4 h.

(4) Intermingling: complete or nearly complete intermingling of the cells resulted when *Hymeniacidon* and *Suberites* cells were reaggregated simultaneously, when *Microciona* cells were added to *Halichondria* cells after 8 h reaggregation, when *Halichondria* cells were added to *Suberites* after 6 h reaggregation and to a considerable degree, though some coating occurred, when *Microciona* cells were added to *Suberites* cells after 10 h reaggregation.

Those pairs of species which sort out completely if reaggregated simultaneously are those in which reaggregation proceeds at different rates. The pair of species which intermingle under such conditions are those which reaggregate at much the same rate. If the patterns of reaggregation in pairs of mixed species are considered it can be seen that there is a sequence (types 1–4), which is shown by those three pairs when the faster reaggregating species is added to the slower after successively longer intervals. With a sufficiently long interval before the addition of the fast type to the slow one both species will presumably show the same type of cell behaviour during the period when sorting out would normally occur and thus they associate together, type 4 behaviour. Thus the pattern of separation of two cell types and even its occurrence at all can be controlled by the timing of some phase of cell behaviour in the two types. The experimental results strongly suggest that if the behaviour shown by two cell types can be synchronized (at least at some crucial stage of the process) they will not sort out. With increasing desynchronization sorting out will produce varying different patterns which show greater and greater degrees of separation (coating, then segmentation, then complete separation). A further point in support of this interpretation is that the pair of species which do not normally sort out can be made to do so by delaying the addition of one species (*Hymeniacidon* and *Suberites*).

Thus a variety of 'tissue' patterns can be built up presumably by difference in the timing at which cells show certain types of behaviour. The four 'tissue' patterns found in these experiments are also widely spread in the animal kingdom, and it is probable that the same specific timing of the appearance of various different types of cell behaviour is responsible for the building of such structures during embryogenesis. It is interesting that one of the patterns is a segmental one, for Deuchar[9] has suggested that aggregation phenomena occur during the process of somite formation. It appears that a mechanism of temporal specificity operates both in tissue separation in amphibia and in species separation in sponges. There is some evidence, due to Gaze and Jacobson[10], that the regenerating optic nerve of the frog re-acquires functionally adequate central connexions by means of some sort of temporal specificity in the timing of the growth and movement of the various optic axons. If the timing of a type of cell behaviour does not coincide in two cell types they will separate out, and this may occur either with cells of different species or of different histological type. This explains why some workers have reported that the sorting-out specificity is displayed on the histological level[6] and others that it is displayed between species[3]. With several cell types of two species mixed together it might be expected that on occasion some of the cells would associate though of differing species and cell type and that others would separate out: there is some evidence that this has occurred in the *Microciona–Suberites* mixtures. The origin of the timing specificity must lie in some physico-chemical property of the cell—a possible suggestion is that the cells contain some class of grouping which reacts with a component of the medium as reaggregation proceeds; differing tissues having differing velocity constants for this reaction.

These experiments have a considerable bearing on the mechanism of cell adhesion. The specific adhesion of cells, or any other individual property of a given cell type, cannot explain these results by any but a most complex theory; for example, *Microciona* cells added after 8 h to *Suberites* coat the latter, but if they are added after the same interval to *Halichondria* cells they intermingle. Thus the sorting out or otherwise appears to be due to a mutual interaction between two cell types. In consequence it seems improbable that there is any mechanism involving the specific adhesion of members of a cell type to one another (egg and sperm may be an exception). If this is so, we can discard hypotheses of cell adhesion such as those of Steinberg[11] or Moscona[12] in which the specificity of adhesion forms the main feature of the theory. Moscona[12] has suggested that there are specific cementing substances which cause like-cell types to adhere together, formed by cell metabolism. Subsidiary experiments suggest that a specific cementing substance is probably not involved in the adhesion of *Suberites* or *Halichondria*. For if such a substance exists it would be dissolved during disaggregation. Washing away the disaggregation medium would remove the substance and if the cells were prevented from reforming it metabolically, for example, by culturing them cold, they should not reaggregate from cooled washed cell suspensions, though cooled unwashed suspensions might contain sufficient substance with which to re-adhere. However, cells of these two sponges will slowly reaggregate at 3° C even after such extensive washing that the concentration of any possible 'cement' would be reduced to 0·00001 of its original concentration.

It can be concluded that: (1) the pattern of separation of cell types or even its occurrence at all in reaggregates of mixtures of two species of sponges can be controlled by a mechanism involving the timing of certain types of behaviour in each cell type; (2) that this 'temporal specificity' can act in both the separation of reaggregating cells of different histological or different species type; (3) that a hypothesis of the 'specific adhesion' of cells does not explain the sorting-out processes in reaggregation; (4) that there is no evidence for the existence of specific materials cementing cells together in the reaggregation of these sponges.

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A MECHANISM FOR THE DISSOLUTION OF GROUND QUARTZ POWDERS IN DILUTE HYDROFLUORIC ACID: AN 'EASILY SOLUBLE CORE' AND ITS RELATION TO THE 'EASILY SOLUBLE LAYER'

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INVESTIGATIONS of the rate of dissolution of ground quartz powders of graded size in the respirable range (particle diameter $<5\mu$ (particle sizes are given in the form of Stokes's diameters)) in dilute alkali[1] and hydrofluoric acid[2] have confirmed that the early stages of dissolution occur at a rate of an order of magnitude greater than that for the remaining material. The material dissolved during these early stages has been termed the 'easily soluble layer'. The amount of easily soluble material present in the powders of diameter $>3\mu$ was small enough (about 0·2 per cent at 4μ) to be explained in terms of a surface monolayer solubilized by hydration. Once the easily soluble layer had been removed, the dissolution rate fell to a low and fairly constant level. With powders of smaller initial particle size, however, the amount of easily soluble material was too great (about 8 per cent at $0{\cdot}3\mu$ diameter) to be explained entirely by surface hydration, and there was no clearly defined transition between successive stages of the dissolution, for the rate of dissolution decreased gradually as the material was etched away.

The examination of these and similar quartz powders by X-ray diffraction[3] has shown an increase in the line-broadening of diffracted X-rays with decreasing initial particle diameter below 4μ; this increase runs parallel to that in the easily soluble layer. With powders of diameter $<1\mu$, the line broadening was not completely removed by etching, even when the greater part of the material had been dissolved. These powders also showed a generally higher dissolution-rate; both this and the line-broadening are believed to be due to disturbance of the crystal structure of the material by the grinding process. The disturbance appears to be intense near the surface, and decreases slowly towards the core.

On this assumption, the quartz powders can be regarded as made up of three types of material: ordered crystalline, disturbed crystalline, and hydrated quartz. The powders of particle diameter $>3\mu$ would consist of a disturbed and afterwards hydrated layer on an ordered core; those $<3\mu$ of a hydrated layer merging into a disturbed crystalline material through which the intensity of disturbance decreased gradually towards the core.

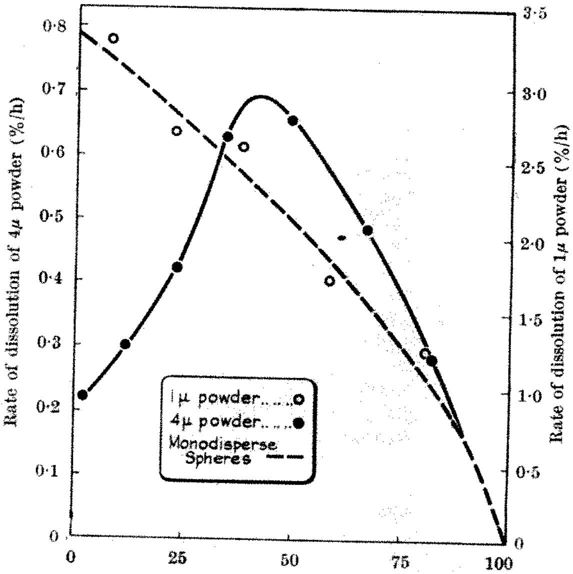

Fig. 1. Variation of dissolution-rate with amount dissolved for 1μ or 4μ powders and for monodisperse spheres (fitted to 1μ curve)

This explanation is considered in the light of further evidence from studies of dissolution in dilute hydrofluoric acid beyond the stage required to remove easily soluble material. These experiments revealed that, for a number of ground quartz powders of particle diameter $> 2\mu$ which had already lost about 5 per cent of their material, the rate of dissolution (expressed in terms of a percentage of the initial amount of material) increased gradually until it had more than doubled, as if the particles had an 'easily soluble core'. Such a dissolution-rate curve is shown for a 4μ diameter quartz powder in Fig. 1. Now, in general, the rate of dissolution should decrease gradually with the depletion of available surface area. For a homogeneous, monodisperse and isotropic powder the dissolution-rate would decrease as the 2/3 power of the volume remaining, unless the dissolution process had resulted in a significant change in the average particle shape. A dissolution-rate curve, calculated for the ideal case of monodisperse spheres, fitted to the experimental points for a 1μ diameter powder, is shown in Fig. 1; the points lie fairly close to the calculated curve. In order to facilitate comparison of the shapes of the dissolution-rate curves for the two powders and the calculated curve, the scales for the 1μ and 4μ powders have been adjusted to bring the curves together at about 90 per cent dissolved.

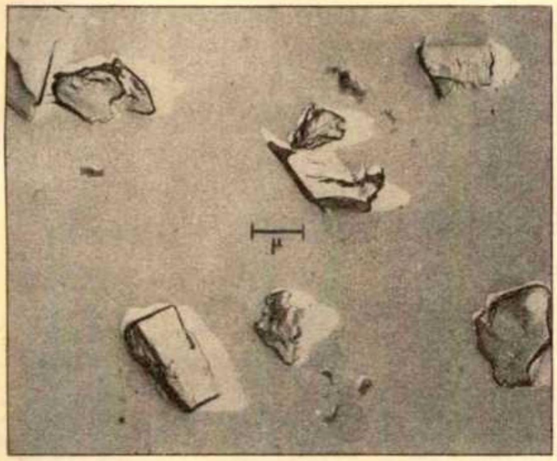

Fig. 2. 1μ diameter powder before etching

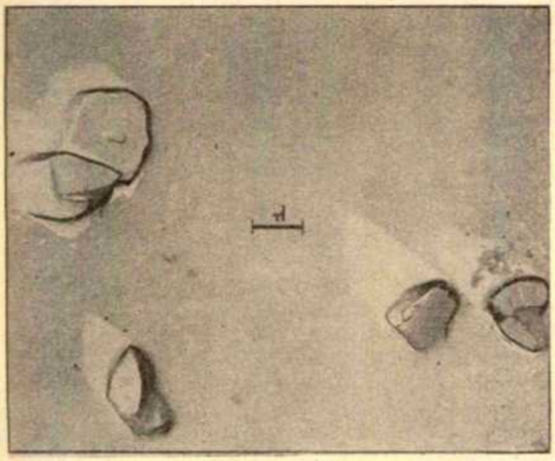

Fig. 3. 1μ diameter powder after etching

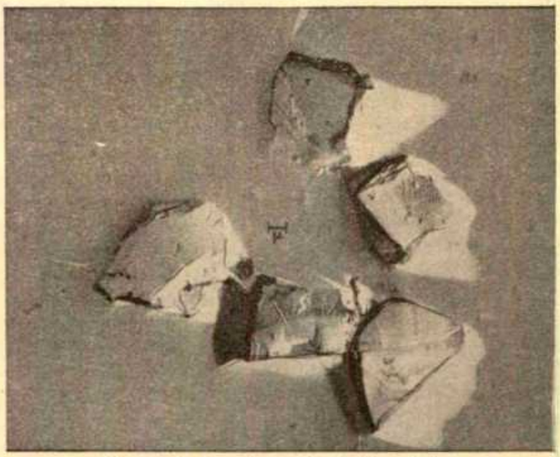

Fig. 4. 4μ diameter powder before etching

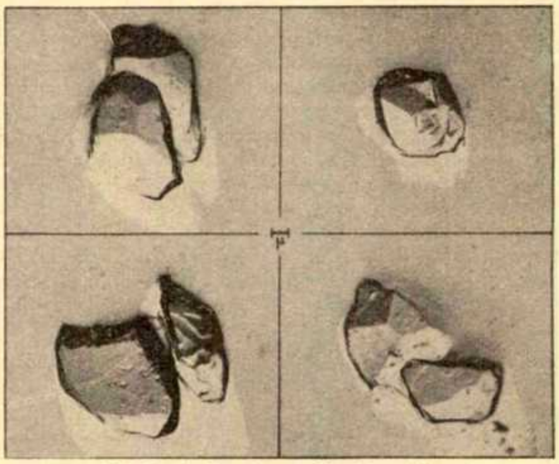

Fig. 5. 4μ diameter powder after etching

The increasing dissolution-rate of the 4μ sample up to 50 per cent lost could conceivably have been due to shape changes; but measurements of the specific surface areas of etched quartz powders by gas adsorption, and of their particle shapes by microscopy, revealed no change in shape after etching sufficient to account for such an increase. Since the increase in rate for these powders was not due to an increase in available surface, the indication was that there must be a change in the mechanism of the dissolution, or in the nature of the dissolving material, once 5 per cent had been dissolved. It was thought that either of these two effects might show up as a change in the structure of the etched surface. The fine detail of the particle surfaces is not revealed by transmission electron microscopy; a technique was therefore developed to prepare replicas of surfaces of the particles for study by electron microscopy.

The powder, in suspension in ethanol, was sprayed on to a glass microscope slide, while another slide was coated with a thin film of gelatin. The two were pressed together, partially embedding a number of particles into the gelatin. The specimen was gold palladium shadowed, and then coated with a carbon film by evaporation[4]. This film was stripped from the gelatin in hot water, and the quartz particles were dissolved in concentrated hydrofluoric acid. Figs. 2–5 show electron micrographs of replicas of quartz powders of 1μ and 4μ diameter, before and after dissolution of about 50 per cent in dilute hydrofluoric acid. The unetched powders (Figs. 2 and 4), show gently curving surfaces with fine stepped striations (characteristic of conchoidal fracture) which are occasionally observed on particles of < 2μ but more frequently seen on particles of ground quartz powders above this size. The etching process has had little effect on the particle shapes of the 1μ diameter powder (Fig. 3), but appears to have led to the development of plane faces with straight edges on the 4μ powder (Fig. 5); often three faces meet at a point, at angles to each other which appear to the eye to be consistent with the trigonal crystal structure of quartz. On such faces occasional etch pits, often triangular in outline, are observed, rather than striations. On the remaining surfaces, the irregularities are unchanged in appearance.

The mechanism of the dissolution of ground quartz powders of particle diameter > 2μ appears, therefore, to be one of preferential dissolution in directions normal to a number of crystal planes. The rates of dissolution of these faces must be similar; otherwise, particles shaped like needles or plates would be formed, and both the specific surface and the average particle shape of the powder would change in an obvious manner. The fact that fine striations characteristic of an unetched surface are still present on some parts of the particle surface even after 50 per cent has been dissolved is consistent with the idea of a much slower dissolution of surfaces not parallel to the preferred crystal planes. All the experiments so far have yielded clear evidence of the formation of these flat etch faces on > 2μ particles. The assumption that these faces are the result of etching in preferred directions seems reasonable and offers an explanation for the increased dissolution-rate associated with their formation. Once the faces were formed, their areas would tend to increase during dissolution and an increasing fraction of the particle surface would consist of preferred faces; hence there would be an increasing rate of dissolution (per unit area) until all the faces consisted of preferred planes and the remainder of the particle constituted an 'easily soluble core'. Thus the growth of etch faces might be the primary mechanism controlling the dissolution-rate of particles > 2μ.

With < 2μ particles the observed results are different and suggest a different mechanism. Etch faces do not form to any marked extent, and the rate of dissolution decreases with time as would be expected of a material dissolving by an isotropic etching mechanism. These results suggest that the disturbance of the bulk of the material, though not intense enough to increase the dissolution-rate appreciably, is sufficient to inhibit the anisotropic mechanism of dissolution, that is, to prevent the development of faces parallel to well-defined crystal planes. Thus, compared with the larger particles, those of < 2μ diameter have a slowly dissolving core.

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THERMAL AND PHOTOLYTIC DECOMPOSITION OF THE LEAD AZIDES IN AIR

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The thermal decomposition of α-lead azide in air has already been investigated by Todd[1] using X-ray diffraction methods. It was shown that the ultimate product was lead oxide and that the decomposition proceeded through a number of intermediates none of which could be further identified at that time. The similarity between the diffraction pattern of the first emergent phase and that of basic lead azide I (several basic azides probably exist) was, however, demonstrated and the relative diffraction data are now collated in Table 1. On this basis it was suggested that the first emergent phase might prove to be a dehydrated basic azide I or oxy-azide, and recent gravimetric work[2] showing a loss in weight of 12 per cent for its formation by oxidation from normal lead azide appears to substantiate the formula $PbN_6.PbO$.

It has not yet been found possible to assign unit cell data to this oxy-azide but the second emergent phase of the decomposition series has now been identified as a tetragonal phase with parameters $a = b = 11{\cdot}3$ Å and $c = 11{\cdot}2$ Å. Pattern spacings and intensities are shown in Table 2. The second phase appears to degenerate to a crystallographically similar phase with a single resolved line at 7·7 Å.

The decomposition sequence has been studied gravimetrically by Stammler *et al.*[2], who have noted arrest-points in the weight-loss curves corresponding with formation of these intermediate phases. They have also characterized a further phase (termed phase 3) not observed in the earlier work and therefore probably occurring between the 5 and 65 h heating arrests at 240° C (Fig. 1, ref. 1). It is interesting to note that the weight-loss arrests at 15 and 15·5 per cent (Fig. 1, ref. 2), and which probably correspond with the second and third emergent phases of the X-ray work, can be explained by transitions of the type:

$$3PbN_6 \rightarrow PbN_6.2PbO.\tfrac{1}{2}H_2O \rightarrow PbN_6.2PbO$$

indicating formulæ for these phases.

Preparation and formula of basic lead azide I. Basic lead azide I may be prepared by the double decomposition of soluble azide and lead salt. Six preparations were attempted as shown in Table 3. M and 2 M solutions of salt and alkali respectively were used, volumes being adjusted to give the equivalents ratio shown. The arrow indicates order of addition.

Table 1. COMPARATIVE DIFFRACTION DATA FOR BASIC LEAD AZIDE I AND THE FIRST EMERGENT PHASE OF THE α-LEAD AZIDE THERMAL SERIES TAKEN WITH COBALT $K\alpha$ RADIATION

Lattice spacings (Å)		Relative line intensities		Lattice spacings (Å)		Relative line intensities	
Basic lead azide I	First emergent phase	Basic lead azide I	First emergent phase	Basic lead azide I	First emergent phase	Basic lead azide I	First emergent phase
6·8	6·8	50	77	2·29	2·29	5	< 10
6·5	6·4	25	79	2·26	—	4	—
6·2	—	13	—	2·22	—	2	—
4·36	4·39	65	33	2·19	2·16	4	< 10
4·28	4·25	15	21	2·15	2·14	4	10
4·19	—	3	—	2·10	2·10	10	< 10
3·95	—	4	—	—	2·09	—	< 10
3·82	3·83	< 1	< 10	—	1·98	—	10
3·75	—	< 1	—	—	1·95	—	< 10
3·66	3·65	< 1	< 10	1·90	1·90	27	< 10
—	3·43	—	20	1·87	1·88	< 1	< 10
3·26	3·28	13	13	1·86	1·86	< 1	< 10
3·13	3·20	9	40	1·84	1·84	10	< 10
3·04	3·06	100	85	1·82	—	< 1	—
2·94	2·94	100	100	1·80	1·80	3	< 10
2·85	2·87	87	38	1·79	—	8	—
2·83	—	4	—	1·77	1·76	1	< 10
2·79	2·80	< 1	23	—	1·73	—	< 10
2·73	—	< 1	—	1·72	1·72	10	< 10
2·68	—	< 1	—	1·70	1·70	< 1	< 10
2·63	2·62	5	25	1·68	1·67	15	< 10
2·55	—	23	—	1·66	1·66	15	10
2·49	2·49	< 1	25	1·64	1·65	2	< 10
—	2·44	—	< 10	1·57	1·59	1	10
2·41	2·40	10	< 10	1·56	—	< 1	—
2·38	2·35	3	< 10	1·53	1·53	1	< 10
—	2·33	—	< 10	1·52	—	1	—
2·31	2·31	5	< 10	1·50	1·50	3	< 10

Diffraction patterns of the six products are shown in Fig. 1. Preparation *BLA* 11 alone appeared to onsist of a relatively pure sample of basic azide I. *BLA* 9 and *BLA* 13 contained amounts of basic lead arbonate and α-lead azide whereas *BLA* 10, 12 and 2*A* contained varying amounts of a phase with diffraction maxima at 7·7, 3·10 and 3·15 Å. This has been prepared in another context[3] by the photo-ysis of α-lead azide and basic azide I in moist atmopheres, and does not appear to be a hydrate of lead xide, most of which we have now examined by X-ray diffraction. Its similarity to the second and hird emergent phases of the α-lead azide thermal series suggests that it may be a further basic azide of formula $\mathrm{PbN_6 . 2PbO} . n\mathrm{H_2O}$.

The empirical formula for basic azide I was obtained by dissolving a sample of *BLA* 11 in aqueous ammonium acetate and estimating lead as lead sulphate, and the azide as silver azide. The respective figures of 80·2 and 15·5 per cent compare with mean figures of 79·2 and 15·3 per cent obtained by Feitknecht and Sahli[4] for crystallographically similar preparations and an empirical formula for basic azide I of $\mathrm{PbN_6 . PbO} . n\mathrm{H_2O}$ is indicated. n is in the range 0·5–1·0.

Thermal decomposition of basic lead azide I. A sample of basic lead azide I (preparation *BLA* 11) was decomposed as in the normal lead azide experiments by heating in air at 240° C and changes studied by diffraction analysis. Certain of the diffraction maxima were observed to weaken and thereafter the phase appeared to decompose directly to the second emergent phase of the normal azide thermal series and thence through the third phase to lead oxide. No diffraction evidence for the first oxy-azide of the normal series was obtained, but the oxy-azide pattern is so similar to that of the basic azide I that small transient amounts could easily pass undetected.

Table 2. OBSERVED DIFFRACTION DATA OBTAINED WITH COBALT $K\alpha$ RADIATION FOR THE SECOND EMERGENT PHASE OF THE α-LEAD AZIDE THERMAL DECOMPOSITION SERIES

Indices of the planes have been assigned as shown on the basis of a tetragonal unit cell with axes $a = b = 11{\cdot}3$ Å and $c = 11{\cdot}2$ Å

Observed lattice spacings (Å)	Relative line intensities	Assigned Miller indices	Calculated lattice spacings (Å)
7·95	33	110	7·95
3·98	< 5	220	3·98
3·74	12	212	3·75
		003	3·73
3·12	100	302	3·12
2·82	< 5	400	2·82
2·79	10	004	2·80
2·71	< 5	104	2·72
2·66	10	330	2·66
		411	2·66
2·63	< 5	114	2·64
2·58	< 5	331	2·59
		313	2·58
2·53	< 5	420	2·53
		402	2·52
2·46	< 5	421	2·46
		412	2·46
2·29	< 5	224	2·29
2·21	< 5	510	2·22
		501	2·22
		431	2·22
1·93	12	503	1·93
		433	1·93
		334	1·93
		305	1·92
1·88 *D*	< 5		
1·81 *D*	< 5		
1·74 *D*	< 5		
1·69 *D*	< 1		
1·66	5	631	1·66
		613	1·66
1·61	8	700	1·61
		623	1·61
		632	1·61

D = Diffuse, indicating group of lines.

Table 3

Preparation	Method		
BLA 9	1 × basic lead acetate	→	2 × sodium azide
BLA 10	1 × sodium azide	→	1 × lead acetate 1 × caustic soda
BLA 11	1 × sodium azide 1 × caustic soda	→	1 × lead acetate
BLA 12	1 × sodium azide	→	1 × lead acetate 1 × ammon. hydroxide
BLA 12*A*	1 × sodium azide	→	2 × lead acetate 2 × ammon. hydroxide
BLA 13	1 × sodium azide 1 × ammon. hydroxide	→	1 × lead acetate

In this connexion, it should be noted that the reverse is also true and small amounts of basic azide I are undetectable by X-rays in the presence of large amounts of emergent phase 1. Whereas no basic

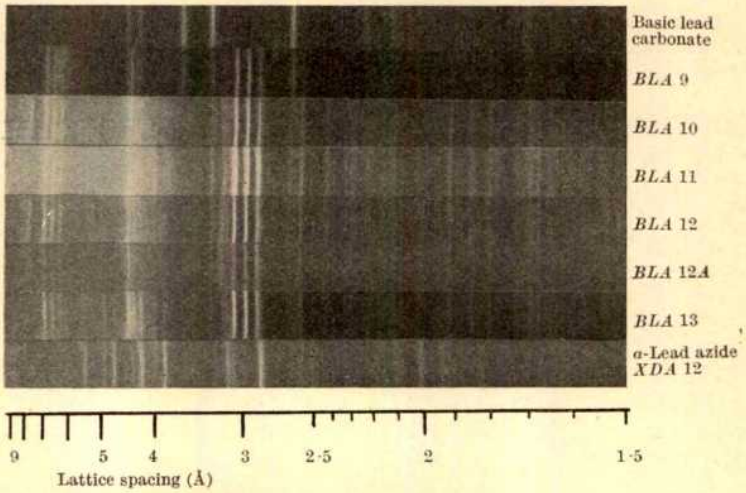


Fig. 1. Taken with a focusing camera using cobalt $K\alpha$ radiation, these diffraction results show the impurity content of basic lead azide samples. *BLA* 9 and *BLA* 13 are heavily contaminated with basic lead carbonate and normal lead azide respectively. *BLA* 10, *BLA* 12 and *BLA* 12*A* contain an unknown impurity with diffraction lines at 7·5, 3·1 and 3·15 Å. (*Crown copyright reserved*)

azide I is detected by X-rays in the thermal breakdown of α-lead azide, there is gravimetric evidence (Fig. 1, ref. 2) for an arrest at a loss of weight of 5–10 per cent corresponding with transient formation of basic azide I.

Comparison of the thermal decomposition of normal α-lead azide and basic lead azide I. In order to avoid confusion, a notation based on the sequence noted in the original paper[1] will be used. If the first, second and third emergent phases of the decomposition of α-lead azide are designated [1], [2] and [3] the three decompositions so far studied may then be stated as follows:

$$\begin{array}{llcl}
(A)\ \text{Normal } \alpha\text{-lead azide} \xrightarrow{\text{heat}} & [?] & \rightarrow & [1] \rightarrow [2] \rightarrow [3]\ \text{etc.} \\
(B) & \begin{array}{c}\text{basic}\\ \text{lead}\\ \text{azide I}\end{array} & \xrightarrow{\text{heat}} & [?] \rightarrow [2] \rightarrow [3]\ \text{etc.} \\
(C) & \begin{array}{c}\text{basic}\\ \text{lead}\\ \text{azide I}\end{array} & \xrightarrow[\text{vacuum}]{\text{heat}} & [1]
\end{array}$$

It is now evident that all sequences can be taken as interpolations of the general sequence *A* in which basic azide I can be inferred as a transient phase undetected in the presence of larger amounts of [1]. Similarly in sequence *B* transient amounts of [1], which is readily hydrolysed to [2], go undetected in the presence of large amounts of basic azide I.

In sequence *A*, on the other hand, [1] is able to accumulate because α-lead azide is a stronger 'getter' for water and frustrates the next hydrolysis stage so long as it is present in any amount.

Mechanism of the thermal and photolytic decomposition of the lead azides in moist atmospheres. Todd and Parry[3] have previously examined the photolytic decomposition in various atmospheres of α-lead azide and basic lead azide I and have shown that the process is essentially through a basic azide sequence to the azide-free product basic lead carbonate $2PbCO_3.Pb(OH)_2$. This in our experience takes the place of the fully hydrolysed and hypothetical $Pb(OH)_2$ in slow-rate reactions where basic lead is the end product.

The thermal decomposition now appears to be of essentially the same type except that oxy-compounds or oxy-hydroxy-compounds take the place of corresponding hydrates unstable at the higher temperatures.

Whatever the electronic detail, the primary process of decomposition of lead azide, both photolytic and thermal, consists of loss of electrons by adjacent ions which then interact to liberate nitrogen and create negative ion vacancies[5]:

$$\underset{\text{(azide ion)}}{2N_3'} \rightarrow \underset{\text{(gas)}}{3N_2\nearrow} + \underset{\text{(electrons—usually trapped)}}{2e}$$

Lead nuclei are formed later in the process when such gas evolutions have occurred at adjacent sites and liberated lead ions are able to collect electrons and collapse to assume the periodicity of lead.

Interface decomposition is, however, only slightly greater than elsewhere in the crystal, and lead nuclei, once formed, show little tendency to grow. The fundamental process still continues as the liberation of nitrogen gas and the creation of negative ion vacancies throughout the crystal.

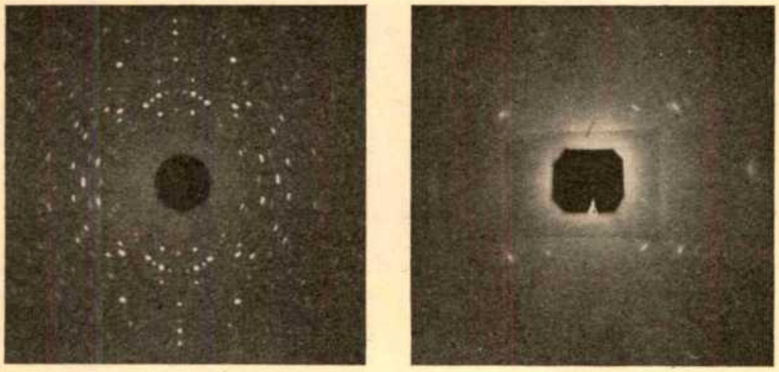
Where decomposition takes place in a vacuum or explosively so that no atmospheric components can reach and react with the decomposing lattice, the products of decomposition can only be lead and nitrogen. However, when water and carbon dioxide are present we show that this is not so, and it is now necessary to suggest a mechanism for the formation of the complex azides which are in fact observed.

Although it is doubtless in principle possible for these complex phases to form as a result of reaction of reactive lead nuclei with other residues in the decomposing crystal, there appear to be a number of arguments against this. For example, if lead azide is decomposed by heat or X-rays in both wet and dry atmospheres, the lead azide diffraction pattern disappears much faster when water is present. If the effect of water were solely to destroy lead nuclei, electron traps would be thus removed and one would expect the rate of decomposition of the lead azide to be, if anything, retarded.

It seems likely, therefore, that metallic lead is not directly involved in the formation of the basic azides, and since the chemical reactivity of *F*-centres is already well known[6] it is suggested that these are in fact the functional groups involved in the generation of basic azides. Although their existence in the thermal case can only be implied theoretically and has not been established experimentally, colour centres can be detected readily in the X-ray case where material removed from the irradiation chamber exhibits delicate colours which fade rapidly in light.

The general reaction of an *F*-centre to water is as follows:

$$e + H_2O \rightarrow OH' + H$$



Before irradiation

After irradiation

Fig. 2. X-ray Laue photographs of the *b*-axis projection of an α-lead azide crystal undergoing X-ray decomposition. Unlike the thermal case (Fig. 2, ref. 1) where the azide appears to decompose directly to a disoriented product the material here shows evidence of disoriented breakdown. (*Crown copyright reserved*)

Gas evolved when irradiated azide is exposed to water has been analysed and found to contain mostly nitrogen with little or no hydrogen. The hydrogen as released is, however, atomic and will not escape as hydrogen gas unless it can find and combine with an equivalent hydrogen atom. It is more likely to react with azide residues and to reduce these to ammonia, nitrogen and hydrazoic acid.

Such an *F*-centre mechanism would explain why both photolytic and thermal decomposition of lead azide via the basic azides, oxy- and otherwise, is so apparently dependent on water, conversion to the oxide being essentially a hydrolysis followed by dehydration. In the photolytic case no dehydration occurs and it is probable that the defect azide lattice may acquire a considerable quota of hydroxy-ion before breakdown and reorientation to the basic azide lattice occurs. Such a situation would lend itself to a measure of oriented breakdown and may account for the anomalous *b*-axis expansion of the decomposing crystal[3] together with certain features revealed by X-ray Laue studies and not found in the thermal case (Fig. 2).

We thank the Ministry of Aviation, Explosives Research and Development Establishment, Waltham Abbey, for the supply and analysis of azide samples.

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IONOSPHERIC EFFECTS OF NUCLEAR EXPLOSIONS

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In a recent communication Dieminger and Kohl[1] described some ionospheric phenomena observed at Lindau, Germany, on October 30, 1961, following a large nuclear explosion at Novaya Zemlya in the Arctic. The Lindau ionospheric recordings were made at ½-hourly intervals and, as the authors point out, this limited the determination of the precise time of the onset of the disturbance. For a large part of the day concerned recordings were made at Aberystwyth at ¼ hourly intervals, and these provide some interesting additional information on the ionospheric effects at a distance of 3,570 km from the source.

Fig. 1 shows the variation in the peak electron density of the *F*2-layer at Aberystwyth on the day of the explosion, expressed as a percentage deviation from undisturbed conditions on a group of six control days. Hourly data only are available until 10 U.T. with ¼-hourly data thereafter until 1745 U.T. It will be seen that between 09 and 10 the peak electron density of the *F*2-layer fell to some 50 per cent below normal and in the succeeding hour rose to 50 per cent above normal. Then followed two further oscillations with a period of about 1 h and with diminishing amplitude. The amplitude of the second peak (marked *b* in Fig. 1) is about 60 per cent that of the first. From about 1445 onwards the electron density remained well below average. The large initial fall in electron density was not observed at Lindau, but it seems probable that this 50 per cent decrease at Aberystwyth is significant and related to the explosion. Support for this suggestion is provided by data for certain other stations and by other data in the Aberystwyth records.

In Fig. 2 corresponding data for Dourbes (Belgium), Prague and Genoa are plotted. It will be seen that all three curves show a depression in *F*2 peak density with a minimum near 10 U.T. and the magnitude of the depression recorded at Dourbes between 09 and 10 is comparable with that observed at Aberystwyth. A decrease of electron density of this sort at temperate latitude stations is typical of a magnetically disturbed condition, but there seems to be no clear evidence for a widespread magnetic disturbance at this time. Further supporting evidence that these changes in electron density were due to the explosion is provided by an analysis which we have made of the electron density/true height ($N(h)$) profiles deduced from our ¼-hourly ionograms. In Fig. 3 the variations in the true height of reflexion of a radio wave of frequency 4·4 Mc/s (corresponding to an electron density of $2\cdot4 \times 10^5$ e/c.c.) on October 30 and 31, 1961, are shown. In this case the oscillations between 10 and

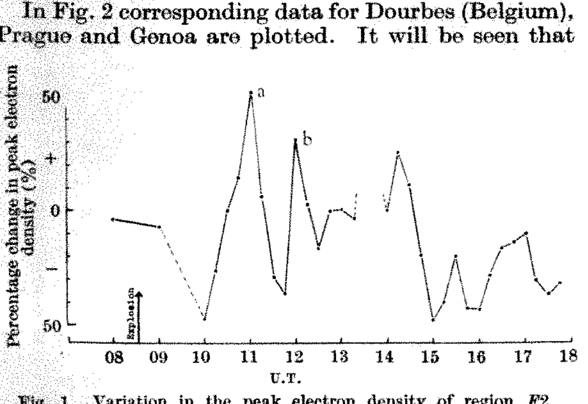

Fig. 1. Variation in the peak electron density of region *F*2 observed at Aberystwyth on October 30, 1961, expressed as a percentage deviation from undisturbed conditions

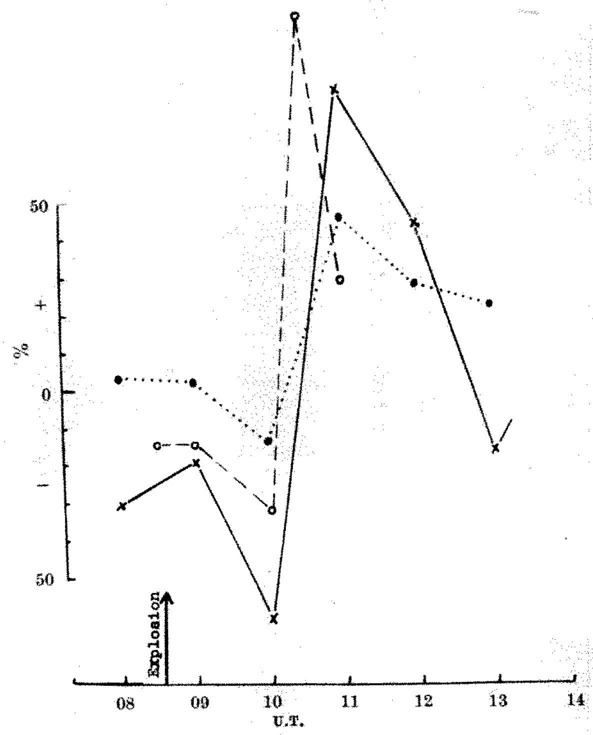

Fig. 2. Variation in region *F*2 peak electron density on October 30, 1961, expressed as a percentage deviation from undisturbed conditions. (The ordinate scales for Prague and Genoa have been displaced upwards for clarity). ×, Dourbes; O, Prague; ●, Genoa

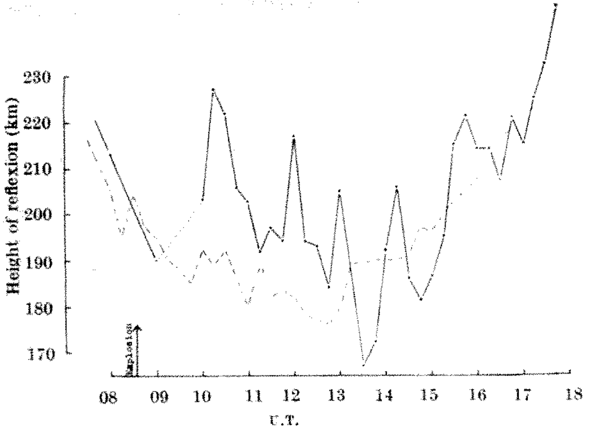

Fig. 3. Variation in true height of reflexion at 4·4 Mc/s observed at Aberystwyth. –, October 30, 1961; – – –, October 31, 1961

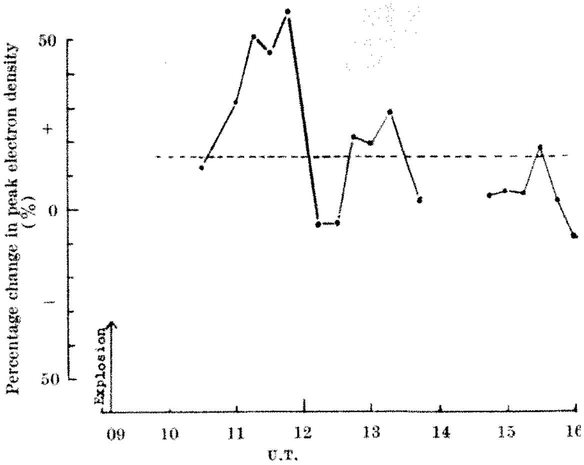

Fig. 4. Variation in peak electron density of region $F2$ observed at Aberystwyth on August 5, 1962, expressed as a percentage deviation from undisturbed conditions on five control days

16 h in the height of reflexion show up very clearly and there seems little doubt that the first peak at 1015 is a real consequence of the explosion and that the onset of the disturbance occurred at, or before, 10 U.T. It is to be noted that Dieminger and Kohl comment on the fact that in their records an unusual $F1$ stratification showed up at 10 U.T., indicating that at Lindau, too, the ionosphere at this level was being disturbed at this time.

We have examined the electron density variations at four levels in the ionosphere above Aberystwyth (220, 200, 180 and 160 km) and find that there is a progressive decrease in the magnitude of the disturbance towards lower levels. On the average, a change of 100 per cent at the 220 km-level is accompanied by changes of 70, 35 and 20 per cent at the 200, 180 and 160 km-levels respectively. This decrease in the magnitude of the disturbance at the lower, denser levels in the ionosphere is confirmed by our normal E layer observations which refer to a level of about 120 km. During the morning period the normal E-layer trace was obscured by sporadic E echoes, but the ¼-hourly measurements between 1230 and 1500 U.T. appear quite normal whereas the F layer was certainly very disturbed at this time.

The marked maximum in the $F2$ peak electron density within 2–2½ h after the explosion (marked a in Fig. 1) is a clear feature in all the data considered here and, for the Aberystwyth observations, the time-interval between this maximum and the explosion corresponds to a travelling disturbance with a mean velocity of about 410 m/sec. We find that when due consideration is given to the uncertainty in precise timing, a mean velocity of this magnitude will fit all the observations of this first maximum by the group of stations considered here. This maximum represents the onset of the first pressure maximum at this level with a time delay of about 150 min, and it is of interest to note that after a further interval of this magnitude the F layer was again very disturbed (between 1315 and 1345). We feel that this second marked disturbance some 5 h after the initial explosion may be significant since we have clear evidence for a very similar sequence of events after a recent Soviet nuclear test on August 5, 1962. Fig. 4 shows the perturbation in peak electron density of the $F2$-layer following this explosion. (The layer electron density seems to have been generally higher than normal on this day and the ordinate scale should probably be displaced upwards as indicated.) Unfortunately, we do not have records before 103 U.T. but between 1030 and 1345 there are two clea oscillations which can be ascribed to the effect of th nuclear explosion. The amplitudes are only abou one-half those observed for the October 1961 test an indicate that the energy of the explosion was considerably smaller. The explosion is reported to have take place at 0908 U.T. and the mean velocity of a disturbance which would give the first observed $F2$ maximu at Aberystwyth lies between 380 and 420 m/sec depending on whether the maximum is assumed to b at 1145 or 1130 U.T. The amplitude of the secon maximum in Fig. 4 is again about 50 or 60 per cen that of the first. The oscillations on October 30 1961, are distinctly sharper in character than thos on August 5, 1962, as might be expected from th relative magnitudes of the two explosions. It wil be noted that in Fig. 4 no values are given betwee 1345 and 1445. This gap in the data is again due t the very disturbed records at this time, which ar particularly characterized by a marked elevation i the height of the $F2$ trace. This disturbance agai occurs some 5 h after the time of the explosion and as before, has a time-delay of approximately twic that of the first pressure wave.

In the case of the large explosion of October 30 1961, we feel that the minimum in electron densit observed some 1½ h after the explosion at four widel separated stations, ranging in distance 3,300–4,25 km from the source, marks the initial onset of th disturbance in the ionosphere. In this connexio it is interesting to note that the time delay involve is just that to be expected for a rarefaction travellin at high level in the ionosphere from above the sourc with a sound-wave velocity appropriate to the mea temperature in the height-range 160–220 km. O the occasion of the test on August 5, 1962, our recordings only commenced some 1½ h after the explosio and consequently any initial minimum may hav been missed. Alternatively, it may be that thi phenomenon in the ionosphere, at large distance from the source, is only a feature of very larg nuclear explosions and it may well depend too o the height at which the explosion occurred.

We thank the International Geophysical Yea World Data Centre C, Slough, for the supply certain ionospheric data.

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LETTERS TO THE EDITOR

ASTRONOMY

Tensional Stress and Lunar Patterns

Warner and Fielder[1] have referred to their conclusion that the lunar surface was under overall tensional stress at the time of cratering. I have approached the problem of the mode of origin of the lunar surface patterns from a different, purely geological point of view, but my conclusions might perhaps be relevant to the conclusions of these authors.

Geological investigations in the rift valleys of Kenya, carried out over the past decade, have led me to the conclusion that in these particular anorogenic zones the lunar patterns are more nearly duplicated than in any other terrestrial field—though the patterns are scaled down and the volcanic products associated with them are not, for the most part, comparable with those likely to have been erupted on the Moon. Firsoff[2,3] has used terrestrial patterns from Tanganyika for comparative illustrations in his penetrating studies of selenology, but, in my view, the patterns in Kenya, to the north, are both more complex and more strikingly 'lunar'. The rift valleys in Kenya seem to have originated as linear undations or volcano-tectonic upswellings, in Miocene times: in central Kenya a second and comparable undation occurred towards the end of the Pliocene. The two undations were each followed by a related recessive phase, during which collapse on normal faults occurred along the zones of undation. Each cycle of activity involved a distension accompanied by vulcanicity followed by either simple subsidence or drawing down of the surface-levels with an actively receding magma-level (as in some calderas[4]). The collapse was mainly on linear fractures, but calderas, associated with the recessive phase, show an integral relationship to the linear fracture patterns in exactly the same way that the larger lunar craters are integrated with the linear trends of the Moon.

The rift valley system as a whole forms a complex continental anorogenic zone: such zones have much in common with oceanic ridges, and indeed the rift valley system of eastern Africa goes out to sea as an oceanic ridge off Mozambique. Further, the geophysical and petrological evidence shows them to be closely related. Chenoweth[5] has recently noted the similarity between ocean floor and lunar surface patterns.

To me it seems that this is not simply coincidence. The concept is entertained that the lunar surface is essentially anorogenically moulded, and expresses volcano-tectonic undation patterns akin to the continental linear anorogenic zones and oceanic ridges. Because of the limitations imposed by the Moon's lower size and density, no atmosphere of light gas or surface hydrosphere could be held at the lunar surface, and this, it is suggested, resulted in complete inhibition of geosynclinal processes—infilling and overdeepening leading to orogenesis. Crustal depressions which were formed complementary to the undational swellings would not have been subject to sedimentation.

The vulcanicity of the Moon is reflected in essentially the same fundamental patterns as are seen in terrestrial volcanic fields; but it is in an extreme senescent stage, and must, when fully active, have possessed a quite different character to the most usual manifestations of terrestrial vulcanism. The differing physical controls operating—lower volume and density, greater curvature of the surface, lack of atmospheric pressure, hydrosphere or continental rafts, etc.—have resulted in much larger crateroid forms[6]. The products of lunar vulcanism were, in all probability, dominantly gaseous. The solid rock at the lunar surface, though almost entirely of volcanic origin, may yet show little resemblance to terrestrial lava fields. Encrustations including sublimates are likely to have predominated—material brought up to the lunar surface by the agency of gases rising up fracture conduits in exactly the same manner as volcanic gases stream up the fracture zones in senescent terrestrial volcanic fields such as the central rift valley of Kenya[7]. The resemblance of the apparently fragile linear and annular ridges of the Moon to the patterns of fumaroles and encrustation in terrestrial solfatara and mofette fields is striking. Water-soluble salts and mineral deposts will have formed permanent rocks on the Moon, and there will have been preserved a more substantial reflexion of gas activity in solid rock form than is the outcome of terrestrial fumarolic activity. Another interesting effect, consequent on the near-vacuum or complete vacuum conditions at the surface, will have been a lowering of boiling-points so that substances molten at the Earth's surface may have been volatile at equivalent temperature on the Moon. Silicate melts may never have reached the lunar surface, and if they did their nature is likely to have been that of vastly expanded filamentous pumices. The dark areas of the lunar surface (lunabase) are frequently equated with basalt, but the existence of such a basic igneous rock type at the lunar surface seems to me highly improbable. Crystallized rocks derived from silicate melts probably have no place there, and the dark areas of the maria and crater bottoms are probably covered with a late stage oozing of a material resembling obsidian: the pattern seen in lunar photographs clearly reflects a plastic response to stress, and the retention of a plastic character is typical of volcanic glasses which have retained some volatiles in solution[8]. The fact that this material seems to have oozed out in the topographic 'lows' of the lunar surface suggests some form of 'hydrostatic' restriction on its uprise.

The major eruptions of the Moon, which produced the great craters, are envisaged as having resulted from the sudden and explosive release of gas from an ascending magma, triggering being effected when the retaining pressure dropped below a critical limit. Such point-focus explosions of a truly magmatic character are evidenced in terrestrial ring complexes of the Kavirondo rift valleys of Kenya[9,10] and of Sweden[11-13]. The volatiles will have burst away from the magma and emerged at the lunar surface through fractures dominantly concentrically arranged, but associated with radial and strongly linear patterns the latter reflecting the overall lunar grid. The point focus explosions will have been deeper than on the Earth because of several physical controls operating—the lack of continental rafts or oceanic cover will in itself have facilitated bursting away of the gas at an earlier stage. A deeper focus leads to cone-fracture patterns of greater diameter at the surface. Cauldron subsidence (of a sort) is clearly reflected in many of the larger lunar craters and this is a logical successor to the positive phase of tumescence, fracturing and explosive gas release. It indicates subsidence or actual drawing down of the surface as the subjacent body of magma contracts. The main body of magma will have congealed in depth, and will not have moved upwards once the sudden bursting-out of the volatiles deprived it of fluidity, but, as already noted, a limited late-stage oozing probably ensued. This ooze may conceivably be of basic composition, but the dark colour is a poor criterion—quartz trachytes in Kenya show deep-black on air photographs where they present absolutely fresh surfaces.

The concept expressed briefly here—that of volcano-tectonic undation—has been derived primarily from terrestrial field and laboratory studies, the results of which have been compared with lunar photographs[14]. It is, of course, not an entirely original concept, and leans heavily on other workers (in particular Jeffreys[15], Spurr[16], Firsoff[2,3], Green and Poldevaart[17]). I have never observed the Moon through a telescope and have little specialized knowledge of astronomy. It is perhaps interesting that this concept, worked out from purely geological and geophysical considerations, is entirely in accord with the deduction that the lunar surface was in an overall state of tension at the time of cratering, made by Warner and Fielder[1] from an entirely different point of view—though perhaps the process is better termed distension, if, as seems likely, it involves dominantly vertical upward pressures.

To me the obvious integration of the crater patterns with the lunar grid alone makes attribution to impact unacceptable, and complex cirques such as Ptolemeus, in which the rim is composed of ridges of short length strongly reflecting the dominant grid trend, and also the annular trace of the cirque at its rim, in which the short ridges are frequently capped by summit craters and must be some form of volcanic mound and in which the floor is so little depressed, seem only explicable in terms of dominantly gas-erupting vulcanism. The existence of cirques in which the crater floor is not depressed and the distinction between floor and surround is only established by an annular line of depression (14, *B7a*, *B8a*, Janssen) again seems otherwise inexplicable.

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In his comments on the morphological comparison between terrestrial and lunar surface features, Dr. McCall has made a number of interesting points. It should be noted, however, that Fielder and I only concluded that the lunar surface was tensionally stressed at the time when certain lunar craters were formed. These craters in question all appear to be 'young'. Full details of our work have now been published[1].

I have recently published work[2–4] relating to the morphology and origin of the lunar grid system. Therein it is maintained that the present alignment of ridges, rilles, crater walls, etc., has been caused by the action of stresses after many craters had been formed, that is, the lunar grid system as seen at present is the result of secondary activity[5]. In the hypothesis of magmatic explosions developing through fractures, as suggested by McCall, the shape of a lunar crater is a property of its origin. The question arises whether this latter theory can account for the differing shapes of lunar craters, and in particular the apparent correlation of percentage distortion with age noted by Fielder[6]. On the other hand, there are difficulties associated with the assumption that the crater walls and ridges were moved bodily after their formation[4].

Working from an observationalist's point of view, I have maintained[7] that the dark material composing the maria and certain crater floors is a superficial covering and should not feature in any theory of the origin of the maria and craters.

Finally, there is evidence indicating that the individual maria and craters were formed by slow processes rather than during catastrophic events[4,6].

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RADIOPHYSICS

Increase in Maximum Usable Frequency for Two-hop Transatlantic Propagation due to Horizontal Gradients

It has frequently been noted that in long-distance high-frequency communication the actual maximum usable frequency (MUF) may be as much as 40 per cent greater than would be predicted from vertical soundings. One explanation for this has been given by Warren and Hogg[1], who showed, by means of an oblique incidence ionogram, that single-hop propagation could occur on a transmission path of 5,300 km, whereupon the resultant MUF exceeds that predicted by a two-hop mode calculation. A calculation by Kift[2] gives good agreement with this experimental result. The possible occurrence of layer tilts and *M* and *N* reflexions has also been advanced to account for the high MUF's obtained in practice[3,4].

An examination of the records of oblique incidence recordings, taken over a 5,600-km link between Ottawa and Den Haag, has revealed that the increase in MUF is strongly correlated with the horizontal ionization gradient. This can be seen from Figs. 1*a* and *b*, which give four typical cases for different times of the year. In making this comparison one-hop modes were rejected from the analysis. Plotted against Greenwich mean time are the critical $F2$ frequencies for vertical incidence as measured at Ottawa (O) and de Bilt (B) (about 60 km. east of Den Haag). It is apparent that, when these cross over, the measured 2-hop MUF is close to the predicted value—as based on interpolated f_0F2 values for the reflexion points. However, as the ratio of these two critical frequencies increases so does the ratio of measured to calculated MUF.

For the month of February 1961 there were 10 good recordings and every one of these has the characteristic shape of the February 26 record shown in Fig. 1*b*. The average curve for this month is shown in Fig. 2. Some 3·5 h before the cross-over point the f_0F2 value of Ottawa is half that of de Bilt. This results in a measured MUF which is 40 per cent greater than the calculated value. About four and three-quarter hours after the cross-over, the Ottawa f_0F2 is double the de Bilt value and now the measured MUF is 30 per cent greater than calculated.

These results can be explained on the basis of 'tilts' in the $F2$-layer due to the horizontal ionization gradients. In the absence of such gradients, each hop of 2,800 km would be made with rays the angle of elevation of which was about 10° and the angle of

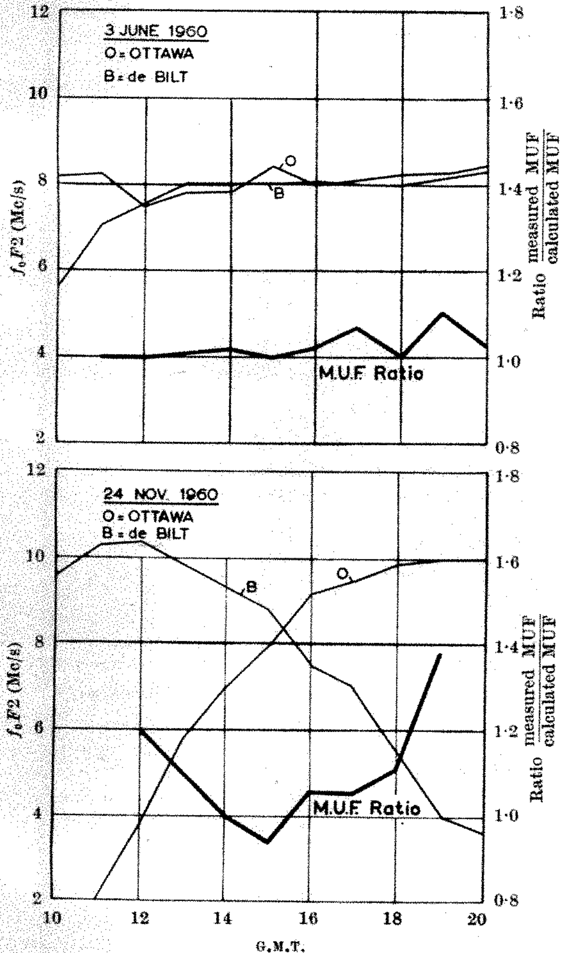

Fig. 1. *a*, Ratio of measured 2-hop MUF to calculated value based on f_0F2 records (Ottawa–Den Haag)

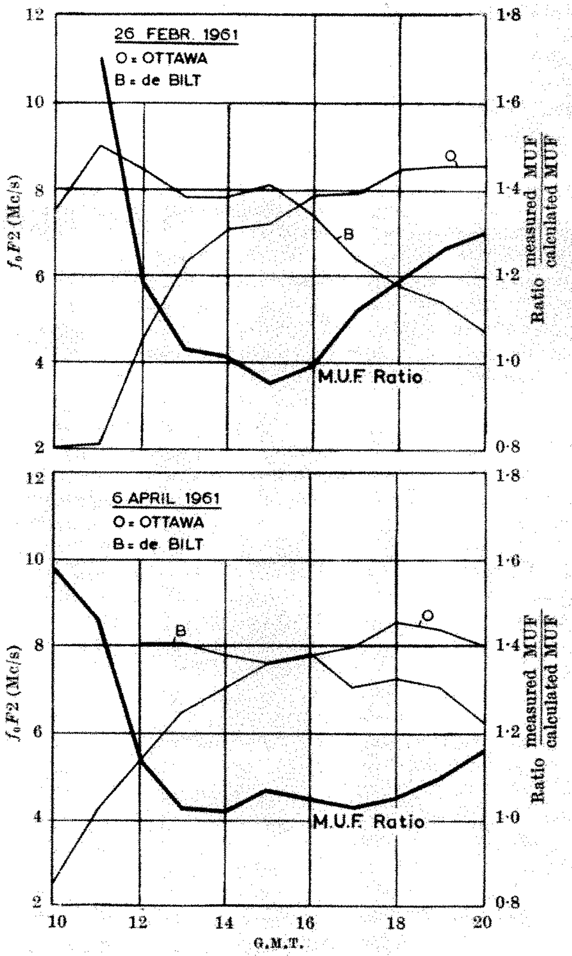

Fig. 1. *b*, Ratio of measured 2-hop MUF to calculated value based on f_0F2 records (Ottawa–Den Haag)

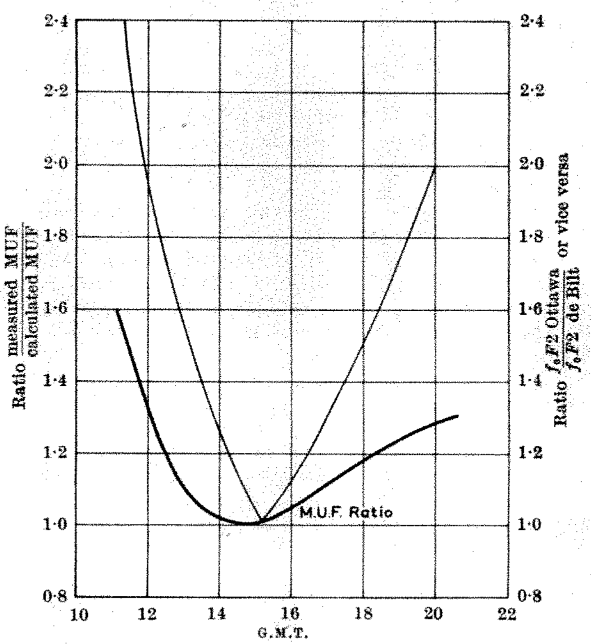

Fig. 2. Ratio of measured 2-hop MUF to calculated value based on f_0F2 records, Ottawa–Den Haag (average of 10 recordings for February 1961)

incidence of which on the ionosphere was about 70°. With a 2 : 1 ratio in the f_0F2 values at the terminals of the link there will be a gradient in the critical frequency of about 25 per cent per thousand km in the middle of the hop with the lower MUF value. Assuming a parabolic distribution of ionization in the vertical direction, this gradient is equivalent to a tilting of the layer by about 6°. Thus the angle of incidence increases by this amount, and application of the secant law shows that the MUF is increased by 23 per cent. The measured values show even greater increases in the MUF, namely, 30–40 per cent.

The foregoing simple argument ignores the fact that the reflexion point will have shifted in the direction of greater ionization and that the hop will now be asymmetrical. Because of the asymmetry the angle of incidence will be slightly reduced (though not to the extent that the angle of elevation of the steeper side is increased). The first of these effects results in an increase in the MUF, but the second will decrease the MUF. Attempts at more refined calculations have not yet proved satisfactory. However, the evidence obtained so far clearly indicates that the substantial increases in MUF found in practice on a Trans-Atlantic route are the results of horizontal gradients in the ionization of the ionosphere.

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GEOPHYSICS

Spread *F* Development associated with a High-Altitude Nuclear Explosion

AT 0900 U.T. July 9, the American Atomic Energy Commission exploded a thermo-nuclear device of megaton yield some hundreds of kilometres in the atmosphere above Johnston Island (16·7° N., 169·4° W.). In order to observe any ionospheric phenomena which might be associated with the blast, the event was monitored at Sydney (34·05° S., 150·66° E.) by using a panoramic ionosonde previously described[1], providing ionograms made by a 15-sec linear scan over a frequency-range 0·5–9·5 Mc/s at the rate of 1 every min. Fig. 1 shows an ionogram taken immediately prior to the explosion, the blast occurring immediately at the end of this particular scan. Fig. 2 shows an ionogram taken 2 min later, the first ionogram following the explosion having been lost by equipment malfunction. It is apparent that *F* region frequency spreading, which before the blast covered a range of 0·2 Mc/s, had extended considerably after the event and in fact on the extraordinary ray there is evidence of spreading over a range of at least 1·5 Mc/s. At 1000 h U.T., frequency spread had decreased to 0·7 Mc/s and was still evident at 1100 h U.T.

A study of ionograms recorded on the previous day shows that spreading over a range of 0·2 Mc/s was present at 0820 h U.T. but took 70 min before increasing to cover a range of 0·6 Mc/s at 0930 U.T. Moreover, rapid-sequence ionograms taken at this laboratory and studied to observe rate of growth of spread *F* have never disclosed a rapid increase comparable with that shown in the figures.

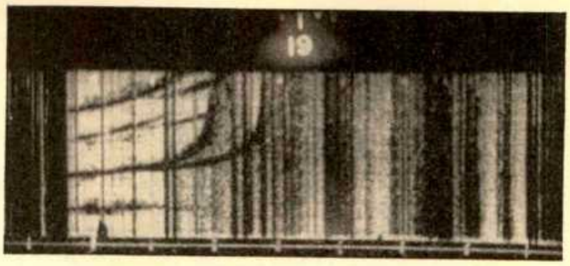

Fig. 1. Ionogram taken at Sydney at 0900 U.T., July 9, immediately prior to the H-bomb explosion

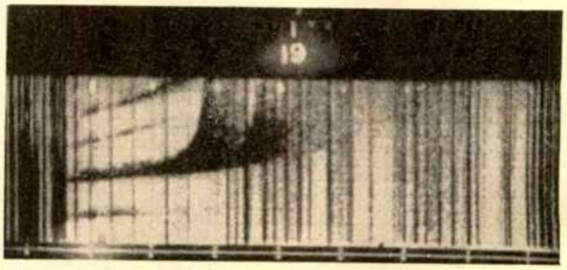

Fig. 2. Ionogram taken at Sydney at 0902 U.T., July 9, showing development of spread *F*

There is every indication, therefore, that the development of spread *F* was directly associated with the nuclear explosion. It is difficult to ascribe an explanation to the phenomenon, but undoubtedly the increased ionospheric turbulence producing the spreading must be related to some geophysical phenomena possessing a high velocity outward from the blast centre. It seems probable that the phenomenon could be associated either with variations in the Earth's magnetic field as observed by Newman[2] during the *Argus* experiments or the influx of high-energy particles into the observed ionospheric region. It is intended to monitor future explosions by means of ionograms with a more rapid sequence in an endeavour to obtain an accurate measurement of elapsed time between *H* hour and the recorded event. It is hoped that this may provide some information on the general mechanism of spread *F* formation.

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A Resonance Property of the Ionosphere due to its Anisotropic Conductivity

SEVERAL years ago A. A. Ashour and V. C. A. Ferraro (private communication, 1955, and following communication) discovered that certain distributions of freely decaying currents in a non-isotropic spherical shell model of the ionosphere would rotate about the geomagnetic axis. More recently we have considered the induction of currents by an oscillating field of external origin in a similar (though not identical) model, and have found that, for frequencies greater than a certain value, the currents induced in the shell are actually greater than those which would be induced in an isotropic shell of infinite conductivity.

In seeking an explanation of this remarkable result, we have been led to examine a particular kind of resonance, which may be produced in the ionosphere by fluctuating magnetic fields of suitable distributions and periods, the periods being of the order of a few minutes. This resonance is caused by the non-isotropic nature of the conductivity and is associated with Ashour and Ferraro's rotation phenomenon.

We treat the ionosphere as a thin spherical shell (of radius a) in which the relation between electric intensity (E_θ, E_φ) and current density is given by:

$$\begin{aligned} E_\theta &= R_{\theta\theta}J_\theta + R_{\theta\varphi}J_\varphi \\ E_\varphi &= R_{\varphi\theta}J_\theta + R_{\varphi\varphi}J_\varphi \end{aligned} \qquad (1)$$

The values of $R_{\theta\theta}$ etc. (in e.m.u.) are assumed to be

$$R_{\theta\theta} = 10^7\,(\xi + \cos^2\theta),\ R_{\theta\varphi} = -R_{\varphi\theta} = 10^7\,\eta\cos\theta,\quad R_{\varphi\varphi} = 10^7\zeta \qquad (2)$$

$$\text{with } \xi = 0{\cdot}5,\ \eta = 7{\cdot}0,\ \zeta = 1{\cdot}5 \qquad (3)$$

These values give a conductivity distribution roughly the same as that produced by a dipole field in a uniformly ionized shell, when the contributions from ions as well as electrons are included. It is similar to that used by Fejer in his discussion of the effect of anisotropy on the magnetic daily variations.

We find that an oscillating magnetic field the potential of which is:

$$\Omega_0 = r^n a^{-n+1}\,P_n^m(\theta)\cos m\varphi\cos\alpha t \qquad (4)$$

gives rise to an induced field inside the shell the potential of which can be written in the form:

$$\Omega_i = r^n a^{-n+1}\,P_n^m(\theta)\,R(t)\cos\{m\varphi + \tfrac{1}{2}(\delta+\varepsilon) + \psi(t)\} \qquad (5)$$

$$\text{where } R(t) = \{\beta^2 + \gamma^2 + 2\beta\gamma\cos(2\alpha t + \varepsilon - \delta)\}^{1/2} \qquad (6)$$

$$\text{and } \psi(t) = \tan^{-1}\left\{\frac{\beta-\gamma}{\beta+\gamma}\tan(\alpha t + \tfrac{1}{2}\varepsilon - \tfrac{1}{2}\delta)\right\} \qquad (7)$$

The constants β, γ, δ, ε are algebraic functions of α, ξ, ζ, η and a.

When the actual Fejer model is used, other spherical harmonics also appear in the expression for Ω_i, but they are small compared with the P_n^m term and disappear if $\xi - \zeta = -1$. To simplify the mathematical problem we have adopted this relation in (3) above.

The expression (5) shows that the resultant field is a P_n^m field of variable magnitude rotating with variable velocity about the dipole axis. The maximum value of $R(t)$ is $\beta+\gamma$, and $\psi(t)$ is then zero. Our calculations show that $(\beta+\gamma)$ rises from zero at $\alpha = 0$ to a maximum which is greater than unity, and then tends to unity as α tends to infinity. Thus for each harmonic there is a resonance frequency which corresponds to the position of this maximum. For a uniform inducing field (P_1^1) the values of $\beta+\gamma$ and $\frac{1}{2}(\delta+\varepsilon)$ are shown in Fig. 1. In this case the maximum induced field is 2·06 times that of the inducing field and the corresponding period ($2\pi/\alpha$) is 7·7 min; the field then makes an angle −152° with the inducing field. Larger induced fields would be obtained for larger values of η.

It follows that the total field inside the shell will also rotate, and its component parallel to the inducing field of external origin will in general be of opposite phase.

The above enhancement of the induced field is associated with the rotation of decaying current systems, discovered by Ashour and Ferraro. For the particular model we have chosen, the decay of the current system corresponding to a single harmonic P_n^m is given by:

$$\Omega_i = r^n a^{-n+1}\,P_n^m(\theta)e^{-pt}\cos(m\varphi - qt) \qquad (8)$$

where

$$\begin{aligned} \lambda p &= (2n+1)\{\zeta - m^2/(n^2+n)\} \\ \lambda q &= (2n+1)\,m\eta/(n^2+n) \\ \lambda &= 4\pi 10^{-7}a \end{aligned} \qquad (9)$$

Thus the pattern of the decaying current system rotates with angular velocity q/m.

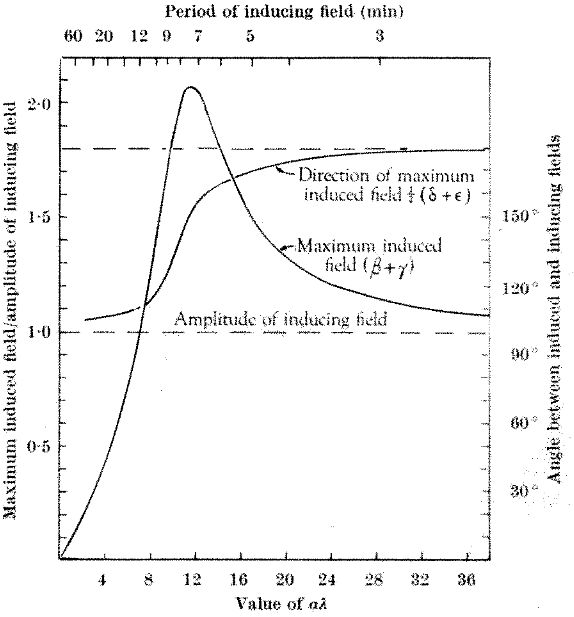

Fig. 1. Maximum induced field and its direction for a periodic uniform inducing field

Considering now a periodic inducing field P_n^m of period $T = 2\pi/\alpha$, the pattern of induced currents, brought into existence by the increase of the field during some short time-interval, will have rotated through an angle $\frac{1}{2}qT/m$ from west to east after time $\frac{1}{2}T$. At this later time the inducing field will be decreasing, so that if the current pattern has rotated through an angle π/m in the half period the induced currents now being produced will supplement those produced half a period ago, instead of subtracting from them, as would be the case if there were no rotation. Hence the total induced currents will build up until a steady state is reached depending on their rate of decay. The greatest enhancement of the induced currents would be expected when $\frac{1}{2}qT/m$ is approximately equal to π/m, that is, when $\alpha = q$. For the uniform P_1^1 field, this makes $\alpha\lambda = 10{\cdot}5$, in good agreement with the value shown by Fig. 1 for this case. The decay factor of the currents during the half period is $e^{-pT/2}$, which for this case has the value 0·41.

The foregoing suggests, and our detailed calculations confirm, that the induced field will have a relatively large amplitude when the period T is given by:

$$T = \frac{2n(n+1)\lambda\pi}{(2n+1)m\eta}$$

and the corresponding value of $e^{-pT/2}$ is not too small. Thus the resonance periods correspond roughly to the times taken by the patterns of freely decaying currents corresponding to each harmonic P_n^m to rotate through an angle $2\pi/m$.

The fact that the intensity of the induced currents is sometimes greater than the intensity of those induced in a shell of infinite conductivity may at first sight seem unacceptable; but there is no actual conflict with physical principles. Energy is absorbed by the shell from the magnetic field and, as with all systems having resonance properties, more energy is absorbed when the period is near one of the natural periods of the system.

It is clear from the above that our general conception of the ionosphere acting as a screen from magnetic fluctuations originating outside it[1] will need considerable modification. It will still shield the Earth from the most rapid fluctuations occurring outside it, but for fluctuations having periods of the order of a few minutes or tens of minutes, there may be no shielding at all, but even an amplification of the outside field. These conclusions may of course be somewhat modified when the low ionization on the night side of the Earth is taken into account. Our detailed calculations will be published elsewhere.

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Ashour, A. A., and Price, A. T., *Proc. Roy. Soc.*, A, **195**, 198 (1948).

Induction of Electric Currents in a Uniform Anisotropic Ionosphere

THE problem of the induction of electric currents in a non-uniform ionosphere is of interest in geomagnetism. It was first studied by Ashour and Price[1] to determine the effects to be expected from the greater conductivity of the ionosphere over the sunlit hemisphere. Some time ago we considered the electric currents induced in a uniform anisotropic ionosphere (thus ignoring the difference in conductivity between the day and night hemispheres). We found that the anisotropy modifies only that part of the inducing field which is asymmetrical with respect to the geomagnetic axis, and that this asymmetrical part rotates about the geomagnetic axis from west to east at a rate depending on the Hall conductivity.

Treating the ionosphere as a spherical current sheet of radius a, and assuming that the current arises mainly from the flow of electrons, we find that the resistivity tensor is of the form:

$$K = \frac{1}{\bar{\sigma}_0}\begin{Bmatrix} 1, \beta\cos\psi \\ -\beta\cos\psi, 1 \end{Bmatrix}$$

where $\bar{\sigma}_0$ is the integrated normal conductivity of the sheet, ψ is the inclination of the local magnetic field to the geomagnetic axis, and β is the ratio of the Hall conductivity to the ordinary conductivity at the pole.

The magnetic potentials, $\Omega^{(e)}$ and $\Omega^{(i)}$, due to the external inducing field and induced currents, respectively, are solutions of Laplace's equation and the induced field is determined from the conditions that, in passing through the current sheet, the normal resolute of the magnetic field and of the tangential component of the electric field are both continuous. Denoting by (r, θ, φ) the spherical polar co-ordinates of a field point, then assuming that an external inducing field derived from a potential:

$$\Omega^{(e)} = a\Sigma E_n^m(t)\left(\frac{r}{n}\right)^n P_n^m(\cos\theta)\exp im\varphi$$

is suddenly set up outside the ionosphere, we find that the total field inside the sheet after time t is derived from the potential:

$$\Omega = \Omega^{(e)} + \Omega^{(i)} = a\Sigma E_n^m\left\{1 - \exp\left[-(p_n^m + iq_n^m)t\right]\right\}\left(\frac{r}{a}\right)^n P_n^m(\cos\theta)\exp im\varphi \qquad (1)$$

where:

$$p_n^m = \frac{(2n+1)}{4\pi a\bar{\sigma}_0} \qquad q_n^m = \frac{(2n+1)m\beta}{4\pi a(n+1)n}\frac{1}{\bar{\sigma}_0}$$

We note that $q_n^m = 0$ if $\beta = 0$ or $m = 0$; in the former case the sheet is isotropic and in the latter case the field is axially symmetric about the geomagnetic axis. In this case, the sheet clearly behaves as if it were isotropic.

The presence of q_n^m in the imaginary part of the time factor shows first that, as was to be expected on general grounds, the Hall conductivity does not affect the rate of decay of the currents and, secondly, that the asymmetric part of the induced field rotates from west to east about the geomagnetic axis. Price and Ferris (private communication) have shown that this result is also true for a more general model of the ionosphere, and that the rotation of the asymmetrical part of the field seems likely to be of importance in causing a resonance effect in the ionosphere.

As a numerical illustration of the orders of magnitude involved, we have taken the case when $n = 1$, $m = 1$ (corresponding to a uniform field), so that:

$$q_1^1 = \frac{3\beta}{8\pi a\bar{\sigma}_0}$$

Then, taking Martyn's value of the integrated conductivity $\bar{\sigma}_0$ (= 5×10^{-8} e.m.u.) and $\beta = 7$, and putting $a = 6{\cdot}5 \times 10^8$ cm (the radius of the Earth) we find that the period of rotation of the asymmetric part of the field induced by setting up of a uniform external field normal to the geomagnetic axis is about 4 min.

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ENGINEERING

Permanent Insulating Duct Walls for Magnetohydrodynamic Power Generation

MOST of the reported experimental work on magnetohydrodynamic generation using hydrocarbon and other flames has been of limited duration owing to the difficulties in providing duct and electrode materials. These must withstand very high temperature (around 2,500° C), high velocity (near sonic), corrosive (a percentage or more of potassium seeding) gas conditions.

A possible method of obtaining permanent electrodes for magnetohydrodynamic power generation under these conditions has already been proposed[1], but the problem of providing electrically insulating duct walls remains. High-temperature materials such as magnesia have been tried with limited success[2]; but they can scarcely be called permanent as yet.

At the temperatures envisaged, the products of combustion contain appreciable amounts of atomic oxygen and hydrogen in equilibrium, as well as other free radicals, and are therefore highly corrosive. All

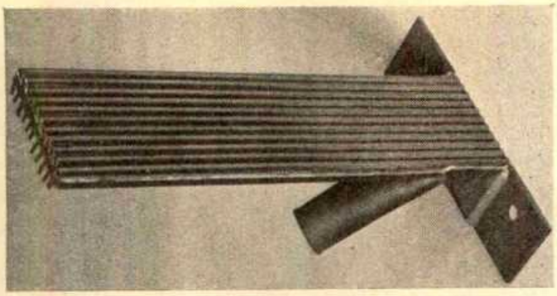

Fig. 1

known refractory materials other than the ceramic oxides (for example, graphite, silicon carbide, boron nitride, zirconium boride, tungsten) are rapidly oxidized, while the high-temperature ceramic oxides (for example, magnesia, zirconia, beryllia, thoria, hafnia) are all fluxed by the alkali metals which are present in fairly high concentrations as seeding in the combustion products. Also ceramics tend to lose their electrical insulating properties at high temperatures.

It therefore seems likely that the duct walls must be cooled, thereby producing very high heat transfer-rates, of the order of 1 MW/m². These high rates of heat loss should not prove embarrassing provided the scale is sufficiently large (of the order one thousand thermal MW), but fairly good thermal conductivity is required through the walls if adequate wall cooling is to be achieved.

The obvious materials to use are metals such as copper, nickel or stainless steel, and these have, in fact, found application in the rocket field where gas conditions are comparable; unfortunately they do not provide electrical insulation.

We have developed and tested a new type of duct wall construction which appears to overcome the difficulties mentioned here. It consists of a set of parallel metal tubes carrying cooling water (or other coolant) and insulated from each other by a thin layer of material such as sprayed alumina. The parts of the tubes facing the flame may be bare metal, and any gas penetrating to the insulating material between the tubes will presumably be cooled before reaching it. The rear faces of the tubes remain cold and can therefore be mounted in any convenient way, to provide a gas-tight seal so that hot gas does not penetrate. Our experimental section of wall consisted of ¼ in. diameter stainless steel tubes bonded with a fibre-glass reinforced epoxy resin backing, as shown in Fig. 1. The test section of wall is 30 cm × 6 cm and the total wall thickness about 1·3 cm.

This section of wall has run for about 20 min in an unseeded kerosene–oxygen flame and for a total of 3 h in a seeded flame. The seeded fuel contained about 4 per cent by weight potassium in the form of an octoate (2-ethyl-hexoate). There is no evidence of any corrosion of the wall, and the resistance between the separate tubes remains substantially unchanged.

During a run with unseeded fuel, the resistance between tubes (2·5 $k\Omega$) is substantially the same as when cold since the gas conductivity is low, but with seeded fuel the resistance drops to about 50Ω, presumably due to conduction through the gas the conductivity of which is around 30 mhos/m. In a practical generator this conduction through the boundary layer will occur even with a perfectly insulating wall, and there is no evidence that insulation between the tubes changes during a run. It therefore seems that the duct wall construction which we have investigated may provide permanent insulating duct walls for magnetohydrodynamic generators.

In conclusion, it may be remarked that an insulated tubular construction may be particularly useful when Hall effects require the electrodes to be segmented and the equipotentials to be at an angle to the direction of flow, since the tubes may be placed along the various equipotentials.

We thank the staff of D. Napier and Son, who constructed the wall, and Messrs. W. F. S. Bugden, P. G. Meier and J. A. Noé for help in carrying out the experimental work at the Central Electricity Research Laboratories. We also thank the Central Electricity Generating Board and the Manager of D. Napier (Luton Division) for permission to publish this communication.

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METALLURGY

Oxidation of Cupro-Nickel Alloys in Steam–Oxygen Mixtures

Wagner and Levin[1] have recently shown that metallic copper separates within the oxide scale formed on a 68/32 cupro-nickel alloy at 850° C. They considered that this was due to a reduction of cuprous oxide by nickel diffusing outward from the parent alloy. In some recent experiments, designed to investigate the oxidation characteristics of a range of cupro-nickel alloys in high-pressure steam, the authors have observed bands of copper in oxide scales formed on specimens oxidized at 275° C. It is our opinion that, at this low temperature, diffusion processes alone could not account for the isolation of such large masses of copper (Fig. 1).

The experimental conditions were chosen to stimulate the type of corrosion that occurs on cupro-nickel feed water heaters in certain generating stations of the Central Electricity Generating Board[2]. Samples of 90/10, 80/20 and 70/30 cupro-nickel alloys were heated for 1,800 h in an autoclave at 275° C containing saturated steam—800 lb./in.², with an oxygen overpressure of 250 lb./in.². Metallographic examination of the samples which had reached differing degrees of oxidation has revealed that the separation of bands of copper was initiated by the formation of a sub-scale beneath the original uniform scale. Typical micrographs illustrating sub-scale growth and copper segregation are shown in Figs. 1, 2 and 3.

It is noteworthy that the copper bands were formed in sections of scale that were directly in contact with sub-scale. It would seem, therefore, that copper is incorporated into the true oxide scale as the sub-scale is itself oxidized. The existence of a fine dispersion of copper within the sub-scale could be expected, because in a limited oxygen supply the nickel component of the alloy would be selectively oxidized owing to the higher free energy associated with the formation of nickel oxide. It is difficult, however, to understand why this dispersion should coalesce into the bands actually observed. It cannot be explained by an electrochemical reaction since the phenomenon has been reproduced under dry conditions, that is, superheated steam, at the same total pressure. Other experiments carried out in dry and wet steam at atmospheric pressure have failed to reproduce this type of oxide scale which suggests that the reaction is dependent on pressure. The authors have drawn the conclusion that under the influence of a large external pressure the copper is extruded into regions of incipient porosity. Such porous regions have been found in association with sub-scale growth on cupro-nickel alloys by earlier workers[3]. Further work is now being carried out to substantiate this hypothesis.

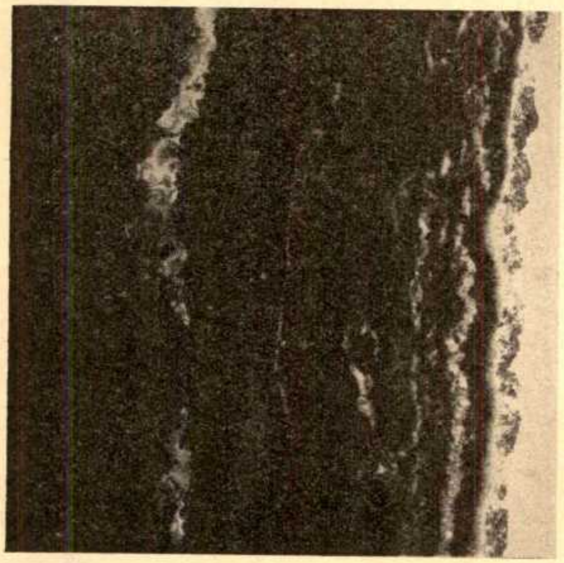

Fig. 1. Micrograph of a 70/30 cupro-nickel alloy illustrating the extent of the copper banding in the oxide scale. (× 100)

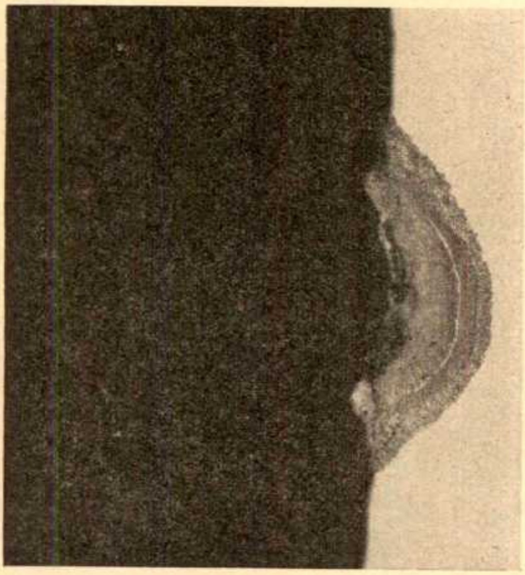

Fig. 2. Micrograph of an 80/20 cupro-nickel alloy illustrating the commencement of sub-scale formation. A thin band of copper has separated in the centre of the sub-scale. (× 150)

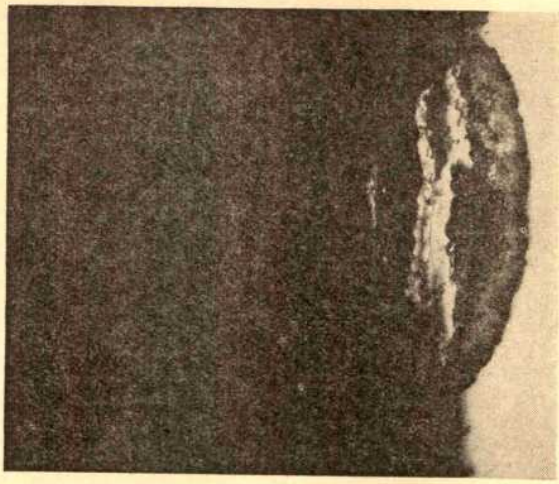

Fig. 3. Micrograph of a 70/30 cupro-nickel alloy illustrating a more advanced stage in the oxidation process than that shown in Fig. 2. The sub-scale formation and copper segregation are again apparent. (× *c.* 110)

We thank Dr. J. S. Forrest, director of the Central Electricity Research Laboratories, Leatherhead, for permission to submit this communication.

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Ternary System Copper–Zinc–Cadmium

AN isothermal section at 500° C of the ternary system copper–zinc–cadmium has been investigated. Previous work by Jenkins[1] had been carried out over a narrow range of composition, and was felt to be of doubtful value on three counts: (*a*) due to the difficulty he experienced in the metallographic identification of cadmium-rich phases; (*b*) the use of ingots from cooling curve experiments for his solid-state determination, when equilibrium had been attained only with difficulty in studies on binary alloys due to low diffusion-rates[2]; (*c*) Laves had reported the existence in the system of a ternary compound[3] which had not been described in Jenkins's studies.

In the work reported here alloys were prepared by sealing weighed amounts of the pure metals in evacuated silica tubes. The alloys were melted and then rapidly cooled to give a fine-grained structure in the alloy. The ingots were then annealed for a minimum period of 28 days (35 days for alloys close to compositions Cu_2 Cd and Cu_4 Cd_3) and water-quenched. Previous experiment had shown this time to be adequate for the attainment of equilibrium. Samples for X-ray diffraction studies were prepared from filings by annealing for 24 h to relieve strain at 500° C in evacuated sealed capsules followed by water-quenching. The average impurity content of the alloys was less than 0·05 per cent total impurities, mainly aluminium, iron and silicon.

The solid solubility of cadmium in the α-solid solution was studied by comparing the lattice parameters of binary and cadmium saturated ternary alloys. The solubility of cadmium in copper at 500° C is reported as being 2·2 weight per cent[4]. The solubility in the ternary phase was calculated on the assumption that the increase in parameter due to cadmium in copper–zinc alloys, and in pure copper, would be equal *pari passu* (Fig. 1).

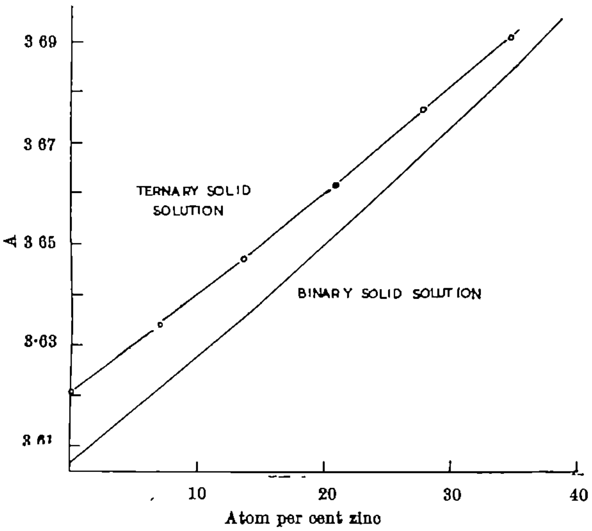

Fig. 1

The compositions of the α-solid solutions in three-phase equilibria with cadmium-bearing intermetallic compounds were determined by interpolating measured lattice parameters in the curve given in Fig. 1. Other phase boundaries were examined metallographically. The results are shown in Fig. 2.

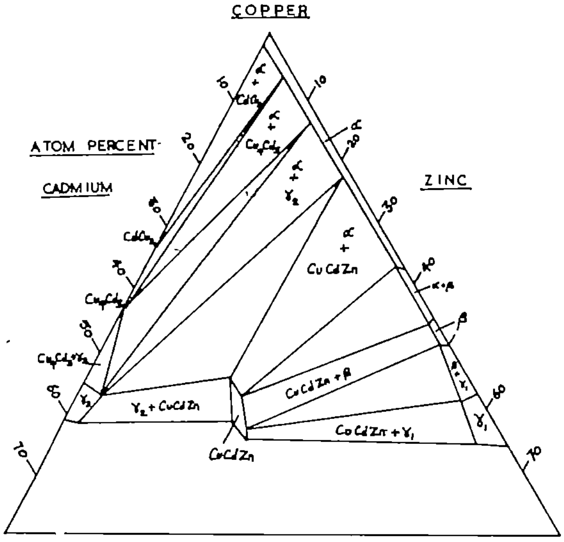

Fig. 2

I thank my former colleagues at the Battersea College of Advanced Technology for their help during this investigation.

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GEOLOGY

A New Upper Old Red Sandstone Fish Locality in Scotland

With the exception of sporadic scales and plates, finds of fish remains in the Upper Old Red Sandstone of Scotland, south of the Grampians, have been limited to the abundant excavation material from Dura Den, Fife, and the more restricted productions of Bracken Bay, Clashbennie, Earlston, Jedburgh, Chirnside, etc. The discovery of two rich fish beds in the Upper Old Red Sandstone in the grounds of Duns Castle, Berwickshire, is therefore a welcome event.

The beds here consist of bright red, sometimes mottled, marls, shales and yellow or pink sandstones. The sandstone ribs have local conglomeratic bases and carry many green clay-galls. Cornstones are absent. The beds are lenticular and do not appear to exhibit the rhythmic profile seen in beds of similar age in other parts of southern Scotland[1].

The lower fish bed is some 3 in. thick and occurs at the top of a red marl. It has yielded a finely ornamented *Bothriolepis* in great abundance and may well be composed of many hundred individual fish. The plates are well articulated although somewhat flattened. The fish show a fairly consistent orientation. Areas of *Holoptychius* squamation have also been found. Unflattened plates of the same species occur in the sandstone immediately above the fish bed; but these plates are disarticulated and apparently of random orientation.

The upper fish bed occurs 20 ft. above the lower in a sandstone rib. It has yielded disarticulated plates of a coarsely ornamented bothriolepid and abundant *Holoptychius* plates and scales. The remains are disorientated and occur along with clay-galls at the base of the sandstone and are also found in the pure pink and yellow sandstone in the upper part of the rib.

It is probable that the lower fish bed has been produced *in situ* by the killing of fish in a drying pool. The remains in the sandstones may well have been washed into their present position by streams carrying the coarser sediment.

The palæontology of the fish beds with a detailed account of the locality will be published by R. S. Miles, King's College, Newcastle upon Tyne, and me. The Royal Scottish Museum thanks Colonel G. H. Hay of Duns Castle for giving access to the site. When the palæontological study is complete the Museum will be prepared to distribute excess material to interested institutions.

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Dinosaur Tracks in the Lower Cenomanian of Jerusalem

As has been announced by the newspaper Press, dinosaur tracks were recently discovered in the vicinity of Jerusalem. Dinosaur foot-prints are relatively rare; at present, they are known in the world probably from no more than about thirty localities. The present discovery is the first not only in Israel but also in the whole Near and Middle East. The nearest foot-print localities are in Caucasian Georgia, the Yugoslav island of Brioni and south Tunisia. In this particular case, the dinosaur foot-prints are of Lower Cenomanian age; no more than five findings are known of this period.

The spot is situated in the village of Beit Zait, a few kilometres west of Jerusalem. It was accidentally discovered by an owner of the plot who, in order to improve his small yard, removed a thin bank of fissured limestone and thus regularly spaced marks on the rock surface were revealed. After some time, this information was passed on to me.

On an area of about 80 m^2 we can see more than 20 foot-prints in a continuous row, almost 20 m long. They belong apparently to a single individual. On both sides of this row, there are more prints, smaller and less distinct. Each of the prints in the row shows three toes, of which the middle one is 24–26 cm long, while the side toes average 20 cm in length. The angle between the toes is about 40°. The distance between the alternate footsteps is about 80 cm so that the distance between one print and another made by the same foot is around 160 cm. Evidently the animal was a bipedal reptile, with long and strong hind-feet and short fore-feet, the latter serving mainly for grasping. On the basis of these data it may be assumed that the hind legs of the reptile were approximately 120 cm high and that the length of entire body with its big tail and expanded neck was 2·5 m or more; in its normally erected posture the animal must have been about 2 m tall.

The age of the track-bearing layer is quite clearly indicated. It is situated below the Mozza marls, which present the lowest part of a fossiliferous series of Middle to Upper Cenomanian; the complex of which the dinosaur beds are part is 200–250 m thick and is underlined by marine layers of Albian age. On the whole, the Cenomanian series was considered to be of marine origin although only few beds of its lower part could be definitely indicated as such. Similarly, not many years ago, the whole upper Cretaceous series of Jerusalem region was believed to be of continuous sedimentation and of marine origin. However, it has been shown that a lacuna exists between the Upper Turonian and the Santonian–Campanian and between the Upper Cenomanian and the Upper Turonian beds[1]. The present discovery of dinosaur tracks below the base of the middle Cenomanian *Acanthoceras* beds shows for certain that at least a part of the 200–250-m thick Lower Cenomanian series is of continental origin. A microscopic examination of the track-bearing rock as well as of that immediately above it shows that both are completely devoid of any organic remnants and their texture is rather lacustrine in character.

The nearest locality which yielded bones of Lower Cenomanian dinosaur is the Baharia Oasis west to the Nile. This occurrence is stratigraphically, facially and palæontologically closely related to those of Tunisia, Algeria and Morocco, forming the upper part of the 'Continental Intercalaire'[2]. This 'Continental Intercalaire' corresponds *grosso modo* to the European Wealdian.

It seems that the foot tracks of Beit Zait belong to a theropode reptile. By the proportions of the footsteps and the calculated measures of the animal it may be hypothetically assigned to the coelurosaurian genus *Elaphrosaurus*, which is common in the deposits of the 'Continental Intercalaire'.

Three species of this genus were recognized in this formation. They are: *El. bambergi* Janensch, *El. iguidiensis* Lapparent and *El. gautieri* Lapparent. The first one seems to be, by proportions of its skeleton, the most appropriate for the Beit Zait foot tracks.

The discovery of dinosaur foot-prints in the region of Jerusalem is not only of palæontological interest; it also leads to important deductions regarding the geological history and structure of the entire area, and stresses its very close relation to the whole north African domain.

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MINERALOGY

Nordstrandite in Soil from West Sarawak, Borneo

Of the three polymorphs of aluminium hydroxide—gibbsite, bayerite, and nordstrandite—only gibbsite has hitherto been known as a natural mineral, and so the discovery of well-crystallized nordstrandite in Sarawak is noteworthy. The mineral was found in red soil and was tentatively identified as gibbsite from its optical properties, but when sent to London for confirmation it was shown by X-ray examination to be nordstrandite. The following preliminary details of this discovery have resulted from field work by the Agricultural and Geological Departments in Sarawak, and laboratory studies by Miss B. B. Fox and Mr. J. A. Bain in the Mineral Resources Division, Overseas Geological Surveys, London.

The nordstrandite was found in soil at Gunong Kapor (gunong = mountain) near Bau in west Sarawak. The isolated, cliff-bounded hill is formed of limestone (kapor = lime), and the soil was collected from the edge of a large sink-hole about 350 ft. above the foot of the hill. The soil fills an irregular crevice, about 10 ft. deep, and appears to be a typical terra rossa. It resembles a dark red clay loam and contains, in the deeper parts especially, small quartz crystals and rounded pellets, some of which consist of limonite and others of nordstrandite.

The origin of the deposit is not fully understood, but it appears improbable that the soil could have been derived solely from the weathering of such pure limestone as occurs here: on the other hand, volcanic ash, which is found in some terra rossas, is unlikely to be present in substantial amounts. Wilford[1] has shown that in this region dacite intrusions and gold deposits commonly occur along the contact of the limestone with overlying shale. Sink-holes, formed in the limestone, have been filled with clay and other weathered products of the dacite, gold ore, and shale. The presence in the soil of minerals characteristic of some of the primary gold-ore deposits of the Bau district affords support for the belief that

Table 1. COMPOSITION OF SOIL CONTAINING NORDSTRANDITE FROM GUNONG KAPOR, WEST SARAWAK

Chemical analysis Soil dried at 25° C	Per cent	Estimated mineral composition	Per cent
Ignition loss, 1,050° C*	19·25	Nordstrandite	48
SiO_2	14 95	Goethite	31
Al_2O_3	31·57	Quartz	11·5
Fe_2O_3†	29 60	Kaolin	7·5
MgO	0·25	Wad and pyrolusite	2·5
CaO	0 70	Ilmenite	2
Na_2O	0 10	Diaspore	0·5
K_2O	0·05	Stibiconite	0 5
TiO_2	1·42	Zircon, magnetite, cinnabar and gold	traces
P_2O_5	0 08		
MnO_2	2·20		
Sb_2O_3	0 41		
As_2O_3	0 002		
Total‡	100 58		

* Including 1·60 per cent water at 105° C.
† FeO not detected.
(Analyst: Miss E. Waine.)
‡ Precious metals. gold, 1·7 p.p.m.; silver, 1·1 p.p.m.
(Assayer: F. Stacey.)

such a process has contributed to the formation of this nordstrandite-bearing soil, even though no remains of dacite or shale have yet been found.

The chemical and mineral composition of the first sample of the soil received in London is shown in Table 1. Ultrasonic dispersion in water and separation of the finer particles (nominal size < 10μ) by hydrocyclone yields a clean granular product from which the constituent minerals (except kaolinite, which is restricted to the < 10μ fraction) are readily separated and identified. The nordstrandite varies from almost colourless to coral pink and reddish-brown, the coloration being largely due to inclusions of finely divided goethite. Smaller particles are typically thick tabular crystals, from < 10μ–75μ, occasionally up to 250μ, across, which lie on the basal plane and are rhombic in plan, with angles near 60° and 120°. A perfect basal cleavage is prominent. Larger grains, up to 1 mm, are polycrystalline aggregates occasionally tubular in form. The measured specific gravity, 2·43, and optical properties, $\alpha = 1{\cdot}580$, β (calc.) $= 1{\cdot}581$, $\gamma = 1{\cdot}613$, $2V = +18°$, and $Z \wedge c \simeq 32°$, all fall within ranges reported for gibbsite. Analysis of a small sample, contaminated by included goethite and a little quartz, showed Al_2O_3 60·0, Fe_2O_3 4·35, H_2O 32·7, CaO 0·66, MgO 0·44, SiO_2 1·24, total 99·39 per cent (analyst P. J. Moore). Material of lower iron content has been obtained by magnetic separation.

X-ray powder data, obtained using a Philips 11·48-cm camera and iron radiation, are listed in Table 2, and are in close agreement with the spacings for synthetic nordstrandite grown from gels reported by previous workers[2–4]. With one exception, discrepancies occur only in weak or very weak lines, and might be due to a small amount of contaminant in the synthetic material. The exception is a line of moderate intensity at 3·18 Å reported in two of the three previous patterns but absent from our own pattern and that of Papée[3]. On the evidence of a diffractometer trace published by Lippens[4], it is not possible to dismiss this as a gibbsite line.

Table 2. X-RAY POWDER DATA FOR SARAWAK NORDSTRANDITE

d (Å)	I	d (Å)	I	d (Å)	I	d (Å)	I
4·78	vs	2 330	vw	1·653	vw	1·250	w/m
4·33	s	2·261	s	1·593	w/m	1·242	vw
4·206	s	2·225	w	1 574	w	1·226	w/m
4·153	s	2·118	vw	1·549	w/m	1·216	w
3·886	m	2 097	vw	1·513	m	1·197	Bm
3·600	m	2·073	vw	1·477	m	1·192	
3·425	m	2·016	s	1·465	vw	1·183	w/m
3·140	vw	1·982	vw	1·440	m/s	1·175	vw
3·023	w	1 959	vw	1·429	w/m	1·162	w/m
2·849	w	1·939	vw	1·405	w	1·116	w/m
2 704	Bw	1·899	s	1 387	vw	1·078	w/m
2 637	w	1·779	m/s	1·373	vw	1·052	w/m
2 479	Bm	1·715	vw	1·353	w	1·010	m
2·445	w	1 704	w	1·305	m		
2·392	s	1 672	w	1 276	vw		

vs, very strong, s, strong; m, moderate; w, weak; vw, very weak; B, broad line. (X-ray data: Miss B. B Fox.)

Of the associated minerals present in the soil, the goethite, kaolinite and manganese oxides are typical of limestone terra rossa, the quartz (in prismatic crystals), stibiconite, cinnabar and gold clearly belong to the antimony–gold mineralization of this area, but the mode of formation of the abundant nordstrandite and minor diaspore (largely euhedral) still requires elucidation. Field and laboratory investigations are continuing, and a more detailed account will be published later.

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Nordstrandite from Guam

NORDSTRANDITE ($Al_2O_3{\cdot}3H_2O$), previously reported only as a synthetic product[1,2], occurs in Miocene limestone on Guam. In south Guam this limestone forms a cap several hundred feet thick, on the Mount Alifan–Mount Lamlan ridge, and overlies late Eocene and early Miocene basalt flows and volcanic conglomerates. The volcanic rocks below the limestone are deeply weathered. The nordstrandite occurs in secondary solution cavities and is concentrated mainly in the basal part of the limestone near its contact with the weathered basalt. In thin section the nordstrandite crystals are seen as flamboyant aggregates reaching lengths of as much as 0·4 mm. The optical properties of the mineral are as follows:

$\alpha = 1\ 580 \pm 0{\cdot}004$ colourless	optic sign = positive
$\beta = 1{\cdot}580 \pm 0{\cdot}004$ colourless	2 V = low
$\gamma = 1\ 596 \pm 0\ 004$ colourless	elongation = negative
$\gamma - \alpha = 0\ 016$	extinction = inclined

The hardness is about 3 and the specific gravity was determined by Edward J. Young to be 2·436.

X-ray diffractometer patterns were made of material hand-picked from the limestone and of material separated by solution of the limestone in acid. The X-ray diffraction data agree closely with those presented by Van Nordstrand *et al.*[1] and Papée *et al.*[3]. The 49 lines observed are given in Table 1.

Table 1

d (Å)	I/I_1	d (Å)	I/I_1	d (Å)	I/I_1	d (Å)	I/I_1
4·789	100	2·450	1	1 7152	<1	1 3866	<1
4·322	12	2·392	9	1 7016	<1	1 3534	<1
4 207	10	2·349	<1	1·6706	2	1·3051	1
4 156	7	2·263	15	1·6518	<1	1·2753	<1
3 887	4	2·148	1	1·5948	2	1·2499	1
3·600	4	2·074	1	1·5722	<1	1·2257	1
3 429	4	2·038	<1	1·5495	1	1·1969	1
3 022	2	2·016	8	1·5134	4	1·1936	1
2·830	2	1·9434	<1	1·4773	8	1·1832	<1
2 704	1	1·9008	8	1 4638	<1	1·1615	<1
2 663	<1	1·8797	<1	1·4395	5	1·1146	<1
2·497	<1	1·8017	<1	1 4273	1		
2·480	3	1·7807	5	1·4037	1		

A chemical analysis (analyst, Sherwood, A.) of the mineral gave the following (per cent): SiO_2, 4·14; Al_2O_3, 63·97; Fe_2O_3, 0·25; H_2O^+, 29·05; H_2O^-, 1·59; Sum total, 99·00. Loss on ignition, (H_2O^-, H_2O^+), 31·92.

Because the voids containing nordstrandite appear to have formed preferentially along fissures, we believe that the deposition of the nordstrandite was a late stage in the history of the limestone, after emergence, lithification, fracturing, and subaerial solution. The nordstrandite may have precipitated from aluminous solutions leached from residual soils where these solutions migrated along the contact between the underlying basalt and the limestone. Nordstrandite would thus tend to concentrate in the basal part of the limestone near its contact with the basalt.

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Infra-Red Confirmation of 6-Fold Co-ordination of Silicon in Stishovite

A SAMPLE of natural stishovite[1] (SiO_2), purified by Dr. D. J. Milton, by extensive HF digestion of Coconino sandstone, from Meteor Crater, Arizona, was studied by infra-red absorption techniques. A marked shift to lower frequencies of 400 cm^{-1} has been observed in the position of the maximum absorption peak when compared with that of the more usual tetrahedrally co-ordinated silica minerals, quartz, coesite, and fused silica.

The identity of the white powder was checked by X-ray diffraction analysis, by matching 28 of the lines on the film with those given by Stishov and Popova[2] in their 1961 paper announcing the synthesis of this high-density polymorph of silica. The line (d = 2·09 Å) was observed from our sample. Three faint lines, corresponding to the major lines of coesite, confirm the continued presence of that polymorph in the acid-leached material. Two other lines were observed.

Optical study of the natural powder (*GC* 3425) reveals that some grains are up to about 50–60μ in size. Refractive indices are close to 1·77 (fast ray) and 1·78 (slow ray). Birefringence is low (0·01) as determined from individual grains. The optical constants differ from those of Stishov and Popova for their synthetic product (N_g = 1·826 ± 0·002, N_p = 1·799 ± 0·002). Intergrown crystals with lower refractive indices appear as fine dust throughout the grains of the stishovite host, making up about 1–5 per cent of the specimen. Some isotropic material is also present with indices markedly lower than the stishovite.

Table 1. X-RAY DATA FOR STISHOVITE SAMPLE (*GC* 3425), METEOR CRATER, ARIZONA

d	*I*	*d*	*I*	*d*	*I*
3·278*	f (spotty)	1·333	w	0·988	w
3·094†	f	1·321†	w	0·985B	f
2·944	vs	1·292	f	0·946	w
2·829†	vvf	1·234	m	0·937	w
2·241	m	1·213	mw	0·935B	f
2·189	vvf	1·184	f	0·882	vf
2·085	vvf	1·124†	f	0·868α_1	f
1·976	S	1·085	f	0·850α_1	f
1·867	m	1·062	f	0·822α_1	f
1·527	S	1·043	f	0·819α_1	f
1·476	m	1·011	f	0·806α_1	f
				0·802α_1	w
				0·797α_1	w
				0·792α_1	w

vs, Very strong; m, medium; f, faint, S, strong; w, weak; B, broad.

* Possibly rutile lines.

† Coesite lines.

Copper $K\alpha$ radiation. nickel filter, corrected for shrinkage, camera diameter 114·59 mm. Film pattern prepared and read by H. H. Johnson, Stanford Research Institute.

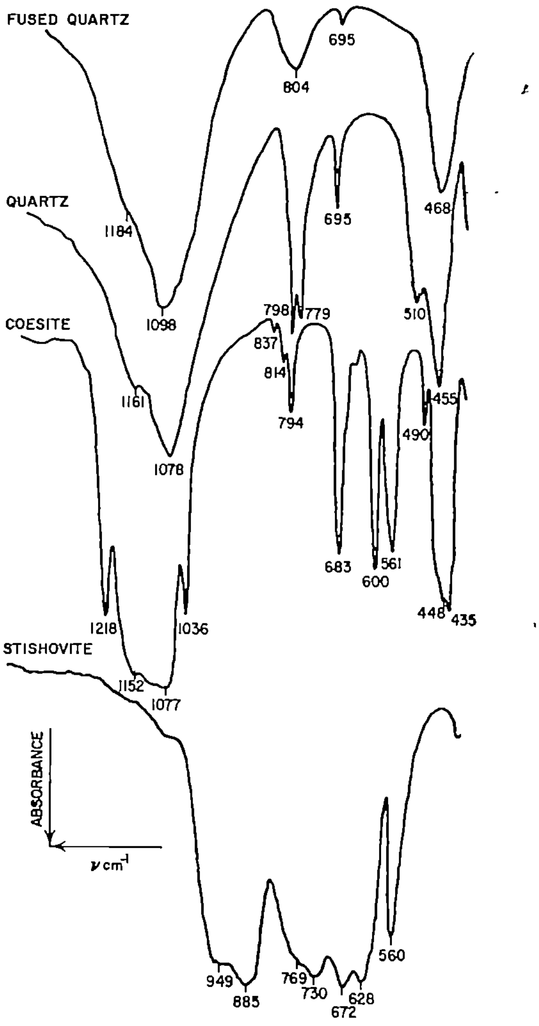

Fig. 1. Infra-red absorption curves for fused silica, quartz, coesite, and stishovite

Natural stishovite, as reported by Chao[1] *et al.* from Meteor Crater material, is in extremely fine grains mostly less than 1μ in size. The mineral has a tetragonal crystal structure, closely related to rutile. Stishovite is considered to be the first mineral discovered in which silicon occurs with oxygen in a 6-fold co-ordination (octahedral) instead of tetrahedrally co-ordinated.

Tarte and Ringwood[3] have recently directed attention to the absence of SiO_6 octahedra in silicate minerals; however, on the basis of other work in XO_4, tetrahedral and octahedral groups predicted the infra-red absorption frequencies for this octahedral configuration to be between 700 and 600 cm^{-1}. The stishovite infra-red absorption curve (Fig. 1) shows a major absorption at 769–628 cm^{-1}, effectively supporting their prediction.

The curves shown in Fig. 1 were prepared at 0·15 per cent level in potassium bromide pellets and extended over the wave-length range 4,000–400 cm^{-1}, on a

Perkin–Elmer model 221 grating-prism instrument, using both sodium chloride and potassium bromide optics. The results show a marked change in the position of the major absorption peaks when passing from the SiO_4 tetrahedral co-ordinations of quartz, coesite, and fused silica (1,098–1,077 cm^{-1}), to the SiO_6 octahedral co-ordination in stishovite (769–628 cm^{-1}; 949–885 cm^{-1}). A parallel to this has been recorded by Dachille and Roy[4] for GeO_2-quartz and GeO_2-rutile structures, which show absorption peak shifts from 870 cm^{-1} for the 'quartz' structure out to 715 cm^{-1} for the 'rutile' polymorph.

No attempt is being made in this communication to assign the various peaks in the stishovite pattern to specific Si–O bonds, particularly in view of the split maximum with the 700 and 900 cm^{-1} bands. Two slight changes in slope in the stishovite pattern around 1,070 and 1,150 cm^{-1} are probably due to the slight coesite impurity indicated on the X-ray pattern.

I thank Dr. D. J. Milton, of the U.S. Geological Survey, Menlo Park, California, for both the coesite and stishovite samples. This work was done on behalf of the National Aeronautics and Space Administration as a part of a continuing research project on the infra-red analysis of silicate minerals.

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CHEMISTRY

Effect of Electric and Magnetic Fields on the Boiling Point of Alcohol

Katti and Chaudhri[1] have reported that strong electric fields depressed the boiling point of some alcohols by a much greater amount than would be expected by simple thermodynamical considerations. At 5 kV/cm they observed depressions up to 2·0° C; but, using similar electric fields and also magnetic fields, we have been unable to find any such effect.

First we examined the effect of a magnetic field by boiling methanol in a Cottrell apparatus, provided with a reflex condenser and Beckmann thermometer, and placed in a thermal bath between the pole pieces of an electromagnet; no effect was found with magnetic fields of up to 3,000 œrsteds. We then decided to repeat the measurements of Katti and Chaudhri on methanol, using this apparatus. Since they did not state the frequency of the a.c. field used, we used the following frequencies and remanent magnetic spectrographic field strengths:

Frequency (c.p.s.)	Field strength (kV/cm)
0	2·7
50	3·5
25	2·2

The field was established between two plates kept in position on the sides of the Cottrell apparatus by an external tight-fitting glass cylinder, which also acted as insulation between the plates and the thermal bath, the temperature of which was electrically controlled. On different occasions the field was applied at the thermometer bulb and at the surface of the boiling liquid. The methanol in most of the determinations was an analytic reagent with stated maximum limits of impurities: water 0·100 per cent, aldehydes and ketones 0·030 per cent, non-volatile matter 0·001 per cent.

On no occasion was any change of the boiling point due to the presence of the electric field found, although any change greater than 0·05° C would have been detected above the fluctuations of this amount, which were probably due to variations in the atmospheric pressure over the periods of up to a few hours during which readings were taken.

The results are in conflict with the previous experimental results[1], but are in agreement with the calculation of Lyon[2], who suggests that an extension of the Clausius–Clapeyron equation to include electrical energy would give an effect of less than 10^{-4} deg. C under these conditions.

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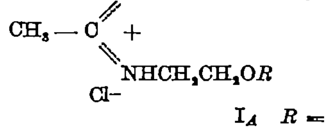
Isolation and Phosphorylation of a New Hydrolysis Product of 2-Methyl-Δ^2-Oxazoline

PORTER *et al.*[1] showed that partially neutralized aqueous solutions of 2-methyl-Δ^2-oxazoline (oxazoline) undergo transformation to yield *N*-acetylethanolamine as the final product. Two mechanisms were postulated, one involving ring opening to give first *O*-acetyl-ethanolamine, which then yielded *N*-acetylethanolamine via 2-hydroxy-2-methyloxazolidine. Based on the work of Martin *et al.*[2] with 2-methyl-Δ^2-thiazoline, a second mechanism was suggested in which oxazoline equilibrated first with 2-hydroxy-2-methyloxazolidine, which in turn is in equilibrium with both *N*- and *O*-acetylethanolamine. Martin and Parcell[3] later elaborated the mechanism derived for 2-methyl-Δ^2-thiazoline and showed that it applied to oxazoline in the *p*H range 0–6.

The decomposition of oxazoline in relatively concentrated aqueous solutions at constant *p*H-levels within the range 0–14 has been examined in these laboratories by means of nuclear magnetic resonance spectroscopy. This work has shown that the kinetic expression derived by Martin and Parcell does not apply above *p*H 6, and that a new hydrolysis product is observed between *p*H 5 and 9. If a 2·5 per cent aqueous solution of oxazoline is kept at *p*H 7 and 30° for 24 h, evaporation of the solution *in vacuo* gives an oil, the infra-red spectrum of which is unchanged after chromatography on 'Woelm IV' acid alumina. Acetylation of this oil, followed by chromatography on the same type of alumina, gave a crystalline product (m.p. 107–109°) for which analytical data indicated the molecular formula $C_{10}H_{10}O_4N_2Cl$. This substance was assigned structure I_B on the basis of nuclear magnetic resonance and infra-red data.



I_A $R = H$
I_B $R = CO.CH_3$

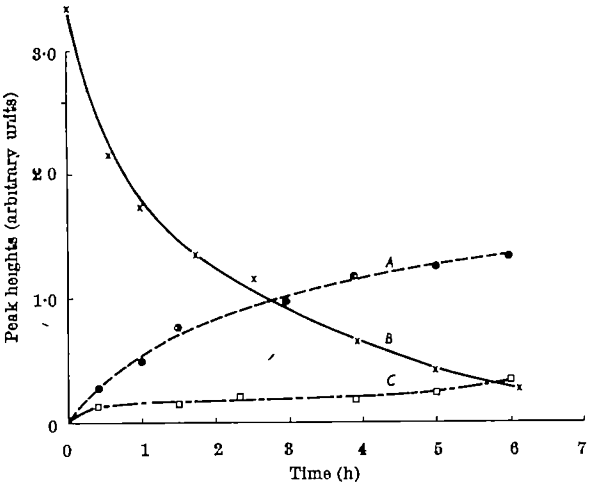


Fig. 1. Decomposition of a 2·5 per cent aqueous solution oxazoline at *p*H 7 and 30°. *A*, Peak height of the $O.CO.CH_3$ protons of *N*-2-hydroxyethyl *N'*-2'-acetoxyethylacetamidate HCL at 2·595 p.p.m up field from water, *B*, 2 × peak height of the centre band of the CH_2 triplet of oxazoline at 2 728 p.p.m.; *C*, peak height of the CH_3 protons of *O*-acetylethanolamine HCL at 2 571 p.p.m.

Comparison of infra-red spectra indicated the original oil to be the monoacetyl derivative, namely, *N*-2-hydroxyethyl-*N'*-2'-acetoxyethylacetamidate hydrochloride (I_A). The rate of formation of I_A at *p*H 7 at 30° was determined using n.m.r. spectral measurements. It is quite evident from Fig. 1 that I_A is the main product under these conditions.

Amidates are formed by the reaction of imidates with the hydrochlorides of primary or secondary amines[4]. If we consider oxazoline to be a cyclic imidate, the amine component of the reaction can be either *O*-acetylethanolamine or 2-hydroxy-2-methyloxazolidine, of which the former is favoured for this reaction. Although *N*-2-hydroxyethyl-*N'*-2-acetoxyethylacetamidate hydrochloride is relatively stable at *p*H 7 compared with oxazoline, it slowly decomposes forming *O*-acetylethanolamine, *N*-acetyl-ethanolamine, *O*,*N*-diacetylethanolamine, acetic acid and ethanolamine.

An earlier communication[5] reported the discrepancy of products obtained when oxazoline was phosphorylated with diisopropyl phosphorofluoridate (DFP) and diisopropyl phosphorochloridate (DClP). Whereas DClP reacts rapidly (1 h) to yield *N*-phosphoryl derivatives, DFP reacts slowly and after 4 days yielded two *O*-phosphoryl compounds. In a control experiment no oxazoline was detectable after 23 h and the main product was *N*-acetyl-ethanolamine. Since we have shown that oxazoline can undergo decomposition by a second mechanism the oxazoline/DFP reaction was re-examined. After 25 h, *N*-2-hydroxyethyl *N'*-2'-acetoxyethylacetamidate was isolated as its acetyl derivative in small yield, but no *O*-phosphoryl compounds were detected by paper chromatography. *N*-2-hydroxyethyl *N'*-2'-acetoxyethylacetamidate hydrochloride will react very slowly with DFP to give at the end of 4 days, 2-acetamidoethyl diisopropyl phosphate and 2-aminoethyl diisopropyl phosphate. It should be noted that under these conditions *N*-acetylethanolamine yields only 2-acetamidoethyl diisopropyl phosphate.

It has been postulated[6] that the *O*-phosphoryl derivatives of ethanolamine isolated from mixtures of oxazoline and DFP in aqueous bicarbonate solution arise from direct interaction of these two materials. However, since these derivatives cannot be detected in the reaction mixture before all the oxazoline has decomposed it would appear that an alternative explanation is required for their formation. The fact that DFP reacts with two of the hydrolytic products of oxazoline to give the observed *O*-phosphoryl derivatives offers such an explanation.

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BIOPHYSICS

Action of Iodine on Strained Keratin

In earlier work on the setting of strained animal fibres in steam or boiling water it was suggested that the strained disulphide bonds of stretched fibres are more reactive than unstrained bonds[1]. Further support for this view has now been obtained in work on the action of iodine on keratin.

The relevant observations are summarized in Fig. 1, where curve 1 is the load-extension diagram of a human hair fibre in distilled water at 22·2° C. Extension was restricted to 50 per cent, and the fibre, which returned exactly to its original length on being released, was re-stretched under the same conditions as before, after a 24-h rest in distilled water. The fibre broke at about 55 per cent extension, as is usual with hair and wool, and the weakening caused by the first extension is shown by the relative positions of curves 1 and 2.

Curve 3 is the load-extension diagram of a second fibre, which was immersed in a 0·1 N solution of iodine in 0·25 M potassium iodide for 24 h at 22·2° C, before being transferred to distilled water and stretched 50 per cent while still containing the large amount of

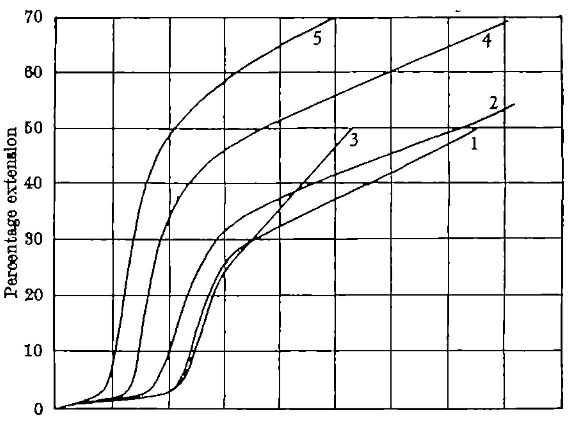

Fig. 1 (1) First fibre stretched 50 per cent in water; (2) first fibre stretched a second time in water (up to break); (3) second fibre stretched 50 per cent in water after iodination; (4) second fibre stretched a second time in water after de-iodination; (5) third fibre stretched a second time in water after iodination, stretching to 70 per cent in water, and de-iodination iodine which is absorbed[2]. Up to about 25 per cent extension, curves 1 and 3 are almost identical, but the modulus of the iodine-treated fibre at higher extensions is very much less than that of the untreated fibre. On being released, the fibre was washed over a period of 4 days in many changes of distilled water, 0·02 N sodium thiosulphate, and then water again, to remove the whole of the loosely bound iodine before being re-stretched in distilled water. Curve 4 was obtained, and it is obvious that the fibre, which broke at 69 per cent extension, is much weaker and far more extensible than if it had merely been stretched first in water (curve 2).

Even when extension is rapid, an iodine-treated fibre, unlike untreated fibres, can be stretched 70 per cent without rupture, and the weakening due to extension is then very severe. The curve for the first extension of such a fibre is not shown in Fig. 1, because it is identical with curve 3 up to 50 per cent extension, and the linear section above 30 per cent extension can be extrapolated to complete the curve. When this third fibre was freed of iodine and restretched in water curve 5 was obtained, breakage occurring at 70·5 per cent extension.

As is suggested by the similarity of curves 1 and 3 up to 25 per cent extension, both the solution of iodine in potassium iodide, and a solution of potassium iodide alone, were found to have little effect on the elastic properties of the fibres, provided that all loosely bound iodine is removed before extension occurs. It is only when the fibres are stretched in presence of the iodine that extensibility is increased and severe weakening induced.

The preceding results suggest that the bonds which oppose extension of animal fibres at extensions above 30 per cent are readily broken—but only under stress—when the fibres contain loosely bound iodine. In agreement with this deduction, paper chromatographic examination of the hydrolysate of a bundle of 100 5-cm lengths of human hair fibres, which had been stretched 65 per cent in water after being impregnated with iodine in the usual way, revealed the presence of much more cysteic acid than in the hydrolysates of similar bundles of stretched untreated fibres (50 per cent extension) and iodine-treated unstretched fibres.

Use is now being made of these observations to establish the amino-acid environment of the bonds which characterize the elastic properties of animal fibres at extensions above 25 per cent.

We thank the International Wool Secretariat and the Wool Research Organization of New Zealand for grants in aid of this investigation.

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Denaturation of Deoxyribonucleic Acid of *Escherichia coli* by X-Irradiation *in vitro*

Deoxyribonucleic acid as a genetic material occupies a key position in the normal and abnormal behaviour of cells. The effect of ionizing radiation on DNA, both inside and outside the cell, is of importance for analysis of the effect of radiation on cells. Nucleic acids have absorption maxima in the range 257–261 mμ and the effect of denaturation induced by radiation would be to increase the value of the extinction coefficient in this wave-length region[1].

This communication is concerned with the effect of *in vitro* irradiation of DNA from *E. coli*, as examined from the ultra-violet absorption of irradiated and non-irradiated samples. DNA containing less than 2 per cent protein and 3 per cent RNA was extracted from stationary phase *E. coli* of 18–20 hr. growth by Marmur's method[2], and this was finally dissolved in 0·002 *M* sodium chloride solution. The concentration of the extracted DNA solution as determined by the diphenylamine reaction[3] was about 300 μgm./c.c. The stock solution was then divided into several parts, some of which were given different doses of X-rays and one was kept as control. Irradiation by 70-kV X-rays was carried out at a concentration of DNA of about 50 μgm./c.c. The dose-rate, as determined with the help of a ferrous sulphate dosimeter, was 1 krad/min.

For ultra-violet investigations both the control and irradiated solution were diluted to the same extent with 0·002 *M* sodium chloride. Ultra-violet absorption in the range 220–300 mμ was determined with a Zeiss *PMQ* II spectrophotometer (Fig. 1).

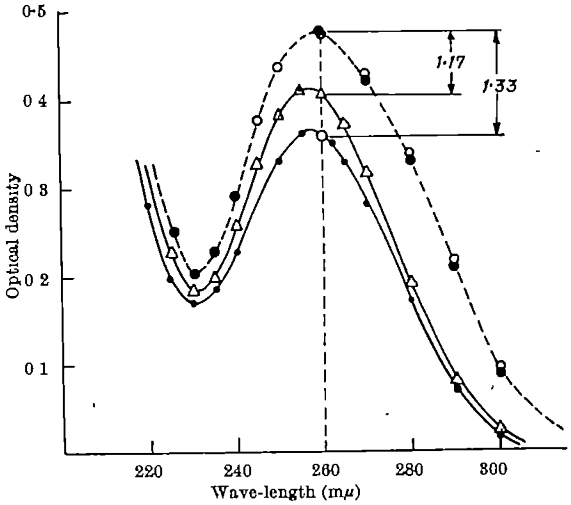

Fig. 1. Absorption spectrum of *E. coli* DNA. •, Control non-irradiated; Δ, irradiated DNA dose 9 krads, ●, control at *p*H 3·0. ○, irradiated at *p*H 3·0

Radiation-induced denaturation was estimated by the method of Fredericq *et al.*[4]. If ε_{RC} and ε_{OC} denote the extinction coefficients at 260 mμ of the irradiated and non-irradiated control DNA, then:

$$\varepsilon_{RC} = x\,\varepsilon_{OC} \tag{1}$$

where $x \geqslant 1$ is the denaturation factor from equation (1):

$$x = \frac{\varepsilon_{RC}}{\varepsilon_{OC}} = \frac{\varepsilon_{OA}}{\varepsilon_{OC}} \Big/ \frac{\varepsilon_{RA}}{\varepsilon_{RC}} = \frac{R_1}{R_2} \tag{2}$$

where ε_{OA} and ε_{RA} are the extinction coefficients of control and radiated DNA after complete denaturation by acid treatment at $p\text{H} = 3·0$ and $\varepsilon_{OA} = \varepsilon_{RA}$.

Typical ultra-violet absorption curves of non-irradiated, irradiated and acid denatured DNA solutions (correcting for dilution caused by addition of acid) are shown in Fig. 1. The value of R_1 for the non-irradiated DNA was 1·33 from 6 independent determinations. The value of R_2 decreased with increasing dose of irradiation. The denaturation factor x at various doses as calculated from equation (2) are shown in Fig. 2. It may be seen that the

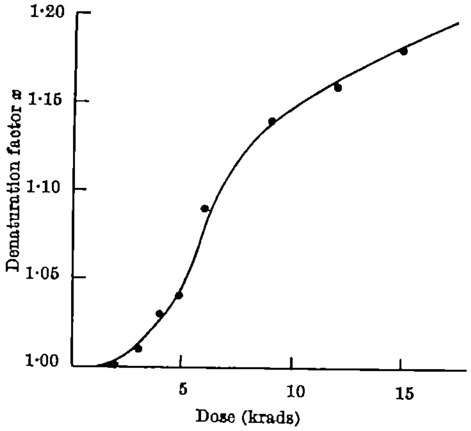

Fig. 2. Variation of denaturation factor with X-ray dose

denaturation factor increased rapidly with dose and reached a value of 1·18 at 15 krads. This was about 54·5 per cent of the maximum possible denaturation observed with acid denatured control.

The experiments recorded here indicate that there is a progressive increase in the disruption of hydrogen bonds as determined from the increase in the extinction coefficient with increasing radiation doses. This result is in good agreement with the conclusions by other workers from the electrometric titration and other studies[5,6].

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BIOCHEMISTRY

Co-ordination Compounds as Resolving Agents

IN the normal chemical resolution of amino-acids using organic bases, one optical antipode—corresponding to the less-soluble diastereoisomeric salt—can generally be obtained optically pure. The more soluble derivative remaining in the mother liquor is contaminated with a small amount of the less-soluble salt and is therefore always very difficult to obtain pure. The usual device of using both forms of the optically active base in turn is not feasible as only the natural occurring antipodes of the alkaloid bases (quinine, brucine, cinchonine, strychnine, etc.) are readily available.

We have now found that metal complex cations can be used successfully for the resolution of amino-acid derivatives. Particularly useful were the *cis*-dinitro-*bis*(ethylenediamine) cobalt(III) ion [I] and oxalato-*bis*(ethylenediamine) cobalt(III) ion [II], which wer prepared from readily available starting material and resolved to give both enantiomorphs[1]. To a aqueous solution of the sodium salt of racemic benzoy alanine[2] was added an aqueous solution of *d*-oxalat *bis*(ethylenediamine) cobalt(III) acetate [II], and th resulting diastereoisomeric salt *l*-benzoylalanine-*d* oxalato*bis*-(ethylenediamine) cobalt(III) [III], whic precipitated almost quantitatively from the solution was removed by filtration. After recrystallizatio from water, recovery of pure *l*-benzoylalanine wa effected by slurrying [III] in water and addin potassium iodide, which precipitated the insolubl iodide of [II]. The filtrate on acidification yielde optically pure *l*-benzoylalanine. The specific rotatio $[\alpha]_D$ was − 35° (0·125 g in 1 N sodium hydroxide 2 diam. tube). From the solution containing th more soluble diastereoisomeric salt, partly resolve *d*-benzoylalanine was isolated which on treatmen with the *l* antipode of [II] yielded optically pur *d*-benzoylalanine of specific rotation + 35°.

Hydrolysis of *d* and *l* benzoylalanine was effecte by refluxing in 6 N hydrochloric acid[3]. The pur amino-acids had a specific rotation $[\alpha]_D = \pm 15$ (1·5 per cent in 6 N hydrochloric acid).

The L-alanine $[\alpha]_D = + 15°$ (assayed biochemically with D-amino oxidase)[4] contained less than one part in 500 of the D antipode.

In an analogous manner phthalyl, tosyl, formyl and benzoyl derivatives of the following α amino-acids have been resolved: valine, serine, phenylalanine, leucine, isoleucine, norleucine, methionine, proline, cystine, tryptophan, aspartic acid, glutamic acid, glutamine, ornithine and tyrosine.

We thank A. H. Ennor and H. Rosenberg for biochemical assays.

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Phospholipase Activity of Staphylococcal Toxin

IN view of an earlier finding that phospholipase *A* had an antagonistic effect on staphylococcal toxin in mice[1], and a later observation that staphylococcal antitoxin gave some protection in mice against phospholipase *A* (ref. 2), the possible phospholipase activity in staphylococcal toxins was investigated.

As a first approach, a batch of staphylococcal toxin derived from an αβ strain, *B.S.M.24*, of *Staphylococcus pyogenes* isolated from a case of bovine mastitis was examined as well as fractions obtained by curtain electrophoresis of the crude toxin. In this communication the action of this toxin on phosphatidyl inositol and lysophosphatidyl inositol will be described in some detail, and evidence for other phospholipase activity of staphylococcal toxins will also be reported.

A commercial preparation of soya bean phospholipids was used as substrate. This was found by paper chromatography and phosphorus analysis[3] to have the following percentage composition: phosphatidyl ethanolamine 22, phosphatidyl choline 28, phosphatidyl inositol 20, lysophosphatidyl inositol 3. Phosphorus compounds other than known phospholipids accounted for the difference between this composition and 100 per cent. The major components were identified by the standard staining procedures[4-6]. The zone migrating with R_F 0·23 was identified as lysophosphatidyl inositol in the following manner. When phospholipase *A* reacted with the soya bean phospholipids and the products were examined by paper chromatography, lysophosphatidyl ethanolamine and lysophosphatidyl choline were identified, a reduction of phosphatidyl inositol and an increase in the component with R_F of 0·23 occurred. In addition, when the area of paper occupied by the latter component was removed and placed on a blood agar plate, lysis of the blood occurred in the area beneath the paper.

The reaction between the toxin and phospholipids was studied in the following manner. Soya bean phospholipid emulsion in water (0·4 per cent) was incubated with both crude toxin and electrophoretic fractions at an optimum *p*H of 4·6 at 37° for times up to 20 h. Aliquots were freeze dried, the products were extracted with chloroform/methanol, 2:1 (v/v), and examined by paper chromatography as previously described. Reduction of the phosphatidyl inositol and lysophosphatidyl inositol spots was observed.

To identify the reaction products, aliquots of the reaction mixture were dialysed in a rubber membrane[7]. Using the method of Stern and Shapiro[8], an increase in the concentration of ester groups in the dialysate of the toxin-treated substrate was found, compared with that of the control.

Other aliquots of the reaction mixture were precipitated with trichloroacetic acid. A portion of the filtrate was extracted with diethyl ether to remove trichloroacetic acid and when examined by paper electrophoresis[9] showed a phosphorus staining[10] zone which moved in the region of an inositol phosphate control. Other portions of the trichloroacetic acid filtrate were analysed for phosphorus by the method of Brown[11]. The release of trichloroacetic acid soluble phosphorus was inhibited by commercial staphylococcal antitoxin. No significant inhibition was obtained by concentrated normal horse serum.

The relationship between water-soluble phosphorus and reaction time followed a typical enzyme reaction curve. These results suggest the following reaction:

$$\text{phosphatidyl inositol} + H_2O \xrightarrow{\textit{enzyme}} \text{diglyceride} + \text{inositol phosphate}$$

A similar enzyme occurs in guinea pig brain[12] and rat liver[13].

Preparations of rabbit red cell stroma[14] and striated muscle of the rat were similarly treated with the electrophoretic fractions of the toxin and action on phosphatidyl inositol was again demonstrated.

Evidence was obtained, however, that the phospholipase of this toxin was not directly responsible for the lethal effect in mice or the hæmolysis of rabbit red cells.

In addition, there was evidence that action on lysophosphatidyl choline was produced by another batch of crude toxin derived from the same strain, *B.S.M.*24. With toxin from strain *C.N.*56 (identical with the Wood strain) phospholipase *A*-like action, namely, conversion of phosphatidyl choline to lysophosphatidyl choline, was demonstrated. Surprisingly, there was no evidence of the action of this toxin on other substrates susceptible to phospholipase *A*. The conditions which determine these activities are to be investigated more fully. Further work on the various phospholipases released by staphylococci in relation to the pathogenicity of the strain appears to be necessary.

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Urinary Excretion of Urocanic Acid and Formimino-glutamic Acid

THE major pathway of histidine breakdown is to glutamic acid, and this occurs via two relatively stable intermediate compounds, urocanic acid and formimino-glutamic acid. A competent folic-acid coenzyme is required to accept the formimino-group in the last stage of this pathway. When there is impaired, folic-acid function formimino-glutamic acid accumulates and is excreted in the urine. This has been widely used as a test of folic-acid deficiency in man[1], the urinary excretion of formimino-glutamic acid being measured after an oral dose of 15 g of histidine hydrochloride. The urinary excretion of formimino-glutamic acid after oral doses of histidine may be accompanied by the excretion of urocanic acid[2]. The purpose of this communication is to direct attention to the importance of urocanic acid in the urine of patients with megaloblastic anæmia and other disease states following oral doses of 15 g of histidine hydrochloride.

A total of 186 urine samples were examined. These were obtained from 11 normal subjects, 27 patients with megaloblastic anæmia (pernicious anæmia 10, post-gastrectomy 2, Crohn's disease 1, pregnancy 3, idiopathic steatorrhœa 6, nutritional 1, hereditary spherocytosis 1, carcinoma 2, associated with disseminated lupus erythematosis 1) and 18 patients with miscellaneous disorders associated with normoblastic hæmopoiesis (hereditary spherocytosis 2, hypochromic anæmia 6, chloramphenicol aplasia 2, carcinoma 2, acute leukæmia 3, chronic myelofibrosis 2, osseous tuberculosis 1). In the patients' serial urine samples were examined before and after treatment with B_{12} and/or folic acid.

The urine passed in the 8 h following an oral dose of 15 g of histidine hydrochloride was collected into a

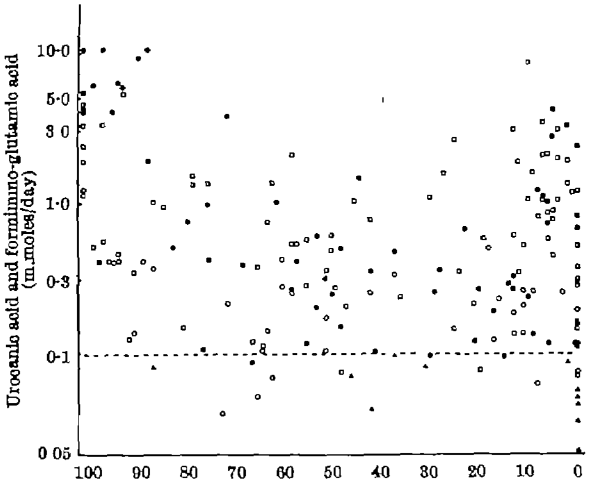

Fig. 1. Each point represents the amount of urocanic acid and formimino-glutamic acid excreted in the urine after an oral dose of 15 g (97 m.moles) of histidine hydrochloride. The proportion of urocanic acid present is shown on the abscissa. ▲, Normal subjects; ●, miscellaneous disorders, megaloblastic anæmia, ○, vitamin B_{12} deficiency; □, folic acid

container to which had been added 3–5 ml. of N/1 hydrochloric acid. An aliquot was retained for estimation of both urocanic acid and formimino-glutamic acid. A second aliquot (4·0 ml.) was brought to *p*H 9·6–10·0 with 1·5 N/1 potassium hydroxide and heated at 10 lb. pressure for 10 min. The alkali added to the urine sample was neutralized with an equimolar amount of 1·5 N/1 hydrochloric acid. This procedure destroyed all the formimino-glutamic acid present but did not interfere with urocanic acid. Recovery of urocanic acid added to urine and treated in this manner varied from 93 to 105 per cent.

Formimino-glutamic acid and/or urocanic acid were estimated by incubating the urine with folic acid, TPN and a chick enzyme preparation. This transferred the formimino group to tetrahydrofolic acid and the end-product was converted to 5–10, methenyltetrahydrofolic acid with hydrochloric acid. The latter was measured by its absorption at 350 mμ (ref. 3). Orocanic acid is fully active in this system because urocanase, the enzyme converting urocanic acid to formimino-glutamic acid, is present in the enzyme preparation.

The total histidine derivatives excreted following an oral dose of 15 g of histidine (Fig. 1) was less than 0·1 m.moles (equivalent to 17 mg of formimino-glutamic acid) in normal subjects and was more than this in all but 11 of the samples from the patients. Eight of these 11 specimens had been obtained after the patient had received either B_{12} or folic acid. The percentage of urocanic acid in these samples is also shown in Fig. 1 and in Table 1. Urocanic acid was present in 163 (88 per cent) of the urine sampl examined. In 79 urines, urocanic acid constitute more than 50 per cent of the histidine derivative present and in 10 urines, only urocanic acid wa present.

Table 1. EXCRETION OF UROCANIC ACID IN THE URINE FOLLOWING AN ORAL DOSE OF 15 G OF HISTIDINE HYDROCHLORIDE, EXPRESSED AS A PERCENTAGE OF THE TOTAL HISTIDINE DERIVATIVES APPEARING IN THE URINE

Diagnosis		$\frac{\text{Urocanic acid (m.mole)} \times 100}{\text{Urocanic acid + formimino-glutamic acid (m.mole)}}$ %						
		0	1–19	20–39	40–59	60–79	80–99	100
Normal	(11)	5	1	2	2	0	1	0
Folic-acid deficiency	(86)	7	31	6	12	9	14	7
Vitamin B_{12} deficiency	(38)	9	0	8	6	8	8	0
Others	(51)	2	12	7	11	7	9	3
Total	(186)	23	58	18	31	24	27	10
	100%	12%	28%	10%	17%	12%	15%	5%

The concentration of urocanase in the liver of foli acid-deficient rats has been found to be abnormall low[4]. Folic-acid deficiency results in an accumulatio of formimino-glutamic acid, and this in turn depresse the production of the enzyme urocanase. The en result is that urocanic acid rather than formimin glutamic acid accumulates and both appear in th urine.

Although urocanic acid is excreted by most patien who excrete formimino-glutamic acid, it is most likel to appear in large amount in patients with lon standing folic-acid deficiency. Thus two patien with hæmolytic anæmia (hereditary spherocytosis an acquired autoimmune hæmolytic anæmia) wh developed megaloblastic arrest both excreted larg amounts of urocanic acid. Formimino-glutamic ac was either absent or present in small amount. Sim larly a patient with myeloblastic leukæmia of months duration who had been treated with steroi and 6 mercaptopurine excreted between 2·3 and 4 of urocanic acid after 15 g of histidine. The excretio declined rapidly when oral folic acid was given.

We conclude that in carrying out tests for folic-aci 'deficiency' in man it is important to estimate th excretion in the urine of urocanic acid as well formimino-glutamic acid. Failure to do so may gi misleading results in many cases.

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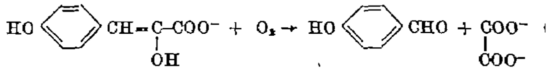
Oxidation of Phenylpyruvates to Aromati Aldehydes and Oxalate

Ewald and Hubener[1] have recently described new method for the determination of tyrosine-ketoglutarate transaminase activity. In their pr cedure, the *p*-hydroxyphenylpyruvate generated the transamination reaction was converted on stan ing in alkali to an unknown 'chromogen' absorbi maximally at 331 mμ with $\varepsilon_{334\,m\mu} = 16{,}200$. T Ewald and Hubener technique represents anoth report of the oxidative cleavage of phenylpyruvates aromatic aldehydes and oxalate according to equ tion (1) and depends on the strong absorption of t phenolate ion of *p*-hydroxybenzaldehyde.



This reaction was recognized in this laboratory[2] and preliminary reports of the Cu++ and Mn+ catalysis of the oxidation have been published[4] For pure *p*-hydroxybenzaldehyde in 0·1 N sodiu hydroxide I have found $\varepsilon^{\max}_{330\,m\mu} = 27{,}200$. Gol schmid[5] reported $\varepsilon^{\max}_{333\,m\mu} = 26{,}800$, and Do reported $\epsilon^{\max}_{330\,m\mu} = 25{,}000$. In my work, the oxidation of *p*-hydroxyphenylpyruvate in 0·1 N sodium hydroxide measured manometrically with air at 30° (0·00167 M substrate) and of 0·01 M keto-acid under oxygen was complete in less than 15 min. These reactions yielded 60–70 per cent *p*-hydroxybenzaldehyde, which is consistent with the extinction coefficient of the Ewald and Hübener 'chromogen'.

It is noteworthy that the oxidation of phenylpyruvates to aldehyde and oxalate has been reported a number of times by different investigators. The oxidation of phenylpyruvic acid was noted by Bougault and Hemmerlé[8] and described by Hemmerlé[9]. Wieland[10] observed the formation of benzaldehyde and oxalate from aqueous phenylpyruvic acid with palladium and oxygen. Cavallini and Stirpe[11] reported the alkaline oxidation of phenylpyruvate to benzaldehyde and oxalate.

The instability of *p*-hydroxyphenylpyruvate in alkali was noted by Bucher and Kirberger[12]. The formation of *p*-hydroxybenzaldehyde from alkaline solutions of *p*-hydroxyphenylpyruvate was recognized by Doy[7], who suggested a mechanism involving hydration of the enol to *p*-hydroxyphenylglyceric acid and hydrolytic cleavage to aldehyde and glycollate, a mechanism which he no longer supports[13]. The presence of *p*-hydroxybenzaldehyde in chromatograms of *p*-hydroxyphenylpyruvate was reported from this laboratory[14]. Billek[15] has also described the oxidation of *p*-hydroxyphenylpyruvate in alkali to *p*-hydroxybenzaldehyde and oxalate.

In my work and in other reports[9-11,15], the participation of the enol as the tautomer undergoing oxidation was reported.

I have found that the oxidation of phenylpyruvate, *p*-hydroxyphenylpyruvate and *p*-methoxyphenylpyruvate occurs at a negligible rate at *p*H 7 or less. The reaction is catalysed by ultra-violet light, indicating its free radical nature. At *p*H 8 and greater, the reaction is rapid enough to monitor spectrophotometrically, and its increase in rate with *p*H indicates that the enolate is the species oxidized.

In view of the numerous reports on oxidation of phenylpyruvates to aromatic aldehydes and oxalate, it is somewhat surprising that this reaction is not more generally recognized.

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PHYSIOLOGY

Passive Transport in the Renal Re-absorption of Inorganic Phosphate in the Dog

DURING an investigation[1] on the renal effects of profound hypothermia (10° C) in the dog, the rates of renal re-absorption of filtered sodium and inorganic phosphate were found to differ markedly in their response to changing body temperature.

The dogs were cooled to an œsophageal temperature of 10° C by means of an extra-corporeal circulation and heat-exchanger. Urine was collected via uretic catheterization. Continuous urine and frequent blood samples were collected during step-wise reduction of temperature. These samples were analysed for sodium, inorganic phosphate and creatinine. The percentage of re-absorption of filtered sodium and inorganic phosphate was calculated as:

$$100\,X\left(I - \frac{U_x\,.\,V}{C_{r}\,.\,P_x}\right)$$

where U_x = urine concentration of sodium or inorganic phosphate; V = urine volume per minute; C_{cr} = creatinine clearance, P_x = plasma concentration of sodium or inorganic phosphate.

The percentage re-absorption of filtered sodium was found to decrease rapidly with a falling temperature. That of inorganic phosphate, on the other hand, showed much less reduction (Fig. 1). In a series of 6 dogs, the Q_{10} of sodium re-absorption

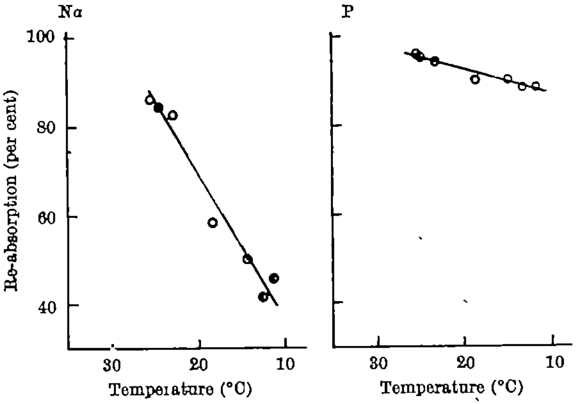

Fig. 1. Typical data for the fall in percentage renal re-absorption of filtered sodium and inorganic phosphate, on cooling a dog to 10° C

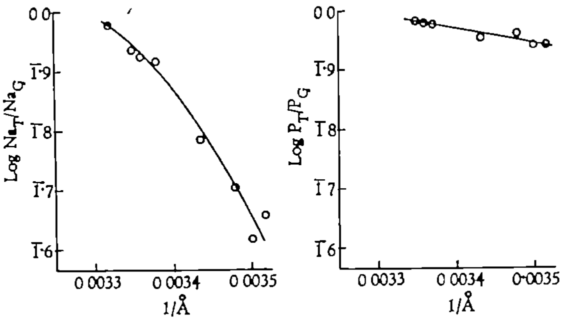

Fig. 2. The logarithms of the (corrected) rates of renal re-absorption of filtered sodium and inorganic phosphate are plotted against the reciprocals of those absolute temperatures at which the rates were determined. The slopes of the resultant curves are a measure of the apparent activation energies of the renal re-absorption of sodium and inorganic phosphate. Na_T or P_T, rate of tubular re-absorption of sodium or inorganic phosphate, Na_G or P_G, rate of glomerular filtration of sodium or inorganic phosphate

averaged 1·67, whereas that of urinary inorganic phosphate averaged 1·08.

The Arrhenius equation, $\log \frac{k_1}{k_2} = \frac{E\ (T_1 - T_2)}{2 \cdot 3\ R\ T_1\ T_2}$, was then applied to these data. (R is the gas constant, k_1 and k_2 the rate constants at the absolute temperatures T_1 and T_2, and E is the apparent activation energy.) Inasmuch as the glomerular filtration-rate, and therefore the filtered load, fell considerably during progressive cooling, the absolute rates of re-absorption were corrected for the alterations in load (Fig. 2). The mean apparent activation energy for re-absorption of filtered sodium, in a series of 6 dogs, was 8,400 cal/mol/deg, whereas that of inorganic phosphate was 1,200 cal/mol/deg.

The latter figure is well below that usually considered the upper limit (5,000 cal/mol/deg) of purely passive processes, such as diffusion[2]. This favours the hypothesis that inorganic phosphate is re-absorbed by a passive transport process in the canine kidney.

This work was carried out in collaboration with the Department of Experimental Surgery, and the Renal-Metabolic Group of the Department of Medicine, University of Cape Town. It was supported by the Council for Scientific and Industrial Research, the Dr. C. L. Herman Research Fund of the University of Cape Town, and the Wellcome Trust.

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Electrophoretic Pattern of the Soluble Proteins of a Pineal Body Extract

THE possible physiological effects of the pineal body and its extracts are being re-investigated[1-4]. As few details are given about the nature of the extracts used, we studied the mobility of the water-soluble proteins of the organ.

A water extract was prepared from fresh acetone-powdered bovine pineal bodies according to the method of Kitay and Altschule[5].

Electrophoresis was performed in a horizontal tank in a veronal buffer at pH 8·6 of ionic strength 0·05 using Whatman No. 1 filter paper and a gradient of 10 V/cm for 5 h. After the run, the paper strip was dyed in amido-schwarz.

For comparison of mobilities, pineal extracts and bovine serum were run side by side on the same filter paper strip. The dyed strips, after impregnation with liquid paraffin, were scanned in an automatic densitometer. The resulting pattern was subdivided in gaussian curves and the areas determined geometrically.

For free electrophoresis, the pineal extract was dialysed against the same buffer. Analyses were performed in a Perkin–Elmer model 38 *A* apparatus, under a gradient of 10 V for two hours.

The extract from fresh pineal bodies revealed the presence of four protein fractions (Table 1 and Fig. 1).

Table 1. ELECTROPHORETIC FRACTIONATION OF WATER-SOLUBLE PROTEINS OF BOVINE PINEAL BODIES

Fraction	Relative concentration (per cent)	Mobility (cm/V/sec)
I	14·7	$6 \cdot 1 \times 10^{-5}$
II	20·8	$4 \cdot 2 \times 10^{-5}$
III	47·0	$2\ 8 \times 10^{-5}$
IV	17·5	$1 \cdot 5 \times 10^{-5}$

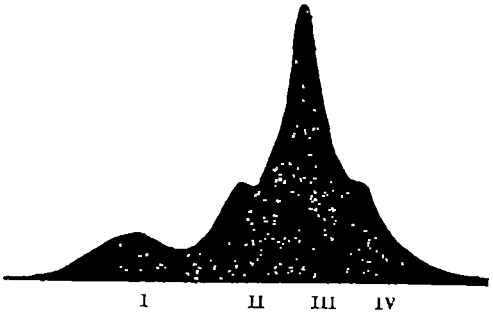

Fig. 1. Electrophoretic pattern of water-soluble proteins of bovine pineal bodies

Another extract prepared from powdered dried glands, obtained commercially, did not contain fraction 4 and only traces of fraction 1.

The fraction 1 has the same mobility as bovine serum albumin.

Immuno-electrophoretic investigation of these fractions in their relation to bovine blood proteins and their physiological actions are in progress.

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Effect of Large Variations in External Potassium on the Oxygen Consumption of Rat Heart Strips

THE link between the electrical state of the cell membrane and the metabolism of the cell is unknown. High external K+, which reduces the membrane potential, causes a five-fold increase in resting oxygen consumption[1] and a twenty-fold increase in resting heat production[2,3] of frog skeletal muscle. The onset of the metabolic response (often called the Solandt effect) is so rapid that it is thought to be due to the change in potential rather than to a raised intracellular K+ (ref. 3). This communication reports our efforts to test for the Solandt effect in isolated trabeculæ carneæ from the rat heart[4].

The 24 muscles used were less than 1 mm in diameter, and about 9 mm in length. Except at one cut end, the cells were intact. Oxygen consumption was measured at 32° C by a previously described volumetric technique that allowed simultaneous measurement of the tension by means of a strain gauge[5,6]. By the time the oxygen measurements were started the tension had equilibrated at about 0·5 g. Except for an occasional test stimulus the muscles remained quiescent.

Starting with a recording of the oxygen consumption in Feigen's solution[7] the K+ concentration was raised successively to the various experimental levels. In the 'hypertonic series' this was done by injecting the proper amount of concentrated potassium chloride solution into the chambers with a micrometer syringe. Oxygen readings were begun within 2 min after injection. In the 'isotonic series' the desired final chamber K+ concentration was achieved by withdrawal of a measured amount of solution and replacement with Feigen's solution in which K+ had been substituted for part of the Na+. Thus, the total osmotic pressure of the latter solution did not change. Recording of the consumption of oxygen was begun 15 min after adding the K+. Initial experiments had shown that relatively small increases in Q_{O_2} were obtained below 20 mM/l., so that the concentrations chosen for the osmotic experiments were between 30 and 90 mM/l. of potassium. The gas phase was 98 per cent oxygen and 2 per cent carbon dioxide controlled at pH 7·4.

The oxygen consumptions for each strip at the various K+ levels were expressed as percentages of the oxygen consumptions of that same strip at 5·63 mM/l. external K+. The oxygen consumptions increased each time the external K+ level was raised (Fig. 1). The maximum increase of 73 or 82 per cent mM/l. external potassium is small compared with that seen in frog skeletal muscle. The average maximum increase in Q_{O_2} was about 2.

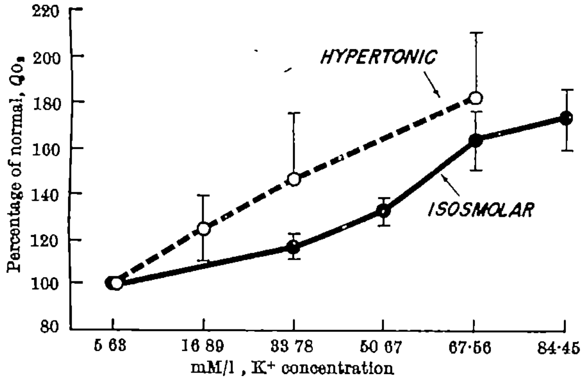

Fig. 1. Effect of variations in the external potassium concentration on the Q_{O_2} of 22 rat trabeculæ carneæ. Each point represents the mean ± 1 *S.E.* (vertical bars) of 10–12 determinations

The chambers used are capable of detecting much larger oxygen consumptions in rat heart strips of the same size[5] so that the limitation of increase in oxygen consumption would seem to have been inherent in the heart muscle strips rather than due to an experimental limitation. It is unlikely that a substantial increment in uptake of oxygen was missed due to the 2-min delay before taking readings, since the lag in the system is about 3 min. There was also little tendency for the oxygen uptake to fall with time. Finally, the small response could not have been due to calcium lack[8] since Feigen's solution[7] contains 5·58 mM/l.

The effects of K+ on metabolism vary markedly with the tissue. Not all have shown the marked increase reported for frog skeletal muscle. Gerard[9] found that high external K+ depressed the consumption of oxygen by nerve. Ashmore, Cahill, Hastings, and Zottu[10] found a depression of glycolysis and glycogenolysis in rat liver slices when K+ medium was used instead of an Na+ medium. On the other hand, Cardot, Faure and Arvanitaki[11] found that consumption of oxygen increased slightly in invertebrate heart muscle when potassium was increased relative to sodium and calcium. Thus, only in frog skeletal muscle have marked effects of the ionic medium on metabolism been seen.

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HÆMATOLOGY

Effect of Carbon Dioxide and Diphenylhydantoin on the Partition of Triiodothyronine labelled with Iodine-131 between the Red Cells and the Plasma Proteins

THE uptake *in vitro* by the red blood cells of triiodothyronine labelled with iodine-131 is a useful method of evaluating thyroid function[1]. It has been shown that under the conditions of the test ^{131}I-triiodothyronine is distributed between the red cells, the α_1- and β-globulins and the binding sites on thyroxine binding protein not occupied by thyroxine (free T.B.P.), according to the relative binding powers of these substances; since the binding power of the red cells and the α_1- and β-globulins appears to be relatively constant, the test is an indirect measurement of free T.B.P. capacity[2]. It is known that an increase in the carbon dioxide tension of the blood increases the proportional uptake of ^{131}I-triiodothyronine by the red cells[3]. The uptake is also increased in patients who are being treated with diphenylhydantoin (phenytoin), an effect which may be reproduced when this substance is added to blood *in vitro*[4]. We have investigated the mechanisms whereby these agents modify the partition of ^{131}I-triiodothyronine between the red cells and the plasma proteins.

The uptake of ^{131}I-triiodothyronine by the red cells was measured in aliquots of blood from the same individual to which increasing amounts of thyroxine had been added in order to obtain a series of values for free T.B.P. capacity. The same experiment was performed: (*a*) with blood to which diphenylhydantoin had been added in a concentration of 10 mg per 100 ml. of plasma; (*b*) with blood which had been allowed to equilibrate for ten minutes with pure carbon dioxide at atmospheric pressure and room temperature. The results of these experiments are shown in Fig. 1, where for each aliquot of blood the reciprocal of the uptake of ^{131}I-triiodothyronine by the

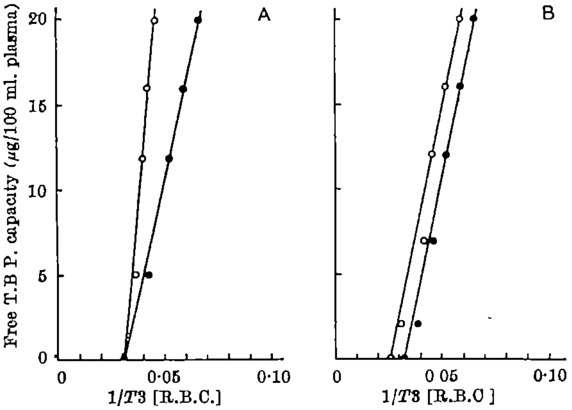

Fig. 1 Values for free T.B.P. capacity plotted against the reciprocal of $T3$[R.B.C.]. *A*, Comparison of control blood and blood to which diphenylhydantoin has been added in a concentration of 10 mg/100 ml. of plasma. The equations for the regression lines may be expressed as.

Control (●), $\frac{1}{T3[\text{R.B.C.}]}$ = 0·00175 free T.B.P. + 0 031.

Diphenylhydantoin (○), $\frac{1}{T3[\text{R.B.C.}]}$ = 0 00071 free T.B.P. + 0·031.

B, Comparison of control blood and blood which had been allowed to equilibrate in an atmosphere with a high carbon dioxide tension. The equations for the regression lines may be expressed as.

Control (●), $\frac{1}{T3[\text{R.B.C.}]}$ = 0·00163 free T.B.P + 0 032.

Carbon dioxide (○), $\frac{1}{T3[\text{R.B.C}]}$ = 0·00160 free T.B.P. + 0 0265.

red cells has been plotted against the corresponding value for free T.B.P. capacity.

The proportion of ^{131}I-triiodothyronine taken up by the red cells ($T3$[R.B.C.]) may be expressed as follows[3]:

$$T3[\text{R.B.C.}] = \frac{\text{binding power of R.B.C.}}{\text{binding power of R.B.C.} + \text{binding power of free T.B.P.} + \text{binding power of } \alpha_2\text{- and } \beta\text{-globulins}}$$

The binding power of free T.B.P. is equal to the product of free T.B.P. capacity and affinity. Hence:

$$\frac{1}{T3[\text{R.B.C.}]} = \frac{\text{free T.B.P. capacity} \times \text{affinity}}{\text{binding power of R.B.C.}} + \frac{\text{Binding power of } \alpha_2\text{- and } \beta\text{-globulins}}{\text{binding power of R.B.C.}} + 1$$

It can be seen from Fig. 1 *A* that the slopes of the regression lines for the normal blood and the blood to which diphenylhydantoin had been added are different, but that they intercept the x axis at the same point, which implies that diphenylhydantoin affects the binding power of free T.B.P. When free T.B.P. capacity is zero, diphenylhydantoin has no effect. Diphenylhydantoin probably competes with both triiodothyronine and thyroxine for binding sites on T.B.P.[4]. In the case of carbon dioxide (Fig. 1 *B*) the regression lines have the same slope but intercept the x axis at different points. According to the above formula, this must be interpreted as an effect of carbon dioxide on the binding power of the α_2- and β-globulins. The proportional effect on the uptake of ^{131}I-triiodothyronine by the red cells is of the same magnitude whatever the value for free T.B.P. capacity.

The uptake of ^{131}I-triiodothyronine by the red cells is only a valid test of thyroid function if the binding power of the α_2- and β-globulins remains relatively constant[3]. Since an increase in the carbon dioxide content of the blood diminishes the binding power of these proteins, it is advisable, when carrying out this test, to allow the blood to equilibrate with the atmospheric carbon dioxide before and during the period of incubation.

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Chemical Difference between Normal Human Hæmoglobin and Hæmoglobin-I

Ingram and I have reported that a specific colour reaction on a 'fingerprint' indicated that the tryptic peptide 23 of hæmoglobin-I (Hb-I) contains tryptophan whereas the corresponding one in the normal hæmoglobin (Hb-A) does not. Ingram reported that the peptide is in the α-chain of the molecule[2]. Hill and Konigsberg[3] found that there is only one tryptophan residue in the α-chain of Hb-A; it is in a pentapeptide with valyl as the NH_2-terminal and lysyl as the COOH-terminal residues. I wish to report that it is the lysyl residue of the pentapeptide which is exchanged with aspartyl in the genetic alteration of Hb-I. It is now known that the pentapeptide is the third tryptic peptide from the NH_2-terminal end of the α-chain[4,5] and that the interchange of lysyl for aspartyl takes place at the 16th from the NH_2-terminus.

The soluble peptides were prepared from chromatographically purified Hb-A and Hb-I as previously described[1]. One-dimensional electrophoresis was adequate for the present investigation for the examination and isolation of soluble peptides. The tryptophan-containing peptides were easily located by fluorescence under an ultra-violet lamp.

Results indicate that there were three tryptophan-positive peptides in both hæmoglobins as shown in Fig. 1. The tryptophan-positive peptide which migrated the fastest at pH 6·4 towards the cathode in Hb-A vanished in Hb-I. (In our previous publication, Fig. 2, 4 tryptophan-positive peptides were shown for Hb-I; this is now known to be due to a trace of Hb-A present as a contaminant.) As it was previously reported the peptide 23 of Hb-I moved more rapidly towards the anode than the normal peptide. The peptide 23 of Hb-I contained tryptophan and an aspartyl residue (the latter accounted for its greater mobility towards the anode), whereas the corresponding peptide 23 of Hb-A did not. Because the tryptic digest of the α-chain of Hb-A contained but only one tryptophan residue the peptide with which it is associated vanished in Hb-I (Fig. 1). Thus

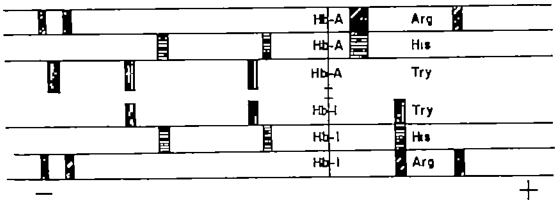

Fig. 1. Comparison by one-dimensional electrophoresis of tryptic digests of the normal and I hæmoglobins at pH 6 4. Three peptides of both hæmoglobins developed colour for tryptophan. Note that the peptide which moves the fastest toward cathode showing positive reaction for tryptophan in Hb-A is absent in Hb-I. It appears on the anode side in hæmoglobin-I

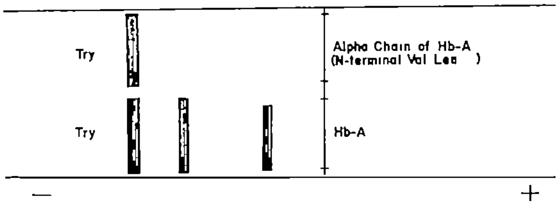

Fig. 2. Tracing of one-dimensional paper electrophoresis of tryptic digest of the normal hæmoglobin (Hb-A) and the α-chain isolated from it. The α-chain contains only one tryptophan-positive peptide. It moves the fastest towards the cathode, it is thus peptide which vanishes in Hb-I. The amino-acid interchange of this genetic alteration takes place in the tryptophan-positive pentapeptide of the α-chain

the specific chemical difference between Hb-A and Hb-I must be located in the tryptophan-containing pentapeptide.

The specific amino-acid composition of the tryptophan-positive pentapeptide of the α-chain of Hb-A was determined by the column chromatography method of Spackman, Stein and Moore[6] and found to consist of $(Ala)_2(Try)(Gly)(Lys)$; where Ala = alanyl, Try = tryptophyl, Gly = glycyl, and Lys = lysyl. In order to preserve tryptophan, leucine-aminopeptidase was used to hydrolyse the peptide which contained it. The NH_2-terminal residue of the pentapeptide was alanyl by the method of Sanger[7]. The amino-acid sequence of the pentapeptide was deduced in the following manner: the peptide was split with α-chymotrypsin and then the peptides were separated by one-dimensional paper electrophoresis; the tryptophan-positive segment behaved essentially neutral; the NH_2-terminal residue was alanyl and the amino-acid composition was $(Ala)_2(Try)$; so the sequence of the tripeptide was Ala-Ala-Try. The dipeptide contained glycine and lysine. The amino-acid sequence of the pentapeptide was deduced to be Ala-Ala-Try-Gly-Lys.

The NH_2-terminal residue of peptide 23 of Hb-I was alanyl, but it was valyl for peptide 23 of Hb-A. The amino-acid composition of Hb-I peptide 23 was identical with the sum of the tryptophan-positive pentapeptide and peptide 23 of Hb-A, however, with one exception—instead of lysyl, aspartyl residue was found. Since the pentapeptide is the third from NH_2-terminus of the α-chain, the tryptic peptide 23 must be the fourth; they are simply linked together in Hb-I through aspartyl residue.

Attempts made in this laboratory to find out whether or not other amino-acids in peptide 23 might be involved in the genetic change have not been successful; the amino-acid sequence appears to be identical with those reported by Braunitzer *et al.*[4] and by Hill and Konigsberg[5].

Schroeder *et al.*[8] reported that human hæmoglobin is composed of four polypeptide chains of two different kinds, referred to as α- and β-chains. In accordance with recommendations for the nomenclature of hæmoglobins[9] $\alpha_2{}^A\beta_2{}^A$ designates Hb-A and $\alpha_2{}^{16Asp}\beta_2{}^A$ could be used to define Hb-I.

I thank Dr. Robert J. Hill and Dr. William Konigsberg, of the Rockefeller Institute, who provided me with the α-chain of Hb-A; they also collaborated with me on the amino-acid analyses of the pentapeptide. I also thank T. Viswanatha and Mr. H. B. Marsh for assistance.

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Hæmolytic Action of Mercurials

IN studying the effect of heavy metal ions on staphylococcal α-lysin it was found that whereas mercuric chloride inhibited hæmolysis at a concentration of 10^{-3} M, it became a potent lytic agent at lower concentrations: it was decided to investigate this problem.

The lytic effect of mercuric chloride, certain organomercurials, and other heavy metal ions was examined on human, sheep, guinea pig and rabbit erythrocytes. The compounds were dissolved in isotonic phosphate or acetate buffer pH 6·8–7·0 and titrated in doubling dilutions in isotonic buffer of the same pH; 0·5 ml. of a 2 per cent suspension of four-times washed erythrocytes were added to each tube. The results were read at various time-intervals after incubation at 37° C; hæmolysis was assessed visually. All chemicals were of B.D.H. 'AnalaR' or 'laboratory' standard.

Using rabbit erythrocytes, lysis began after 30 min at a concentration of $6·8 \times 10^{-5}$ M mercuric chloride. At 1 h, lysis had spread to higher and lower concentrations; the upper limit was $2·5 \times 10^{-4}$ M, the lower $1·7 \times 10^{-5}$ M, with usually a trace of lysis at $8·4 \times 10^{-6}$ M. Above 5×10^{-4} M the erythrocytes became agglutinated. On prolonged incubation lysis spread to 5×10^{-4} M and 4×10^{-6} M.

The erythrocytes of other species were found to be less susceptible than those of the rabbit. After 1 h no hæmolysis was observed. At 2 h human erythrocytes were incompletely lysed, between 5×10^{-4} M and $6·8 \times 10^{-5}$ M of mercuric chloride and traces of hæmolysis were seen with sheep erythrocytes between the same concentrations: guinea pig erythrocytes were incompletely lysed at $2·5 \times 10^{-4}$ and $1·25 \times 10^{-5}$ M. Prolonged incubation for 4–5 h led to further lysis, that of human erythrocytes spreading to almost the same limits as of rabbit erythrocytes. Mercuric chloride thus exhibits selective lysis similar to some extent to that shown by staphylococcal toxins.

The lytic effect is shared by organomercurials: *p*-chloromercuri-benzoate, ethyl mercuric chloride and thiomersal. In contrast with mercuric chloride no upper limit of hæmolysis was observed at the concentrations tested. After 1-h incubation with rabbit erythrocytes *p*-chloromercuri-benzoate and ethyl mercuric chloride caused lysis down to a concentration of 10^{-3} M and thiomersal to a concentration of 4×10^{-3} M; at 2–3 h *p*-chloromercuri-benzoate caused lysis at $1{\cdot}25 \times 10^{-4}$ and thiomersal at 5×10^{-4} M. Those compounds also lysed human and sheep erythrocytes although again not as rapidly as rabbit erythrocytes. The order of susceptibility of erythrocytes was rabbit > human > sheep.

The ability to lyse seems to depend on the number of free positive mercury charges on the compound. Mercuric chloride, which has two free positive charges, is more potent than either *p*-chloromercuri-benzoate or ethyl mercuric chloride, which have one free positive charge. These in turn are more potent than thiomersal, which although it has no free positive charge in the native state is thought to split in the presence of tissue sulphydryl groups to reveal 1 free positive charge.

Other heavy metal ions, such as copper and lead, are also hæmolytic, but less potent than mercury. Barium ions were inactive even after 19 h of incubation; it would thus appear that hæmolytic activity is not a general property of heavy metal ions.

The conditions under which lysis occurs and the nature of the hæmolytic system suggests that the mechanism of lysis probably involves the formation of a chelate or co-ordination complex of the heavy metal ion with a reactive group on the surface of the red cell membrane causing instability and subsequent lysis.

I thank the Rankin Research Fund for a grant towards the expenses of this work.

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Effect of Methylene Blue on Red Cell Glutathione

An unusually high value for red cell reduced glutathione (GSH) was encountered while performing a glutathione stability test, using Beutler's[1] technique, on blood from a 2-year-old girl who developed methæmoglobinæmia and hæmolysis following the accidental ingestion of an unknown quantity of diaminodiphenylsulphone ('Dapsone'). Our range for red cell GSH in normal subjects is 78–104 mg per 100 ml. red cells (mg per cent): in the patient a value of 160 mg per cent was found. After incubation of the blood in the presence of acetylphenylhydrazine, the GSH-level was 119 mg per cent (normal range 60–94 mg per cent); red cell glucose-6-phosphate dehydrogenase activity[2] was normal.

It seemed unlikely that either the 'Dapsone' or the subsequent hæmolysis was responsible for this high value for GSH. It was demonstrated that the addition of 'Dapsone' to blood *in vitro* did not interfere with the estimation of GSH, and no difference was found in the GSH content of young and old red cells following their separation by centrifugation in bovine albumin as described by Prankerd[3]. Furthermore, normal red cell GSH-levels have been found in patients receiving 'Dapsone', even when prescribed in high dosage with resultant hæmolysis[4]. Our patient had received five intravenous injections, each of 20 mg methylene blue (1·5 mg/kg body-weight), during the 24 h preceding the withdrawal of blood for GSH estimation. We therefore investigated the effect of methylene blue on the level of red cell GSH. Two rabbits were used: the first received 10 mg (3·1 mg/kg body-weight) methylene blue, intravenously, twice daily for two days. The initial red cell GSH-level was 126 mg per cent: 18 h after the last injection this had risen to 190 mg per cent. The second rabbit received 10 mg (3·4 mg/kg body-weight) twice daily for four days. The resulting changes in the level of red cell GSH are shown in Fig. 1. It can be seen that the GSH rose from an initial level of 137 mg per cent to a maximum of 432 mg per cent; this peak was reached 18 h after the last injection of methylene blue. The red cell GSH afterwards fell, returning to its initial level 10–12 days after the last injection of methylene blue. The red cell glucose-6-phosphate dehydrogenase activity was 109 units initially, and 135 units when the GSH-level was at its peak.

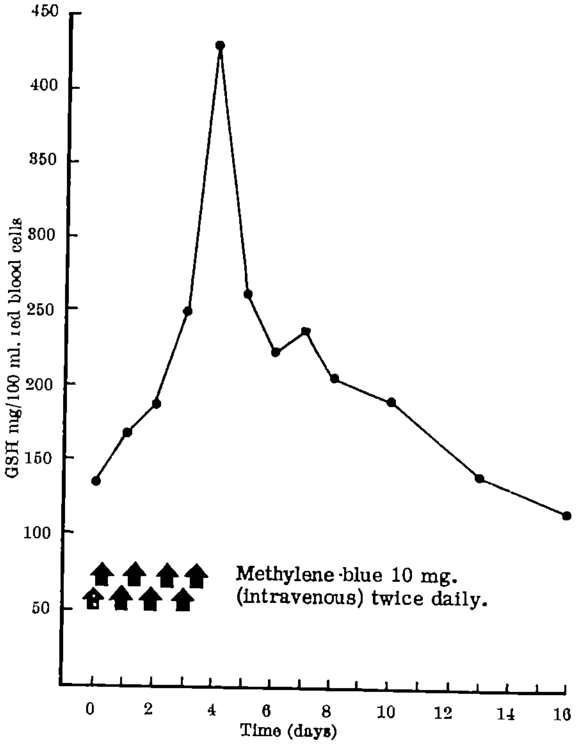

Fig. 1

It is generally accepted that oxidized glutathione (GSSG) in the red cell is reduced by the enzyme glutathione reductase, the presence of reduced triphosphopyridine nucleotide (TPNH) being essential for the reaction to proceed[5]:

$$\text{GSSG} + \text{TPNH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{TPN}$$

An adequate level of red-cell TPNH depends on the aerobic breakdown of glucose to carbon dioxide and water through the hexose monophosphate pathway. In normal circumstances, however, this pathway accounts for only a fraction of the glucose utilized by the red cell: little oxygen is consumed, and the main energy source appears to be derived from the anaerobic breakdown of glucose to lactate. When red cells are incubated *in vitro* in the presence of methylene blue, their oxygen consumption is greatly increased[6]: this indicates increased activity in the hexose monophosphate pathway, and as a result, increased TPNH production and consequent reduction of further GSSG to GSH might be expected to follow. Whether our observed high GSH-levels can be explained on this basis must depend on the amount of GSSG present in red cells and available for reduction. Very few observations have been made on the level of red-cell GSSG: however, Jocelyn[7] found that less than 6 per cent of the total glutathione in normal human red cells was present as GSSG. It therefore seems highly improbable that the substantial increases in red cell GSH observed in the present study can result from the reduction of pre-existing GSSG: indeed, it is almost certain that there is also a substantial increase in the total glutathione content of the red cells. We suggest, therefore, that in the presence of methylene blue there is an increase in the synthesis of glutathione in red blood cells: this may be a consequence of the increased activity in the hexose monophosphate pathway, or the result of the influence of methylene blue on another metabolic pathway in the red cell.

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Erythropoietic Stimulation in the Hypertransfused Guinea-Pig following Injections of Human Urinary Erythropoietin

CONSIDERABLE evidence has accumulated in recent years that supports the concept of a humoral component as part of the mechanism for control of erythropoiesis. The humoral agent has been termed erythropoietin or erythropoietic stimulating factor, and has been obtained from plasma and urine of various anæmic animals and from urine of patients with various hæmatological disorders. Erythropoietin does not appear to be species specific; thus, human, rabbit and sheep erythropoietin stimulate erythropoiesis in the mouse, rat and rabbit.

Reports of the effectiveness of erythropoietin obtained from various sources in stimulating erythropoiesis in the guinea pig have been variable. Gordon[1] reports that injections of urine from a child with thallassæmia major did stimulate erythropoiesis in the guinea pig, but that other urine samples which were quite erythropoietically active in rats failed to stimulate guinea pigs. Osmond[2] failed to observe erythropoietic stimulation in guinea pigs following the injection of plasma extracts from anæmic ponies. Osmond *et al.*[3], on the basis of quantitation of marrow cellularity, found that injections of human urinary erythropoietin into normal guinea pigs were not only not erythropoietic but actually granulopoietic. Van Dyke[4], using the iron-59 assay, found an absence of response in the guinea pig to human urinary erythropoietin. Keighley[5] is reported to have found that a filtrate of anæmic rabbit plasma, erythropoietically active in other species, was inactive in guinea pigs. In general, these workers conclude that failure of guinea pigs to respond to injections of extracts which are erythropoietically active in other rodents may be due to the presence in the extracts of antigens to which the guinea pig is more responsive than the other animals studied. Possibly these antigens indirectly influence the erythropoietic response by stimulating the cellular proliferation of other cell lines such as granulocytes, lymphocytes and plasma cells. This increased proliferation may compete with erythropoiesis for stem cells or essential nutrients. This competition, if it does occur, should be less in assay animals not undergoing active erythropoiesis prior to the administration of exogenous erythropoietin. Thus, in the present experiments the responsiveness of guinea pigs to human erythropoietin has been reinvestigated using hypertransfused guinea pigs.

Male guinea pigs of the Hartley strain initially weighing about 400 g were used. On each of the first two days of the experiment the animals were given intraperitoneally 10 ml. of a 60 per cent suspension of washed red cells obtained from donor guinea pigs. This was followed by 5 days of treatment with 2 mg daily of erythropoietin. The erythropoietin contained 16 cobalt units per mg dry weight. The daily dose was given in 0·5 ml. of saline, half the dose being given intraperitoneally and the other half subcutaneously. The control animals were injected in the same manner with saline. The erythropoietin was obtained from the urine of a patient with aplastic anæmia and was extracted by ultrafiltration as described elsewhere[6]. The day following the last injection of erythropoietin the guinea pigs were injected intraperitoneally with 0·5 μc. iron-59 in 0·5 ml. of saline. The animals were anæsthetized with ether 24 h later, and approximately 10 ml. of blood withdrawn from the aorta into a heparinized syringe. An aliquot of this blood was counted for radioactivity and, using a value of 5 per cent of the body-weight for the blood volume, the radioactivity in the total blood volume was calculated. This figure is given as a per cent of the iron-59 injected. Microhæmatocrits and reticulocyte counts were done on the same sample of blood. A total of 1,000 cells were counted in smears made of blood, previously incubated 20 min with 1 per cent brilliant cresyl blue in saline, for the reticulocyte determinations. The spleen was fixed in formalin, embedded in paraffin, sectioned, and stained with hæmatoxylin and eosin. Smears of the bone marrow were also examined.

The 24-h iron-59 incorporation and the reticulocyte response in the erythropoietin and saline injected hypertransfused guinea pigs is shown in Table 1.

Table 1. RESPONSE OF HYPERTRANSFUSED GUINEA PIGS TO HUMAN URINARY ERYTHROPOIETIN

	No. of animals	Weight (final) (g)	Hæmatocrit (per cent)	24-h ^{59}Fe red cell incorporation (per cent)	P*	Reticulocytes (per cent)
Control (saline)	10	472	60	2·1		0·01
Erythropoietin (2 mg/day for 5 days)	8	380	62	11·0	< 0·001	1·01

Guinea pigs transfused with a total of 11·54 ml. of packed red cells during the two days prior to treatment.

* t test.

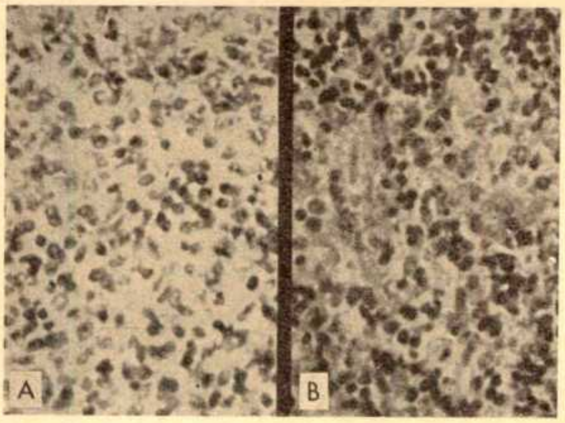

Fig. 1. *A*, Spleen of saline injected hypertransfused guinea pig; non-erythroid. *B*, Spleen of hypertransfused guinea pig injected with human urinary erythropoietin; active erythropoiesis

We observed, as did Osmond *et al.*[3], that a loss of weight occurred in the guinea pigs receiving erythropoietin. The estimated blood volume is obviously low because of the hypertransfusion and weight loss in the erythropoietin injected animals, so that the estimated uptake of iron-59 in the calculated blood volume indicates only qualitatively the nature of the erythropoietic response. These results and a comparison of the cytological changes in the marrow and the histological changes in the spleen (Fig. 1) indicate that injection of human urinary erythropoietin can specifically stimulate erythropoiesis in the hypertransfused guinea pig, that is, the marrow or spleen devoid of erythropoietic foci shows marked erythropoietic activity after erythropoietin injections.

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Clotting of Fibrinogen with Aqueous Fibrin Extract

IN a previous communication[1], a clotting factor was reported to occur in aqueous fibrin extract. Chemically, it appeared to be a steroid glucuronide. It was differentiated from thrombin by greater heat stability, inactivation by oxalate and β-glucuronidase, by producing urea-insoluble fibrin clots in oxalated plasma without calcium chloride and reacting with *C* reactive protein antibody.

For a more thorough examination of certain properties of the factor, particularly its relationship to thrombin and fibrinogen, it was desirable to obtain it in higher concentration. To this end, it became necessary to let the clots decompose spontaneously. This took about 2–3 months at 5° C. In 1 per cent solutions of sodium chloride and other salts, clots broke down much more rapidly, but much of the active material remained then adsorbed to the protein residue. The aqueous fibrin extract was heated at 56° C for 30 min, a procedure which invariably destroys thrombin. Heating also precipitated out of solution certain breakdown products of fibrin. The supernatant was dialysed against distilled water to free it of any traces of calcium chloride. The dialysed solution reacted with bovine fibrinogen, 0·1 ml. producing a clot with 0·1 ml. of a 0·3 per cent solution of the substrate in 20–30 sec. Addition of 0·03 ml. of 0·1 M sodium oxalate produced a precipitate and delayed clot formation corresponding to a loss of 70–80 per cent of the original activity, but no more, regardless of length of time of exposure. The clots differed from thrombin clots in their tendency to retract readily.

Lorand[2] postulated that a heat-labile fibrin stabilizing factor present in serum and calcium ions was required to render fibrin insoluble in 30 per cent urea, but did not present data as to the relative stability of different clots in this solvent. In the present work it was found that clots made with clotting factor and fibrinogen dissolved in urea in about 45 min; with oxalated clotting factor the time was reduced to 5 min; on the other hand, when 0·03 ml. of 0·1 M calcium chloride was added to the two reactants, clots remained stable in their entirety for 2 h and parts of clots up to 8 h. The addition of serum, whether unheated or heated at 56° C for 30 min, and calcium chloride to clotting factor and fibrinogen, kept clots quite stable for at least 24 h. It would seem then that clotting factor functions in aggregating fibrinogen molecules, that calcium ions strengthen certain internal chemical bonds—merely adding calcium chloride to fibrinogen produces increased turbidity—and that serum proteins somehow give greater cohesion to the clot.

The action of clotting factor on fibrinogen is in conformity with observations by Godal[3,4] on the binding of heparin to fibrinogen in a stoichiometric relationship. In both cases, attachment to the protein may be by means of the glucuronic acid moieties of the molecules. Clotting factor has been shown[1] to counteract directly the anticoagulant effect of heparin. This suggests a competitive relationship between the two compounds for fibrinogen. The same is bound to exist between clotting factor and lytic factor, the 17-ketogenic steroid glucuronide obtained from extracts of digested fibrin[1]. It has been postulated that in the physiological process of blood coagulation, lytic factor is converted to clotting factor by the enzymes of the thromboplastin complex. So far there has been no clue to the chemical configuration of the steroid part of the clotting factor molecule. In view of its association with calcium, a relationship to vitamin D may be worth consideration.

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HISTOCHEMISTRY

Histochemical Demonstration of Non-specific Esterase in Mast Cells of the Albino Rat with Properties Similar to Mast Cell Enzyme activated in Anaphylaxis

RECENT investigations suggest that release of histamine from isolated mast cells of the albino rat by an antigen–antibody reaction is due to an enzymatic process[1,2]. Further experiments with inhibitors which are more selective reveal that certain specific chymotrypsin substrates or inhibitors prevent the reaction, whereas substrates and inhibitors of carboxypeptidase, leucine-aminopeptidase or trypsin do not[3–5]. Toxic organic phosphorus compounds such as diisopropylfluorophosphate (DFP) or 'Paraoxon' also inhibit in relatively high concentration[6]. By virtue of these findings we have concluded that the enzyme responsible for the antigen–antibody reaction of isolated tissue mast cells of the albino rat is a chymotrypsin-like peptidase having pronounced esterolytic properties[5].

By means of histochemical techniques a number of enzymes can be detected in mast cells of the albino rat; in particular, one finds strong activities of leucine-aminopeptidase and adenosine-triphosphatase[7]. Benditt and Arase[8] have also demonstrated a chymotrypsin-like enzyme using naphthol-AS-chloroacetate as substrate.

I have endeavoured to discover which of the histochemically demonstrable enzymes displays properties similar to those of the enzyme responsible for the anaphylactic release of histamine. Normal isolated mast cells of the rat[9] were spread on slides and fixed by formalin vapour for 20 min. Following Pearse[10] and Löffler's[11] methods, the following histochemical tests were carried out on fixed smears: leucine-aminopeptidase according to Nachlas, Crawford and Seligman; acid phosphatase according to Gomori and Pearse; adenosine-triphosphatase according to Wachstein and Meisel; non-specific esterase according to Pearse (α-naphthyl-acetate), to Gomori (naphthol-AS-acetate), to Moloney *et al.* (naphthol-AS-D-chloroacetate) and to Holt (5-bromo-indoxyl-acetate). Comparative control tests were performed after heating slides to 90° C and with incubation mixtures lacking substrate. Several preparations were always examined simultaneously. The experiments with the inhibitors were performed according to the methods of Pearse[10], and Hess and Pearse[12].

The results are set out in Table 1. This shows that the mast cells of the albino rat, in accordance with the findings of Eder and Schauer[7], display pronounced activity of leucine-aminopeptidase, acid phosphatase and adenosine-triphosphatase. Histochemical detection of these enzymes is not influenced by eserine, DFP or 'Paraoxon' (2×10^{-6} and 10^{-3} M). The detection of non-specific esterase is unsuccessful or unsatisfactory with the substrates α-naphthylacetate and naphthol-AS-D-chloroacetate. On the other hand, the use of naphthol-AS-acetate or 5-bromoindoxylacetate results in an intensive staining which is clearly localized in the granules of the mast cells. This activity is not influenced by eserine (2×10^{-6} M), DFP (10^{-6} M) or 'Paraoxon' (10^{-6} M); however, incubation with higher concentrations of these substances (10^{-3} M) results in virtually complete loss of enzymatic activity. In addition, copper sulphate (10^{-3} M), lead nitrate (10^{-3} M), silver nitrate (10^{-2} M), and β-phenylpropionic acid (10^{-2} M) are also inhibitory. Histochemically the enzyme thus appears to be a non-specific organophosphate-resistant esterase. In so far as comparative experiments are available, its properties are identical with those of the enzyme responsible for the anaphylactic release of histamine.

Table 1. EFFECT OF VARIOUS INHIBITORS ON HISTOCHEMICALLY DEMONSTRABLE ENZYMES IN MAST CELLS OF THE ALBINO RAT

	Untreated normal mast cells	Mast cells pre-incubated with: Eserine (2×10^{-6} M)	DFP or 'Paraoxon' (10^{-3} M)		
			(10^{-3} M)	(10^{-4} M)	(10^{-6} M)
Leucine-amino-peptidase	+ + +	+ + +	+ + +	+ + +	+ + +
Acid phosphatase (Gomori)	+ + +	+ + +	+ + +	+ + +	+ + +
ATPase	+ + +	+ + +	+ + +	+ + +	+ + +
Esterase					
Naphthol-AS-acetate	+ + +	+ + +	–	(+)	+ +
5-Bromo-indoxylacetate	+ + +	+ +	–	(+)	+ +

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PATHOLOGY

Established Bone Marrow Cell Cultures with High Degree of Iron Incorporation

THE literature contains numerous reports of cell strains derived from normal or pathological human bone marrow or from elements of peripheral blood[1]. In the great majority, cell lines of such derivation are known to transform into fibroblastic or epithelial forms after a certain period of time[2]. Their experimental application is extensive, but almost exclusively confined to the field of virology[3].

Theoretically, it is possible to develop a permanent population of cells which would retain the basic properties of the primitive elements of normal bone marrow. With this possibility in mind, attempts were made to culture under varied conditions human bone marrow derived aseptically from surgically resected ribs. Of 19 such cultures, 17 were discarded, because of degeneration or fibroblastic transformation. The two remaining cultures derived from two young persons operated on for diaphragmatic hernias showed signs of stability.

The cells (*RI*) in these two lines were originally grown in T-flasks (60 ml.) with 25 ml. of culture medium. The culture fluid used consisted of Eagle's minimum essential medium, inactivated horse serum 10 per cent, glutamine 1 per cent, and antibiotics; it was supplemented with 0·5 ml. of folinic acid ('Leucovorin' Lederle, 3 mg/ml.) and 1 ml. of vitamin B_{12} ('Rubramin' Squibb, 3 μg/ml.). Since after 25 transfers the folinic acid and vitamin B_{12} were tested in

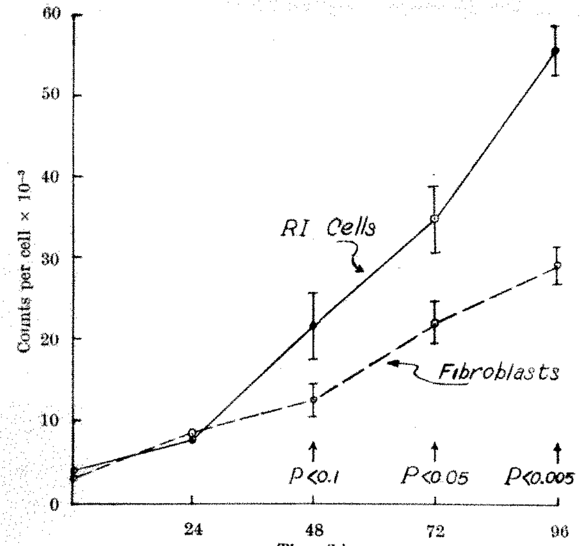

Fig. 1. Incorporation of iron-59 by *RI* cells and fibroblasts. Average of three experiments ± 1 *S.E.*

duplicate experiments, and found unnecessary, they were henceforth omitted.

Both these lines of cells grew at the same rate with a generation time of 22–24 h. The cells were best adapted in monolayer cultures. Attempts to grow them in spinner flasks gave erratic results. Cells frozen slowly kept for a period of 7 weeks at −70° C were successfully recovered. Under the phase-contrast microscope the cells appeared as round or polyhedrical elements with a large nucleus and granular, non-vacuolated cytoplasm. Numerous distinct nucleoli were present. In preparations treated with Wright's stain, the morphological characteristics were reminiscent of immature reticulum cells.

In order to study possible residual functional activity pertaining to normal bone marrow, the iron uptake of the *RI* cells was tested in short-term cultures. The cells were suspended in the described medium at optimum concentration of 7·5–10 × 10^4/ml. and incubated in a water bath shaker under a gas mixture of 5 per cent oxygen, 5 per cent carbon dioxide and 90 per cent nitrogen flowing at 2–3 lb./min. An atmosphere containing 13 per cent oxygen, 5 per cent carbon dioxide and 72 per cent nitrogen was found to be less effective for cellular growth. The *p*H was stable throughout the period of culture in a range of 7·2–7·3. Iron-59 in a citrate salt (sp. act. 20–25 mc./mg) was added in amounts of 1 microcurie per culture flask. At the end of the culture time, the radioactivity of the cells, after three washes, was counted in a well-type scintillation counter. All the cultures were run in triplicate samples. Fibroblasts from an established line were used as controls. In three experiments, the results calculated in counts/cell show (Fig. 1) that both cell lines had similar iron uptake at 24 h and were not significantly different after 48 h of culture. At 72 and 96 h, however, the *RI* cells showed significantly higher incorporation of iron. These findings were readily reproducible.

It is of interest that the *RI* cells were derived from normal bone marrow and after 85 passages had not transformed into the usual fibroblast form. The reticulum cell-like morphology was reminiscent of the stage III transformation of the Detroit line described by Berman[2], which was also derived from bone marrow. The factors arresting the usual fibroblastic dedifferentiation and stabilizing the *RI* cells are not known. It is possible that the initial addition of folinic acid and vitamin B_{12} played a decisive part. It is also possible that additional unknown factors, pertaining to either the bone marrow itself (age of the donor, state of the bone marrow at the time of implantation, etc.) or to the environmental characteristics of the culture, were equally responsible for the successful establishment of the *RI* cells.

A second point of significance is the high iron uptake of the *RI* cells. Such a quality of a cell line derived from normal human bone marrow has not been reported. At present, attempts are being made to enhance the rate of iron incorporation through hæmopoietic substances (erythropoietin) and to establish the fate of the incorporated iron. If the high rate of iron incorporation implies a retention of normal bone marrow function, the *RI* cells can prove a useful tool for future hæmatological research.

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Distribution of Tritiated Thymidine about a Capillary Sinusoid in a Transplanted Mouse Tumour

DURING an investigation of the uptake of tritiated thymidine by mouse tumour tissue, fortuitous sections of tumour were found showing a large capillary sinusoid cut along its long axis for a distance of 2,000μ. Autoradiographs of these sections showed concentric zones of labelled cells, unlabelled cells morphologically intact, and necrotic tumour cells, at increasing distances from the capillary.

The tumour was a mammary adenocarcinoma which occurred spontaneously in our *C3H* mouse colony. It was designated as mammary adenocarcinoma *M2*, and has been maintained through multiple passages. It was grown subcutaneously to a diameter of approximately 0·6 cm. One hour after a tumour dose of 400 r. (250 kV constant potential, 15 m.amp, 2 mm aluminium filter added), tritiated thymidine (specific activity 0·36 c./mM) was injected intraperitoneally in the amount of 5 mc./g. The animal was killed 45 min after the injection. The tissue was cut into 5-μ sections, hydrolysed in N hydrochloric acid, and stained with Feulgen reagent. Autoradiographs were prepared by dipping in Kodak *NTB2* emulsion. They were developed after an exposure of ten days.

Figs. 1 and 2 are photographs of the autoradiographs of one of these tumour sections. The labelled cells extend on an average of 30–45μ from the capillary wall. Within this volume, cells closest to the capillary show a higher percentage of labelled cells and more label per cell. Surrounding the shell of labelled cells, a second volume is distinguishable containing unlabelled cells which show intact morphology and staining. These unlabelled intact cells lie between 45–70μ from the capillary. Beyond

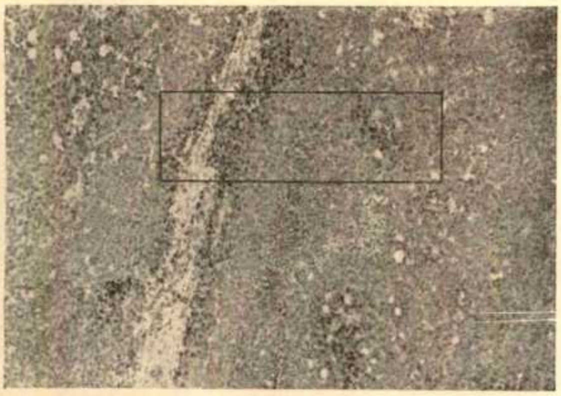

Fig. 1. Autoradiograph of mouse tumour section showing a portion of the long capillary sinusoid (just to the left of centre) surrounded by heavily labelled cells. Other capillaries cut on cross-section show the same distribution

about 70μ from the capillary are masses of necrotic cells. At greater distances from the large sinusoid and beyond the area of necrosis, other zones of morphologically intact cells without label are present. Still further, labelled cells are seen immediately surrounding other capillaries. This distribution is repeated throughout the tumour.

This observed distribution of labelled cells appears to represent a pattern of diffusion. It may be simply the diffusion of the thymidine in the 45 min following the single injection. However, Mendelsohn[1] was unable to label all morphologically intact tumour cells after continuous infusions of tritiated thymidine in tumour-bearing animals. These infusions lasted for periods up to 8 days, and autoradiographs were exposed for as long as 200 days. Cells capable of synthesizing DNA are dependent on an adequate supply of metabolites and oxygen. Therefore, the distribution in our sections may represent limited diffusion of oxygen and other metabolites. The examination of the sections themselves showing the progression from morphologically intact cells to necrosis as one proceeds peripherally from the blood vessel supports this thesis. It fits very well the diagrammatic map of a solid experimental tumour drawn by Thomlinson[2]. He attributed this progression to the drop in pO_2 as oxygen diffused through the tissue. Indeed, Johnson and Bond[3] doubled the depth of *in vitro* labelling of tumour tissue with tritiated thymidine by bubbling 95 per cent oxygen and 5 per cent carbon dioxide through the media.

We believe, with Mendelsohn, that this observed distribution of tritiated thymidine in our experimental tumour demonstrates some of the potential problems of cancer chemotherapy, and in particular the use of analogues of DNA precursors to increase radio-sensitivity of all the cells in a tumour. Also, the uneven distribution points to another pitfall in the evaluation of data obtained by autoradiography.

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Leukæmia in the Progeny of Rats inoculated with a Leukæmogenic Virus

VERTICAL transmission of virogenic leukæmia, that is, the occurrence of leukæmia in the untreated progeny of animals inoculated with a leukæmogenic virus, has been observed with four different viruses of murine origin[1-5]. The phenomenon is not a uniform one. For example, Moloney's virus is transmitted in some strains of mice with little, if any, change of incidence through successive generations; in other strains, equally susceptible to inoculated virus, it is not transmitted at all[4,6]. Gross's virus in one strain passes with undiminished incidence to the first generation, but incidence falls off rapidly in subsequent generations; in other susceptible strains it is not transmitted[7-9].

Because of the potential value of vertical transmission as an aid in the interpretation of spontaneous leukæmia in man, it is important to know whether it occurs in genera other than the mouse. All the murine viruses referred to produce leukæmia when injected into rats as well as mice; but vertical transmission in rats has not hitherto been reported. Recently an unmistakable example of vertical transmission of leukæmia in rats occurred in this laboratory. The virus investigated was Moloney's, originally derived from mouse sarcoma 37 (ref. 10). A lyophilized sample obtained from Dr. Moloney was

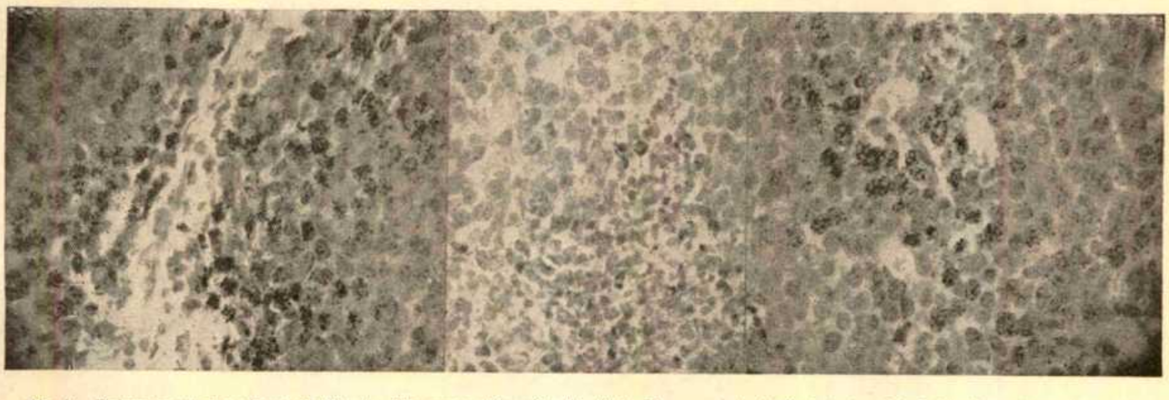

Fig. 2. Enlargement of enclosure in Fig. 1. The zones of heavily labelled cells, morphologically intact unlabelled cells, and necrotic cells can be seen at increasing distances from the capillaries on the right and left extremes of the illustration

passed twice in mouse embryo tissue cultures, and once in mice[11]. A cell-free filtrate of lymphomatous tissue homogenate from a leukæmic mouse, inoculated in infancy with infected tissue culture fluid, was injected into new-born rats of the Chester Beatty Institute albino strain (*CB*). Of 11 rats which survived for 2 months or more, 10 became leukæmic between the 11th and 20th week. A female of this group was mated when 13 weeks old to an untreated male of the same strain: she had a litter of 10, and died of leukæmia 5 weeks later. Of this litter 8 became leukæmic between 14 and 29 weeks of age. The two survivors appear healthy at 34 weeks.

The incidence of spontaneous leukæmia in the *CB* strain is very low—certainly less than 1 per cent up to 15 months of age.

The pathological changes in mice, following inoculation with Moloney's virus, are those of a generalized malignant lymphoma[12]. In rats, and in the uninoculated progeny of both genera, the changes are similar. Among the leukæmic rats of the present series, in 3 of the inoculated and in 4 of the progeny gross enlargement of the thymus (25–35 mm diameter) was the sole macroscopic abnormality. In the rest there was also enlargement of the spleen (40–60 mm long) and sometimes also of superficial and deep lymphatic nodes. Microscopically the affected organs were seen to be replaced by a dense infiltration of stem cell type, showing very numerous mitotic figures. In several, liver and kidneys were infiltrated by similar cells.

Vertical transmission of Moloney leukæmia in mice is through the maternal, not the paternal, line, and predominantly via the milk[4,6]. It is noteworthy that in the present case transmission was from the inoculated mother. Reciprocal fostering, and other tests, are in progress, with the object of determining the route of transmission in rats.

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RADIOBIOLOGY

Estimation of the Total-Body Load of Cæsium-137 in Japanese People

ANALYSIS of post-mortem samples for cæsium-137 has been carried on since 1958 at this Institute. Samples were selected because a whole-body counter was not available in Japan at the start, and now such counters are limited in number and are not mobile. Breast muscles from autopsies are used for analysis and the activity of cæsium-137 is measured by a low-background β-counting technique[1] after radiochemical separation[2].

Table 1. DISTRIBUTION OF COMMON CÆSIUM IN HUMAN BODY

	Cs (mμgm/gm fresh)	Cs/K, ($\times 10^4$)
Muscle	29·5	1·32
Soft tissues	12·7	0·98
Bone (with marrow)	15·7	0·91
Total body	20	1·0

For the estimation of the total-body load, the relation between the concentration of cæsium-137 in muscle and the total-body amount must be known. It has been accepted in general that the cæsium unit (μμc. of cæsium-137/g potassium) in muscle multiplied by the total-body potassium makes the total-body load of cæsium-137 in μμc. This is not likely to be true since examination of the distribution of the stable cæsium in the human body[3] revealed the different ratios of cæsium and potassium in different tissues (Table 1). It suggests the necessary introduction of a correction factor of 0·76 in the foregoing calculation, so far as the distribution of cæsium-137 is assumed to be similar to that of the stable element. The results in Table 1 also afford another means of estimation if the body-weight of 56 kg is assumed as the average for Japanese adults; concentration of cæsium-137 in μμc./g fresh muscle multiplied by 38 gives the total-body load in mμc.

Either method of estimation can be used; however, the latter is preferable since the errors involved in the former are likely to be larger. In Table 2 are shown the estimates of the total-body load of cæsium-137 from autopsies obtained in Tokyo. The details for samples April–June 1960 are given in a previous report[4]. Measurement by a whole-body counter was made for the first time in Japan in April 1961[5] at the Japan Atomic Energy Research Institute, Tōkai, Ibaragi Pref., and the results were in good agreement with the values estimated from muscle analysis as presented in Table 2 in spite of the differences in subject and method.

Evaluation of mean levels of cæsium-137 in the human body can also be made indirectly by analysis of the diet. As previously reported[6], the factor between the total-body amount and the daily intake of the stable cæsium is 110; then the daily intake of cæsium-137 (μμc.) multiplied by 0·11 will give the total-body load (mμc.). The results of the analysis of actually consumed total diet samples collected from several sites in Japan in November 1961 showed the values of daily intakes ranging from 19 to 45 μμc. for urban adults (average, 29 μμc.) and from 28 to 52 μμc. for rural adults (average, 40 μμc.). The estimated values for the total-body load, 3·2 and 4·4 mμc., respectively, for urban and rural adults are in good agreement with the value of 3·8 mμc. in January 1962 which was obtained from muscle analysis.

In Table 2, a trend of increase in the total-body load can be observed towards February and March.

Table 2. ESTIMATES OF THE TOTAL-BODY BURDEN OF CÆSIUM-137 IN JAPANESE PEOPLE

Time		^{137}Cs in muscle (μμc./g fresh)	Total-body burden (mμc.) Estimated from muscle analysis	Estimated from diet analysis Urban	Rural
Apr.–June 1960	(27)	0·13	4·9		
Apr. 1961	(11)		4·1*		
Nov. 1961				3·2	4·4
Dec. 1961	(26)	0·10	3·8		
Jan. 1962	(20)	0·099	3·8		
Feb. 1962	(19)	0·145	5·5	5·6	5·3
Mar. 1962	(9)	0·20	7·6		
Apr. 1962	(25)	0·124	4·7		

* Measurements with a whole-body counter (ref. 5).
The figures in parentheses represent the number of samples.

This trend was also found in the daily intakes. The diet samples collected in February, 1962, showed considerable increases in the daily intakes, the values being 34–82 μμc. (average 51 μμc.) for urban adults and 40–64 μμc. (average 48 μμc.) for rural adults. This time-trend probably reflects the increase in fall-out cæsium-137 originating from the series of nuclear explosions by the U.S.S.R. in the autumn of 1961.

Examination of the distribution of the stable cæsium in the human body and diet provided a simple means of estimation of the total-body load of cæsium-137 irrespective of potassium concentration or the cæsium unit under equilibrium or near equilibrium conditions. The expression of the concentration of cæsium-137 in terms of potassium may lead to confusion because since this has been introduced another question on the discrimination between cæsium-137 and potassium arises in the various phases of biological systems.

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Effect of Exposure to Ionizing Radiation on Creatine Concentration in Human and Rat Erythrocytes

ALTHOUGH several reports concerning changes in the concentration of creatine in plasma, serum, and whole blood under various pathological conditions[1] as well as after exposure to ionizing radiation[2] have been published, to our knowledge no published results with regard to changes in the creatine concentration in erythrocytes are available.

Six groups of rats of the Sprague–Dawley strain were used; each group consisted of 4 rats and each rat weighed 300–350 g. Each rat was given a single pelvic irradiation of a midline dose of 0 (sham-groups 1 and 2), 500 (group 3), 1,000 (group 4) or 1,500 r. (groups 5 and 6), through an anterior and posterior arrangements of ports measuring 4 × 4 cm. The radiation was delivered from an X-ray tube operated at 220 kV and 20 m.amp without filtration. The midline dose-rate through each port in a rat phantom of tissue equivalent substance, 3·5 cm thick, was 102 r./min. During the entire experiment the rats were kept in individual metabolism cages and on 'Purina' animal diet and water *ad libitum*. Approximately 0·3–0·4 ml. of blood was obtained at various times by transection of the tail (Fig. 1) and collected in heparinized pipettes. After separation of the blood sample into plasma and packed cell fractions, the cells were washed twice with 2·5 ml. of saline. Creatine was determined in 0·05 ml. of plasma or packed cells by the 1-naphthol-diacetyl procedure[3] after the precipitation of protein with barium hydroxide and zinc sulphate[4]. Urinary creatine and creatinine were determined by the method used by Gerber[5], but with slight modifications, namely, the *p*H of the citrate buffer was 3·9 ± 0·1 instead of 3·3 ± 0·1 and 50 mg of 'Norite' charcoal was used instead of 200 mg.

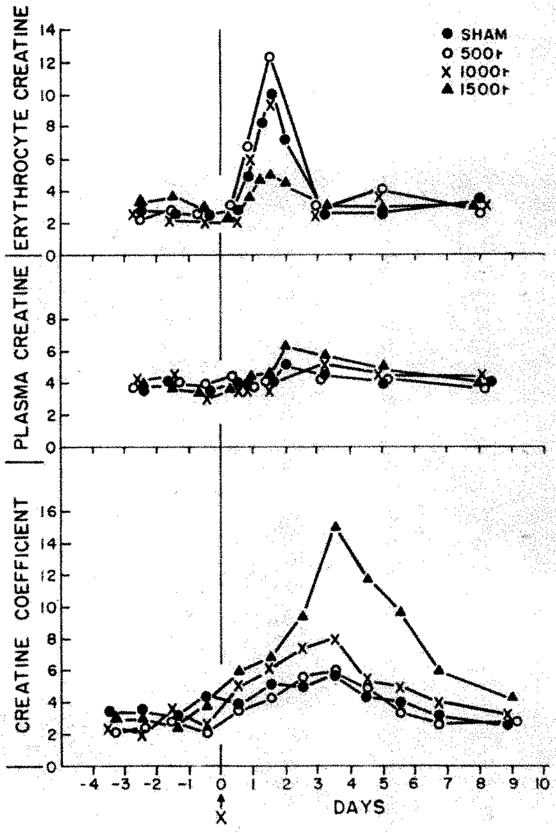

Fig. 1

In order to show that changes in erythrocytic creatine concentration also occur in irradiated human beings, serial determinations of creatine in red blood cells were made on blood samples obtained from patients before, during, and after their course of radiotherapy for various malignancies (Table 1).

Fig. 1 shows plots of erythrocyte creatine concentration, plasma creatine concentration, and urinary creatine coefficient (the ratio of the urinary creatine to creatinine concentrations) as a function of time at different doses of radiation. The means of the maximal values of the concentration of creatine in erythrocytes of the sham-irradiated group is significantly higher than that of the 1,500-r. irradiated group, whereas comparable values for the urinary creatine

Table 1. CREATINE CONCENTRATION IN ERYTHROCYTES OF PATIENTS GIVEN RADIATION THERAPY

Interval after initial irradiation (days)	Creatine concentration in mg/100 ml. of packed cells Patient No. 1	Patient No. 2	Patient No. 3
1–10	5·0	—	5·6
11–15	—	10·1*	11·2*
16–20	5·4	8·5*	6·1
21–25	7·3	8·1*	6·8
26–30	6·6	10·0*	11·0*
31–35	10·4*	*9·2**	7·3
36–40	*13·9**	—	*8·8**
41–45	19·1*	11·1*	8·4*
46–50	6·8	—	6·5
90–100	6·0	6·0	5·5

Patients No. 1 and 2 were given cobalt-60 rotational pelvic therapy with daily tumour doses of 200 r. for bladder and rectal cancers, respectively; patient No. 3 was given 2 MeV fixed beam thoracic therapy with 30 daily tumour doses of 150 r. for Hodgkin's disease.

3–4 blood samples were obtained on successive days prior to initiation of therapy from each of these patients. The mean ± standard deviation of these base-line values were: 6·32 ± 0·34 (patient No. 1), 5·80 ± 0·20 (patient No. 2) and 6·08 ± 0·38 (patient No. 3).

* Significantly elevated values at the 5 per cent confidence-level.

Figures in italics represent the value of the last sample obtained during the period of therapy.

coefficient are higher in the group irradiated with 1,500 r. than in the sham-irradiated group. The means of the maximal value of the plasma creatine concentration in the sham-irradiated and X-irradiated rats do not differ significantly at the 5 per cent level.

The results of serial determinations of creatine concentration in the erythrocytes of three patients given radiation treatments for various forms of malignancies are shown in Table 1.

These observations indicate that under the conditions of radiation therapy used here, the concentrations of creatine in erythrocytes rises after 2–3 weeks of therapy.

In the animal experiments the erythrocytic creatine concentrations rise only in the sham-irradiated rats and in rats exposed to 500 and 1,000 r., whereas in rats exposed to 1,500 r. a marked fall in the erythrocytic concentration of creatine occurred in comparison with the sham-irradiated rats. The response observed in sham-irradiated rats is probably due to the restraint imposed on the rats during sham-irradiation.

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Biosynthesis of Creatine by the Kidney

THE chemical nature of the precursors contributing to the biosynthesis of creatine has been established as the result of *in vivo* experiments by Bloch *et al.*[1,2] and DuVigneaud *et al.*[3] as well as by the experiments with tissue slices and homogenates by Borsook and Dubnoff[4–6]. The last-named investigators also demonstrated that, in the mammal, the kidney is the major site of glycocyamine formation[4,5]; a slight transmethylating activity was also detected at this site. Baker and Miller[7] also reported that the kidney is able to synthesize creatine although at a level exceeding that noted by Borsook and Dubnoff. It appears to be the present prevailing point of view that the kidney does not contribute significantly to the formation of creatine from glycocyamine[8]. Evidence to the contrary, derived from experiments with perfused rat kidneys and eviscerated rats, is presented in this communication.

Rat kidneys were perfused according to a modification of the procedure devised by Weiss *et al.*[9], except that the vena cava and aorta rather than the renal artery and vein were cannulated. Eviscerated rats were prepared according to Ingle's technique[10]. L-Arginine-guanido-^{14}C and glycine-2-^{14}C were used as metabolic precursors of creatine. Creatine and glycocyamine were isolated from blood and kidneys by deproteinizing with picric acid and subsequently separating arginine, creatine and glycocyamine by paper chromatography. Arginine was separated from glycocyamine and creatine in a solvent system containing butyl acetate/acetic acid/ethanol/water, 3 : 2 : 1 : 1 (by volume)[11], and the arginine spot was cut off and eluted after development of the chromatogram. The remainder of the paper strip containing the composite spot of creatine and glycocyamine was then rechromatographed in a solvent system composed of phenol/water/acetic acid (plus a few crystals of versene), 80 : 20 : 0·6 (by volume). The creatine and glycocyamine spots were excised and eluted. The specific carbon-14 activities of the 3 metabolites thus separated were then determined. The chemical concentration of arginine and glycocyamine was determined by the method of Van Pilsum *et al.*[12] and that of creatine by a previously published method[13]. The isotope distribution in creatine and glycocyamine was ascertained as follows. (*a*) Glycocyamine was degraded to glycine, carbon dioxide and ammonia by an adaptation of the method described by Miller and Bale[14] for the degradation of the guanido group of arginine. Glycine was isolated from the reaction mixture by paper chromatography and eluted from the developed paper strip for the determination of the specific activity of carbon-14. The chemical concentration of glycine was determined by means of the ninhydrin reaction. The guanido carbon atom (carbon dioxide) was isolated from the reaction mixture as barium carbonate. (*b*) Creatine was degraded to oxalic acid and methyl guanidine by a method published elsewhere[15].

The data shown in Table 1 indicate that the activity of carbon-14 derived from the methylene carbon atom of glycine and the guanido carbon atom of arginine is incorporated by the kidney into creatine in both types of experimental preparations. The ratio of the specific activity of the guanido carbon atom to that of the methylene carbon atom is nearly identical for glycocyamine and creatine, thus lending further support to the view that the activity of carbon-14 found in creatine is the result of *de novo* synthesis. In all the experiments shown in Table 1 the specific activity of glycocyamine is several times that of creatine. This difference in specific activities, at least in the case of the kidneys, is probably largely attributable to the considerably greater size of the creatine pool (6·0 ± 3·3) as compared with the size of

Table 1. INCORPORATION OF CARBON-14 INTO CREATINE AND GLYCOCYAMINE

Type of experimental preparation	^{14}C-labelled amino-acid used	Source of creatine and glycocyamine	Creatine: Creatine	Creatine: Glycine moiety	Creatine: Guanido carbon atom	Glycocyamine: Glycocyamine	Glycocyamine: Glycine moiety	Glycocyamine: Guanido carbon atom	Arginine
Eviscerated rat	L-Arginine-guanido-^{14}C (5 μc.)*	Kidney	2,470			9,370			
		Blood	65			8,600			5,520
Perfused kidneys	L-Arginine-guanido-^{14}C (3 μc.)	Kidney	2,400			9,600			5,127
	Glycine-2-^{14}C (15 μc.)†	Blood	3,100	13	57	1,130	59	240	
	L-Arginine-guanido-^{14}C (6 μc.)‡	Blood	870	< 4	700	4,100			2,200

All values shown represent specific activities of carbon-14 expressed as c.p.m./μmole.
* This experiment was terminated 105 min. after the administration of carbon-14-labelled arginine.
† This experiment was terminated 75 min after the administration of carbon-14-labelled amino-acids.
‡ This experiment was terminated 95 min. after the administration of carbon-14-labelled arginine.

the glycocyamine pool (0·75 ± 0·24). (The values for the pool sizes are given as µmole/g net weight of tissue.) Different rates of isotope incorporation may also contribute, although to a much smaller extent, to the difference in specific activities. In comparing the two types of experimental preparations, it is noteworthy that the ratios of the specific activity of creatine to that of glycocyamine are of a similar order of magnitude.

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BIOLOGY

Total Mammectomy in Female Mice and Rats

THE total removal of the mammary glands in small laboratory animals has repeatedly been tried, however without much success[1–3]. This communication describes a method for the complete ablation of all mammary glands in female mice and rats.

Female mice, 19–23 days old of the inbred strains $C_{57}BL$, WLL, O_{20}, DBA_f and the hybrids (♀ O_{20} × ♂ DBA_f)F_1 were used. The rats used were 18–20 days old F_1-hybrids between the inbred strains *U* and *R*, Amsterdam.

Bromethol in saline was used for anæsthesia with the addition of ether if necessary. The anæsthetized animals were fixed, ventral side up, on a cork board and the skin was drenched with 70 per cent alcohol. The incision was made along the ventral midline, from just above the clitoris to the root of the neck. Two lateral incisions were then made. The first was about 1 cm long from the upper end of the median incision and almost perpendicular to it, well above the first nipple, towards the right forelimb. The second started with the prolongation of the lower end of the median incision, around the vaginal and anal openings and as close to them as possible, to the base of the tail; from there laterally along the dorsomedial aspect of the thigh to about the midpoint of the hind leg. One at a time the resulting skin flaps, with all mammary glands attached, were carefully separated from the body-wall by blunt dissection. In removing the skin in the inguinal region, special attention has to be paid to the sexual fat pad. The dissection of this fat tissue from the underlying muscle layer has to be done very carefully, without damaging the very thin muscle layer, otherwise a hernia will develop. Another point which deserves careful attention is the fat tissue in the anal region. Incomplete removal of these parts of the fatty deposits may be the main source for unsuccessful operations. Finally, the fat tissue attached along the dorsomedial line of the thigh was cut off and the skin flap freed completely. This flap was then pinned to the cork board, exposing area of the nipples in the inguinal region as well as the larger part of the fat pad. The method of DeOme *et al.*[4] for clearing the fat pad of the fourth mammary gland was now used with slight modifications. The incision through the fat pad was made ventrally to the lymph node. No cauterization of the blood vessel was done; a slight pressure before cutting proved to be sufficient to avoid bleeding. The area containing the fourth and fifth glands, including the adjacent portions of the fat pads and the surrounding loose connective tissue, was now excised *en bloc* with fine scissors. The nipples were removed at the same time, leaving tiny holes in the skin.

The next step was to expose the first, second and third mammary glands. The skin flap covering these mammary glands was carefully separated from the underlying tissues. The median branch of the thoracic blood vessel was then ligated and severed. The ablation of the first mammary gland does not present difficulties, providing that the cutaneous incision is made at the proper place, that is, high enough. The second and third mammary glands were then removed *en bloc*. For this purpose an almost rectangular incision was made. Two longer arms of this incision run along and immediately ventrally to the thoracic lateral and thoracic media blood vessels, so that the latter one remains included in the area to be excised. Two shorter arms of the incision were made perpendicular to the first ones; the upper incision well above the second nipple and the other below the third nipple. The previously ligated blood vessel was removed together with both nipples, the loose connective tissue and the fat tissue from this region.

After ablation of all mammary glands from one side of the body, the exposed skin flap was moistened with saline, and put back in place. Ablation of the mammary glands on the other side of the body was then done in the same way. A non-sterile white cotton was used for sutures. No special post-operative care was necessary.

The surgical procedure in rats was essentially the same as in mice. In view of the different anatomical situation with three pairs of mammary glands in the inguinal region, a greater amount of the fat tissue had to be removed in order to assure a completeness of the operation. The incision through the inguinal fat tissue was made about 1 cm laterally from the fourth nipple. Still greater care was necessary to remove as completely as possible all the fat tissue from the sexual and anal region.

The completeness of the mammectomy was controlled at the end of the first pregnancy using the hormonal stimulus during pregnancy for the development of rests and of possible rudimentary mammary glands.

At autopsy a careful inspection for rests of the mammary glands was made. Suspected areas were dissected, stained with hæmatoxylin and examined under the dissecting microscope.

The results of mammectomy in mice and rats are summarized in Table 1. Here it can be seen that the mammectomy was complete in all but one mouse. In this case a small fragment of mammary tissue was left in the region of the third mammary gland. In the unsuccessfully operated rat, the removal of the third mammary gland was incomplete.

Table 1. RESULTS OF MAMMECTOMY IN FEMALE MICE AND RATS

Strain of mice	No. of animals operated	Successful mammectomy	Mean age when killed (days)
$C_{57}BL$	11	11	84
WLL	10	10	64
O_{20}	10	10	75
DBA_f	12	11	64
(♀ O_{20} × ♂ $DBA_f)F_1$	9	9	96
Total	52	51	
Rats			
(♀ U × ♂ $R)F_1$	10	9	81

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Relationship between *in vivo* Rumen Gas Composition and Feed Efficiency of Calves

THE efficiency of feed utilization by cattle is important because feed amounts to 80 per cent of the cost of beef production[1,2]. There is a need for an improved method of estimating the efficiency of feed utilization by ruminants[1,3] because direct determinations by traditional feed efficiency studies are expensive[4,5] and subject to large errors[5,6]. Attempts have been made to estimate the efficiency of feed utilization by correlating it with physiological criteria such as blood type and composition[1,3] and *in vivo* volatile fatty acid production[7]. However, such criteria have not been adopted for one or more of the following reasons: low heritability, low association with efficiency, difficulty in obtaining and preserving samples and the cost of analyses.

Rate of digestion has been related to gas composition *in vitro*[7] and rapid digestion has been associated with high bloat incidence[12]. Therefore, when bloat scores were found to be correlated with the carbon dioxide and methane (r = 0·898 and − 0·905, P = 0·01) content of rumen gas[9], it appeared that rumen gas composition might be related to efficiency of feed utilization.

Over a two-year period, twenty-six animals, some grade beef but mostly pure-bred Jerseys of diverse genetic origin, were feed-tested from 180 to 240 days of age. The daily ration consisted of alfalfa hay fed *ad libitum*, grass and legume silage at three times the amount of hay consumed plus five pounds of mixed grain concentrate. Pounds of dry matter consumed by each animal were determined and the total digestible nutrient consumption calculated using Morrison's table[5]. Efficiency was calculated as gain in weight per pound of total digestible nutrient consumed.

At the conclusion of the feed test period the animals were fasted for 12 h and then fed alfalfa hay. One hour later a gas sample was withdrawn from the rumen at the left flank by reduced pressure and analysed by gas-solid chromatography[10]. After gas collection the animals were given silage and grain. Gas samples were taken daily from each animal for five consecutive days, and carbon dioxide, methane, nitrogen, oxygen and hydrogen were determined. Correlation coefficients were calculated between feed efficiency and carbon dioxide, methane, the carbon dioxide–methane ratio, and the carbon dioxide–methane difference. Hydrogen, nitrogen and oxygen did not show any relationship to efficiency of feed utilization.

Simple correlation coefficients between rumen gas characteristics and feed efficiency are shown in Table 1. The difference and the ratio between the carbon dioxide and methane contents were calculated, but there was no advantage from the additional calculations.

Table 1. SIMPLE CORRELATION COEFFICIENTS (r) BETWEEN RUMEN GAS CHARACTERISTICS AND FEED UTILIZATION EFFICIENCY FOR 26 6-MONTH OLD CALVES

Rumen gas characteristic	r*
Methane	−0·428
Carbon dioxide to methane ratio	0·425
Carbon dioxide minus methane	0·458
Carbon dioxide	0·458

* Significant at the 5 per cent level.

The reasons for the significant association between rumen gas composition 1 h after feeding commenced and feed efficiency have not been determined in this work. However, during the first 2 h after feeding a rapid increase in the carbon dioxide content of rumen gas[4,9] and a concurrent decrease in rumen pH, which was accelerated by feeds of higher digestibility[9,11], have been observed. The increased rate of pH drop was undoubtedly caused by increased acid production, which in turn would be accompanied by greater carbon dioxide evolution due to higher fermentation-rate[4], decreased solubility and reaction with carbonates. Accordingly a higher carbon dioxide content of rumen gas 1 h after feeding would indicate a higher rate of digestion. A higher fermentation-rate could allow an animal to increase feed intake due to a decrease in retention time, but in this group the more efficient animals had lower total digestible nutrient consumption (r = − 0·537, P = 0·01). Therefore they appeared to extract more nutrients from each unit of food consumed rather than to have consumed more feed and hence to have reduced the proportion of energy used for maintenance. Somewhat the same reasoning would apply to the association between methane and feed efficiency because the principal components of rumen gas are carbon dioxide and methane. Because the volume of methane produced followed the same pattern as the proportion of methane[4] it is possible that animals with a higher percentage of methane also produced a greater volume of methane. Accordingly some of the association between gas composition and feed efficiency might be due to a higher loss of energy as methane in the less efficient cows.

This is believed to be the first report of a significant correlation between *in vivo* rumen gas composition and efficiency of feed utilization. The collection of rumen gas from intact animals has distinct advantages over the collection of gas from artificial rumens because gas production occurs under normal conditions and hence few assumptions are required and many possible errors are eliminated. Though the heritability of rumen gas composition was not determined, the animals differed significantly ($P = 0{\cdot}01$) and gas composition rankings did not change when groups of animals were sampled at widely spaced intervals[9]. The large inherent errors in determining the efficiency of feed utilization[5,6] and factors other than digestion which play an important part in the physiology of feed utilization undoubtedly reduced the correlations.

Rumen gas analyses could be used to replace traditional feed efficiency tests on breeding animals if a higher correlation could be obtained. This would permit screening of much larger populations than is feasible with the present expensive and inaccurate method. Because rumen gas can be readily collected from intact animals, and because it can be conveniently stored and inexpensively analysed with precision, it is anticipated that this measurement will be useful in conjunction with studies concerning the efficiency of feed utilization and the causes of differences between animals.

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Energy Expenditure of Grazing Sheep

THE results of several field experiments[1] have indicated that the energy expenditure of sheep at pasture in equable climates is 1·1–2·0 times larger than that of stall-fed animals. The distances travelled by grazing sheep and the energy cost of locomotion have been measured[2], and it appears that walking would increase the daily energy expenditure by 5–10 per cent. This leaves the energy cost of grazing *per se* and of the animal's reactions to its environment to be accounted for; the first measurements of the former can now be reported.

Two sheep were allowed to graze normally for a week. A patch of turf 1·0 × 1·5 m was then dug up and relaid on the floor of a respiration chamber. The sheep, which had both been trained for such work, were then, one at a time, placed in the respiration chamber (ambient temperature 22° C; water vapour pressure 10–15 mm mercury; still air) and their respiratory exchange measured by the confinement method[3] during five successive 30-min periods. The sheep were restrained from grazing during the first and third periods, usually rested voluntarily during the fifth period, and grazed during the second and fourth periods. Thus any changes of the metabolism due to time of day or to metabolism of ingested food would not affect the measurement at issue. Afterwards, the grass on a 1·0 × 1·5 m patch was cut and given to the sheep in the chamber over the course of an hour.

Five different types of pasture have been used. These ranged from one containing dry mature grass (*Paspalum dilatatum*) with a few small green clover plants (*Trifolium repens*), which was grazed slowly with an intake (estimated by clipping) of about 40 g dry matter per hour while in the respiration chamber, to one containing lush young grass (*Poa annua*), which was grazed avidly with an estimated intake of 130 g dry matter per hour.

The 'resting' metabolism of the sheep was not dependent on whether they were restrained or rested voluntarily, nor on the level of carbon dioxide in the apparatus (never more than 3 per cent). The energy cost of grazing was calculated[4] from the mean increments of carbon dioxide production and of oxygen consumption between the 'resting' and grazing periods; methane production and nitrogen catabolism, which together contribute less than 2 per cent to the calculation of heat production, were ignored.

The energy expenditure of the sheep always increased during grazing and fell again during the subsequent rest period, but there was no significant change when the pre-cut grass was given to the sheep. The grazing increment did not vary significantly from sward to sward, and had a mean value of $0{\cdot}62 \pm 0{\cdot}04$ kcal/h/kg body-weight for a 29-kg Merino ewe and of $0{\cdot}84 \pm 0{\cdot}12$ for a 53-kg Merino wether. When considering the daily expenditure of a sheep at pasture these values must be reduced to allow for the fact that only one-third of the day is spent in grazing[5]. They then amount to 12 and 18 per cent of the heat produced by the small and large animal, respectively, when hand fed.

These experiments are being continued with other sheep and pastures, but it may be concluded tentatively that the energy expended by a sheep in walking and grazing is not likely to be more than one-quarter of its heat production when stall-fed. This expenditure would not wholly account for the apparent additional energy requirements of grazing sheep[1] mentioned here.

Laboratory experiments[6] have shown that the metabolism of sheep can be increased two-fold when they are placed in an environment to which they are not accustomed. It remains to be determined whether climatic factors or the social environment can stimulate the metabolism of grazing sheep similarly and so increase their energy requirements substantially.

I thank Messrs. J. Taylor and R. Raabe for their assistance.

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Effect of Mucus on the Swimming-Rate of Sperms

It has been shown that bull sperm swim more slowly in mucus from the cow's uterine cervix than they do in saline[1]. This slowing-down might be an effect of hydroxyl ions, since the pH of mucus is rather high[2], and it has been claimed that media above pH 8·3 are very harmful to sperm[3]. Alternatively, it might be due to the presence of inhibitory substances in solution, or to the visco-elasticity imparted to the secretion by very large flexible molecules.

Our present experiments indicate that probably it is the last of these factors which is the most important.

We have carried out experiments on the effects of varying the pH of simple saline/phosphate mixtures on the swimming-rate and survival of bull sperm. In the majority of individual experiments, and in the mean for all experiments pooled, it appeared that initially both the swimming-rate and percentage of motile sperm were higher at pH 8·5 than at 7·5 and 6·5 or in unbuffered saline. Although this advantage was transitory, its existence precludes explanation of the slowing down of sperm by mucus as a simple effect of pH.

Œstrous mucus may be readily separated into a gel and a supernatant by centrifugation at 44,000g for $1\frac{1}{2}$ h; the viscous supernatant yields a further precipitate after 4 h, at 103,000g [4]. The final supernatant is essentially non-viscous, but contains the soluble material, mainly protein and low molecular weight substances, present in the native secretion[4]. The behaviour of spermatozoa in this supernatant was examined.

The pH, after addition of a suitable amount of semen, was in the range 7·4–7·7. Supernatants from two separate samples of mucus were tested. The first of these was used as diluent for three samples of semen, and the sperm in each case were found to swim at about four-fifths the saline rate at the same cell concentration, as compared with about two-fifths the saline rate in the whole mucus. In the second experiment two samples of semen from different bulls were made up each at two dilutions, 20,000 and 5,000 sperm per µl., in saline and mucus supernate. Rates were measured at the beginning and also at the end of 1 h, and at the end of 3 h. At the beginning of the experiment the rate was considerably higher in mucus supernate than in saline at both dilutions, about 20 per cent higher at 20,000 and 30–60 per cent higher at 5,000. This difference persisted at the end of an hour, but at the end of 3 h the rates in mucus supernate were similar to those in saline.

Finally the supernatant from a large pool of a number of separate samples of mucus was tested. The rates of sperm from five samples of semen from five different bulls were higher in this material than in saline, in one case nearly twice as high. Although the difference diminished at the end of an hour the rates in mucus supernate were all still higher than those in saline.

It appears, therefore, that neither the high pH nor the presence of soluble-inhibiting substances can be considered as an explanation of the slowing down of sperm by mucus. The gel which may be spun out of the native mucus contains, after repeated washing with saline, very little of the soluble substances in the original secretion, but after appropriate dilution with saline retains the rheological properties of the mucus. It appears to be a complex of sialomucopolysaccharide and protein[5]. In this material the spermatozoa behave as they do in native mucus, both as regards their velocity[1] and their orientation along strain lines[6].

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Abnormal Crystals in *Amoeba lescherae*

For the elucidation of a problem that I was investigating, it was necessary for me to have the developmental stages of *Amoeba lescherae*. A quick method of obtaining these is to use material from a culture grown under natural conditions[1] containing a large supply of mature amoebæ. It will be remembered that in such a culture, or in a catch procured from a natural source in summer, there will be found[1]: (1) mature and ageing adults; (2) encysted developmental stages of every variety; (3) spores; (4) possibly actively moving developmental stages. In the mature individuals there will be spores and differentiating spores. A sudden change in the pH (ref. 2) brought about by any agent not detrimental to the culture will kill off all the adults and so release any spores present in those adults. A study of the phenomenon is best made in Petri dishes (4 in. × 1 in.). After the lapse of a varying period of time (a week, more or less) subsequent to the changing of the pH, a search with the No. 7 objective water immersion lens will reveal large numbers of hatching spores, and creeping amoebulæ of various ages. But all these have a tendency to encyst, after a feed, especially at night, so the best time to search the Petri dish for active specimens is towards noon and early afternoon.

I chose for my source of material a set of three amoeba cultures grown in shallow Petri dishes (7 in. × 1 in.) which, with a hand lens, were seen to be luxurious. Because of what I found when I examined the adults under higher magnification, it will be necessary to state the history of these Petri dish cultures. The top culture was started on February 12, 1961, in the usual way by putting adult amoebæ plus culture fluid into Glasgow tap water containing boiled wheat grains. Food was renewed on June 12, 1961. On September 1, 1961, the culture was passed through a butter muslin net sieve to remove old grains and matted mould growth. The filtrate was so luxurious in adults that it was divided into three sub-cultures.

In January 1962 the phenomena to be described were discovered. I began by pipetting material from the top dish of the three stacked cultures for examination under a Greenough binocular. There was luxuriant growth, but the amoebæ had a yellowish tinge unlike the usual bluish one. Many looked very young; therefore, very transparent. Seen in a rectangular glass block with a dish-like bottom under No. 3 objective by transmitted light they presented a startling appearance. Some of the transparent ones had a mottled look, others were heavily packed with cytoplasmic inclusions. The most conspicuous cytoplasmic inclusions of adult *A. lescherae* are crystals and nutritive spheres (= excretory spherules = refractive spheres), the size of each being proportional to the age of the amoebæ, large nutritive spheres betokening adults. These specimens, however, contained abundant and abnormally large crystals, many times the size of those normally present; the largest, cube-shaped crystal measured 11μ in diameter, and apparently no nutritive spheres. They moved rapidly, quickly attaching themselves to the substratum, and fed readily.

So the questions arise: Has the nucleus stopped functioning? Is the absence of nutritive spheres due to a failure of the nucleus to emit chromidia for the production of spores (in the *proteus* group of *Amoebæ* spores are formed by the utilization of nutritive spheres by chromidia)? A detailed examination of many nuclei showed them to be perfectly healthy. *Amoebæ* were found with two nuclei, even with four; chromidia were present in some, as were blocks of chromatin around the contractile vacuole: all perfectly normal and healthy.

Fortunately crystals are easily dissolved in alcohol, and acetic alcohol is especially effective in clearing the cytoplasm of crystals. Therefore it was easy to find out if the nutritive spheres were hidden by the excessive masses of crystals. After all the crystals had been dissolved out, the cytoplasm looked ghostly —there were no nutritive spheres, the nucleus stained well in aceto-carmine.

The amoebæ of this culture were then excreting plentifully by their large and numerous crystals, but were failing to make any provision for their spore formation cycle. The fission cycle, so far as I have been able to observe, seems unimpaired. Further observations are being made to find out if this can go on indefinitely. So far, sub-culturing of the culture has been successful.

Of the remaining two Petri dishes the second down in the stack of three contained a few normal individuals possessed of ordinary crystals and nutritive spheres. These normal ones can easily be picked out under No. 10 magnification.

The lowest culture in the stack contained many more normal individuals which could be induced to produce hatching spores, etc., by the alteration of the *p*H.

The abnormal conditions obtaining in these three cultures are evidently of cytoplasmic and not nuclear origin. The anabolic products are being used to maintain this abnormality instead of to form nutritive spheres, a prerequisite for spore formation, and the excretory products of this are going to the formation of these large crystals.

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Microscopical Examination of Living Cells

MICROSCOPICAL examination of living cells such as small marine eggs, amœba and isolated cells from larger organisms frequently involves the construction of suitable chambers. After years of struggle with fragments of coverslip, paraffin wax, 'Plasticine' and polythene film, a very simple and versatile design of microscope slide chamber has been developed. The method is to cut paraffin wax sections on a microtome at the required thickness and then to mount the sections of wax on a microscope slide from warm water. The paraffin blank can be cast with a cylindrical hole to give a non-perfusing chamber, as in Fig. 1, or otherwise shaped to give perfusion chambers as in Fig. 2. The mounted sections should be dried at room temperature in a desiccator and then warmed slightly to consolidate the section to the slide. A selection of chambers from 2μ to 35μ can be easily prepared and stored. I have found chambers with enough wax to support a 1½ in. × ⅞ in. coverslip satisfactory. The skill necessary to fill a chamber 10μ deep without bubbles or wastage is easily acquired. Fragments of coverslip, overlapping the ends of the coverslip and the slide, provide reservoirs for perfusion.

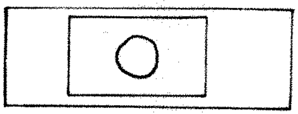

Fig. 1

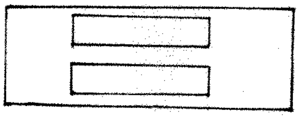

Fig. 2

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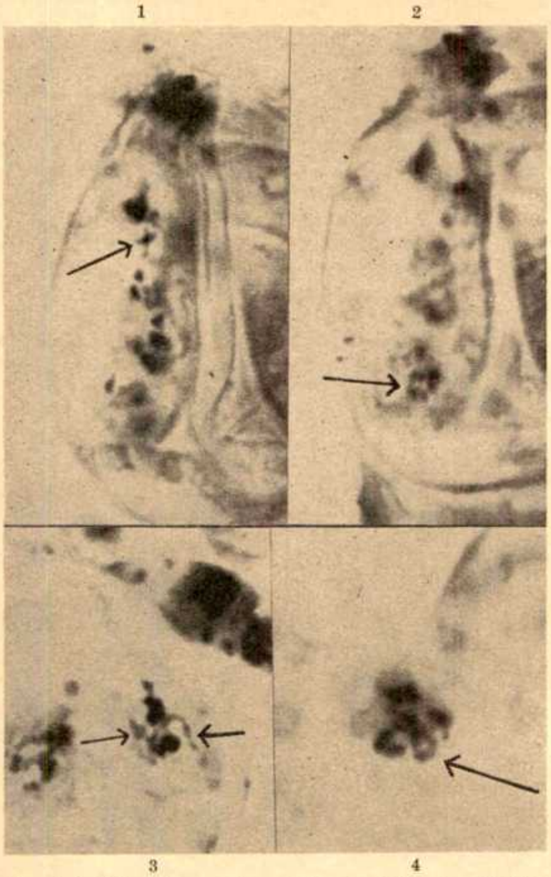
Use of Camphor-induced Polyploidy to determine the Place of Meiosis in Fungi

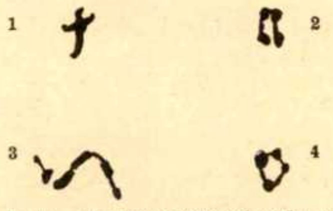
NUCLEAR divisions in the oogonia and antheridia of oomycetes have previously been reported to be mitotic[1]. Since the chromosomes are very small, it is not easy to determine whether they are meiotic bivalents or highly condensed mitotic chromosomes. It appears that it has not yet been possible to count the chromosomes in vegetative divisions because the chromosomes do not normally condense in these divisions.

In *Pythium debaryanum*[2], a ring of four chromosomes was observed in a late metaphase in the oogonium. This was taken to be conclusive evidence that the nuclear divisions in the oogonium are meiotic.

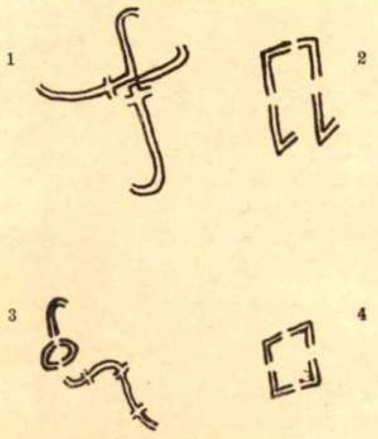
Observations on *Phytophthora cactorum* showed metaphases in the oogonium and antheridium with approximately ten chromosomes. In order to obtain evidence as to whether these divisions were mitotic



Figs. 1–3, *Phytophthora cactorum*; Fig. 4, *Achlya* sp., all after camphor treatment. Figs. 1 and 2, part of a tetraploid oogonium at two different foci showing quadrivalents (arrowed) (× *c.* 2,400). Fig. 3. Nucleus from tetraploid oogonium showing two multivalents (arrowed) (× *c.* 2,400). Fig. 4. Tetraploid nucleus from antheridial branch showing ring of four chromosomes (arrowed) (× *c.* 3,800)



Figs. 1*a*–4*a*. Drawings of arrowed configurations



Figs. 1*b*–4*b*. Possible interpretations

or meiotic, cultures of *Phytophthora cactorum* were treated with natural camphor. Cytological preparations were made using the aceto-orcein squash technique. Some of the oogonia and antheridia obtained after treatment with camphor had larger nuclei than untreated oogonia and, under favourable conditions, configurations of more than two chromosomes could be recognized (Figs. 1, 1*a*, 1*b*, 2, 2*a*, 2*b*, 3, 3*a*, 3*b*).

In *Phytophthora cactorum*, therefore, meiosis occurs in the oogonia and antheridia as in *Pythium debaryanum*.

The same method of inducing or selecting polyploid nuclei by camphor treatment was applied to a species of *Achlya* isolated from a local pond. Again, multiple associations were obtained in the oogonia and antheridia (Fig. 4, 4*a*, 4*b*), thus providing evidence that these divisions are meiotic.

Conclusive evidence has thus been obtained of meiosis in the oogonium and antheridium in two species belonging to the Peronosporales and one species belonging to the Saprolegniales. It seems probable, therefore, that this is the regular condition in the members of these orders, which must therefore be diploid in the vegetative phase. Similar evidence regarding the nature of these divisions might also be obtained by inducing structural changes by chemical or irradiation treatments.

Genetical observations on the Oomycetes are scanty, but such as they are, they are in accordance with the present cytological observations. Of particular relevance is the work of Buddenhagen[3] on induced mutations in *Phytophthora cactorum*. He obtained a number of mutants after ultra-violet irradiation, some of which were unstable when single zoospores were planted out, segregating into more extreme mutant types and also types approaching to the standard type. Such results could be explained if his original mutants were heterozygous for partially dominant genes. Somatic segregation by chromosome non-disjunction or somatic crossing-over could lead to the segregation of wild-types and the homozygous mutant types.

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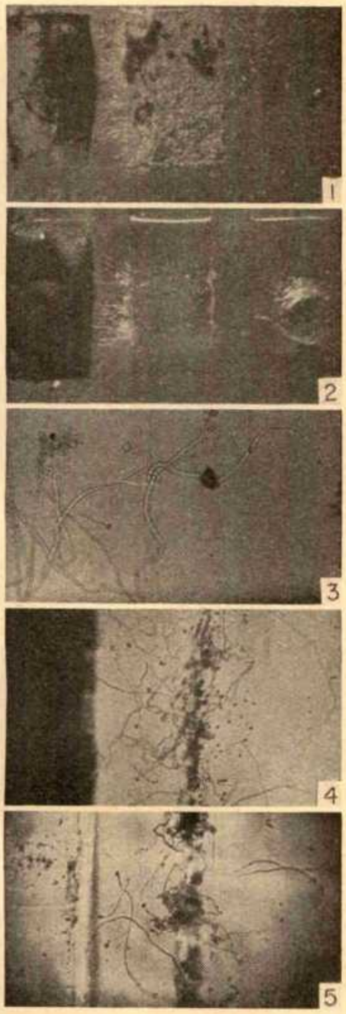
Chemotropic Response of *Antirrhinum majus* Pollen to Calcium

A RECENT review[1] on pollen tube chemotropism tentatively suggests that the chemotropic factor may be the same chemical entity in many plants. Figs. 1–5 are the evidence that the pollen tubes of *Antirrhinum majus* grow tropically towards a source of calcium. A stainless-steel mould was used to make sets of three depressions in nutrient agar[2] with each depression separated from the other by an agar wall 1 mm thick. The depressions are 3 mm wide, 5 mm long, and 3 ml. deep, with a 2-ml. thick layer of agar, underneath the depression. In Fig. 1 dry pollen was placed in the centre or pollen depression, nothing was added to the right or control depression, while solid calcium carbonate (used because of its low solubility) was placed in the left or test depression.



Figs. 1–5. Chemotropic response of pollen tubes of *Antirrhinum* to calcium. The calcium carbonate is in the left depression in Figs. 1, 2 and 4 and below the pollen tubes in Fig. 3. No calcium carbonate is present in Fig. 5

Pollen tubes grew well into the test wall, while few grew into the control wall. This procedure is referred to in the rest of this communication as the bioassay. In Fig. 2 single rows of pollen were placed in the pollen chamber adjacent to the test and control walls. Virtually all the pollen tubes on the test side of the pollen chamber grew into the test wall, whereas those on the control side remained within the pollen depression. In Fig. 3 pollen was placed on the surface of the nutrient agar in a line 1 mm from the outer wall of one of the depressions. Solid calcium carbonate was placed in the depression against the outer wall (out of view below picture). Once again, almost all the pollen tubes grew towards the calcium carbonate while very few grew in the opposite direction. Figs. 4 and 5 are essentially the same as Fig. 3, except that the pollen was placed in a slit made in the agar. With calcium carbonate against the outer wall of the depression (left side of picture), the pollen tubes grew towards the source of calcium (Fig. 4), whereas in Fig. 5 with nothing in the depression the pollen tubes grew in both directions. These demonstrations of a chemotropic response to calcium require careful timing of pollen tube germination and growth in relation to the establishment of the concentration gradients of calcium.

Using the bioassay, it was found that calcium carbonate can be replaced by the nitrate, sulphate, chloride, gluconate, malate and caseinate. On the other hand, no response was obtained to the chlorides of magnesium, barium, strontium, sodium or potassium, or as reported earlier[2] to a large number of organic compounds, such as sugars, amino-acids, organic acids, plant growth hormones, and so forth. The response to calcium appears to be specific.

Calcium functions here as a chemotropic agent even though it also enhances the growth of pollen tubes as observed by Kwack and Brewbaker[3] and by us. The effect of calcium, however, is not simply an enhancement of the growth of those tubes which happen to start their growth in the direction of the source of calcium. In addition to the evidence presented in Figs. 1–5, it can be observed microscopically that pollen tubes which protrude from the pollen grain in the opposite direction to the source of calcium change their direction of growth until they are growing towards the calcium. Further, neither sucrose nor yeast extract, both of which enhance the growth of pollen tubes, induces a chemotropic response when sub-optimal concentrations are provided in the nutrient medium and additional amounts in the test chamber of the bioassay.

The gynœcium of *Antirrhinum* contains the following amounts of calcium, expressed as a percentage of the dry weight: upper one-third of the style, 0·51; middle one-third of the style, 0·50; bottom one-third of the style, 1·23; ovary wall, 1·3; and ovules plus placenta, 2·17. All these tissues, as reported elsewhere[2], elicit a strong chemotropic response in the bioassay described here. On the other hand, petals and stamens do not effect a chemotropic response; neither do they contain calcium in the amounts found in the gynœcial tissue. Thus, as a percentage of the dry weight, the amount of calcium in petals is 0·18 and in stamens 0·36. These figures, showing a relatively high level of calcium in the ovules, lend support to the conclusion that calcium is the chemotropic factor in this plant.

The pollen of the following species has also been found to respond chemotropically to calcium when tested in the bioassay: *Narcissus pseudonarcissus* and *Clivia miniata*. Although the evidence is not available, it seems possible that calcium is the chemotropic factor generally in flowering plants. This would explain the lack of specificity referred to in the introduction. The chemotropic response of pollen to calcium could also explain the unconfirmed reports[1] of the growth of pollen towards such substances as sodium malate, compressed yeast, diastase, and egg albumin on the basis that these preparations may have contained concentrations of calcium sufficient to induce a chemotropic response.

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MICROBIOLOGY

Relationship of Fibrin Formation to Serum Bactericidin

In a previous communication[1] a clotting factor present in aqueous fibrin extracts was described. It was antigenically related to *C* reactive protein, which is found in the sera of patients suffering from acute infectious diseases. No antibacterial effect could be demonstrated with *C* reactive protein or aqueous fibrin extract. The bactericidal activity of serum has recently been reported to be localized in its β-lipoprotein fraction[2], and since in serum clotting factor becomes associated with β-lipoprotein[1], an attempt was made to relate this factor to serum bactericidin.

Pooled normal human serum was used. Dilutions were carried out with 0·9 per cent sodium chloride. Bacteria were grown in heart infusion broth at 37° C. One loopful of a 24-h culture was placed in 1 ml. of saline, and one loopful of the dilute suspension was used for inoculating 0·2 ml. of serum or dilutions thereof. Tubes were kept at room temperature (25–28° C). Aliquot amounts were streaked on heart infusion agar plates after 2 and 4 h. The plates were incubated at 37° C for 24 h. The bactericidal titre of a serum was established as the reciprocal of the dilution, killing 95–100 per cent of the bacteria after 4-h incubation.

It was found that Gram-positive bacteria, such as *Bacillus* and *Staphylococcus*, were more resistant than Gram-negative organisms of the genera *Aerobacter*, *Alcaligenes*, *Escherichia*, *Proteus*, *Salmonella*, and *Shigella*. Most sensitive proved to be a strain of *Escherichia coli*, which was afterwards used in all experiments. The inoculum of the dilute suspension contained $1{\cdot}5 \times 10^4$ *E. coli*. The bactericidal titre for this number of organisms of several sera used was 20–40. A saline control was included in each series of experiments.

Bactericidal activity was removed from serum by passing it through a Seitz filter pad, by heating it at 56° C for 30 min, and by incubating equal volumes of serum and of heparin or lytic factor[1] for 24 h at 5° C. Fifty units of heparin were used for 0·1 ml. of serum. The measure of potency of the lytic factor preparation was the ability of 0·1 ml. to prevent the clotting of an equal amount of oxalated plasma on the addition of one drop of 0·1 M calcium chloride. Lytic factor inactivated by heating at 70° C for 10 min was ineffective.

Potent clotting factor preparations elevated the bactericidal titre of some sera slightly, of others not at all, and were unable to restore the bactericidal property to heat-inactivated or Seitz-filtered sera. This may be explained by assuming that the amount of active material formed is contingent on some factor of limited availability, such as hæmolytic complement, which would be removed by filtration or heating.

As the result of combination of clotting factor with β-lipoprotein certain changes may be expected to arise. Thus, serum bactericidin is more heat-labile than clotting factor. Also, in the formation of the complex, clotting factor appears to be bound in such a way that it can no longer react with *C* reactive protein antibody. Serum freshly obtained from the recalcification of oxalated plasma produced a visible aggregate with this antibody, but the same serum, on standing for 2 h at room temperature, failed to react. The counteracting effect of heparin and lytic factor would seem to present the most convincing evidence for visualizing clotting factor as part o serum bactericidin.

Hæmolytic complement is apparently not required for bactericidal activity. Sera kept at 5° C for 3– weeks remained unchanged in bactericidin wherea only traces of complement were retained. No complement was left in serum on incubation at 37° C for 24 h, yet its bactericidal titre was lowered only from 20 to 10.

A relationship of fibrin clot production to bactericidal action has long been recognized. Gengou[3] found that uncoagulated cell-free plasma, obtained without the aid of anticoagulants, was not bactericida whereas the serum from the same blood was active Under normal physiological conditions there would be no need for such a substance, but when an injury is sustained and fibrin is laid down to wall off the area involved, the simultaneous release of a bactericide would certainly represent a device of great usefulness for the defence of the body.

This investigation was supported in part by a grant from the Kansas Heart Association.

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Prophylaxis of 'Wet-Tail' in Hamsters

THOUGH the Syrian hamster (*Mesocricetus auratus*) is generally free from spontaneous disease[1] many laboratory workers have observed in their colonies outbreaks of fatal diarrhœa colloquially known as 'wet-tail'. This disease has been prevalent in the Wellcome Laboratories of Tropical Medicine, particularly in hamsters subjected to spleen biopsy during the course of screening compounds for leishmanicidal activity[2]. Indeed in some tests more than half the animals succumbed, most of them dying on the third, fourth and fifth post-operative days. In the early stages of the disease the animals are fractious and anorexic. Diarrhœa soon begins and causes the moist caudal area from which the disease gets its name. Death usually occurs within 48 h.

Autopsies performed on hamsters dying of 'wet-tail' revealed that in each animal the ileum, jejunum, colon, and rectum contained merely a little yellow semi-fluid material and some gas. Many animals had inflammatory lesions of the terminal ileum, often associated with similar lesions in the cæcum. In a few cases the cæcal mucosa was found to be ulcerated. No other lesions were consistently found. In healthy animals the small intestine was always filled with partly digested food, the cæcal contents were much thicker, and the colon was loaded.

Examination of the intestinal contents of both sick and healthy hamsters revealed comparable infestations with dwarf tapeworm (*Hymenolepis nana*) and with one or more of several species of protozoa (*Trichomonas muris*, *Giardia muris* and *Balantidium coli*)[3]. Dark-ground microscopy showed vast numbers of bacteria in gut contents removed from all parts of the alimentary canal. Large non-motile bacilli and spirochætes predominated. When agar, blood agar, and McConkey plates were seeded with gut contents from affected hamsters, heavy growths of *Escherichia coli* were invariably obtained. In contradistinction, similar cultures made from healthy animals grew only occasional colonies of *E. coli*, *Staphylococcus* and *Bacillus* spp. Tube agglutination tests were made on boiled suspensions of 11 of the *E. coli* isolates, using the commercially available polyvalent *E. coli* antiserum: 026, 55, 11, 119, 125, 126, 127 and 128. None of the strains was agglutinated.

Table 1. EFFECT OF NEOMYCIN SULPHATE ON THE MORTALITY OF HAMSTERS AFTER SPLEEN BIOPSY

	Treated		Untreated	
	No.	Per cent	No.	Per cent
Wet-tail	4	5·7	29	41·4
Drug toxicity	3	4·3	2	2 9
Other deaths	8	11 4	10	14 3
Survivors	55	78·6	29	41·4
Total	70	100 0	70	100 0

Supporting experiments showed that the growth of the *E. coli* strains isolated from hamsters dying of 'wet-tail' was inhibited *in vitro* by concentrations of neomycin sulphate greater than 8 μg per ml. Furthermore, it was found that hamsters of 40 g weight were able to tolerate daily doses of this drug as great as 50 mg, and that its administration had no effect on the leishmania infection. Starting on the day of spleen biopsy, therefore, half the hamsters used during a period of five weeks were given a daily intragastric dose of 10 mg of neomycin sulphate dissolved in 0·25 ml. of water. Those animals not given the drug received only water. The results of this test are shown in Table 1. The 78·6 per cent survival-rate in treated animals compares favourably with the 41·4 per cent in the untreated group.

Since April 1960, all hamsters used in the leishmanicide screen have been dosed daily with 10 mg of neomycin sulphate. The first dose is administered intragastrically in 0·25 ml. of water, immediately prior to spleen biopsy; the subsequent doses are given in the form of cheese pellets. These pellets are prepared in bulk, and each contains 10 mg of neomycin sulphate in 0·5 g of cheddar cheese. One pellet is dropped into each cage each day. A total of 2,724 hamsters has been used, and of these 2,353 (86·3 per cent) have survived. Only 29 (1·4 per cent) of the deaths observed were due to 'wet-tail' and these frequently occurred in hamsters which refused cheese. The remaining deaths were due to drug toxicity (7·7 per cent) and various other causes (4·6 per cent).

The results obtained in this work show that 'wet-tail' in hamsters resembles in many respects infantile diarrhœa, scouring in piglets and chickens, white scours in calves[4], and the recently described mucoid enteritis of rabbits[5]. The many similarities between 'wet-tail' in hamsters and similar conditions in other animals make it reasonable to suppose that in hamsters, too, *E. coli* can be the cause of fatal diarrhœa. This view is strongly supported by the effectiveness of neomycin sulphate in the prophylaxis of 'wet-tail'.

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Alterations in the Carotenogenesis in *Staphylococcus aureus* after Adaptation to Pyrithiamine

IT has been reported recently[1] that *Staphylococcus aureus*, when cultured successively in media (glucose, 3·0 g; hydrolysed vitamin-free casein, 4·0 g; dipotassium hydrogen phosphate, 5·0 g; cysteine hydrochloride, 0·02 g; nicotinic acid, 0·02 g; thiamine hydrochloride, 10 μg; dist. water, 1 l.; pH 7·0) containing increasingly higher doses of pyrithiamine, acquires resistance to this antivitamin. After this adaptation, the bacteria can grow well in the presence of pyrithiamine (1 μg/ml.) even in the absence of thiamine with some changes in the metabolic pattern. Of the major biochemical alterations as a result of adaptation, severe depression in glucose utilization with the concomitent stimulation in the utilization of acetate has been noticed. By measuring the rate of formation of carbon dioxide labelled with carbon-14 from glucose-1-^{14}C and glucose-6-^{14}C, it has been observed that the percentage reduction in $^{14}CO_2$, formation from glucose-1-^{14}C by the adapted organism is much more than that obtained from glucose-6-^{14}C, causing thereby a lowering in the glucose-1-^{14}C/glucose-6-^{14}C metabolic ratio after adaptation. Relative to the normal strain the adapted strain utilizes acetate-^{14}C at a much faster rate both in the formation of $^{14}CO_2$ and also in the incorporation of carbon-14 into the protein and lipid fractions of the bacterial cells. It has further been noted that as a result of adaptation to pyrithiamine the colour of the pigment of the organism is changed from orange-yellow to lemon-yellow.

Previous studies show that there is either complete suppression of carotenogenesis by *S. aureus* as in the case of adaptation to tetracyclines[2], or an alteration in the nature of the pigments as in the case of adaptation

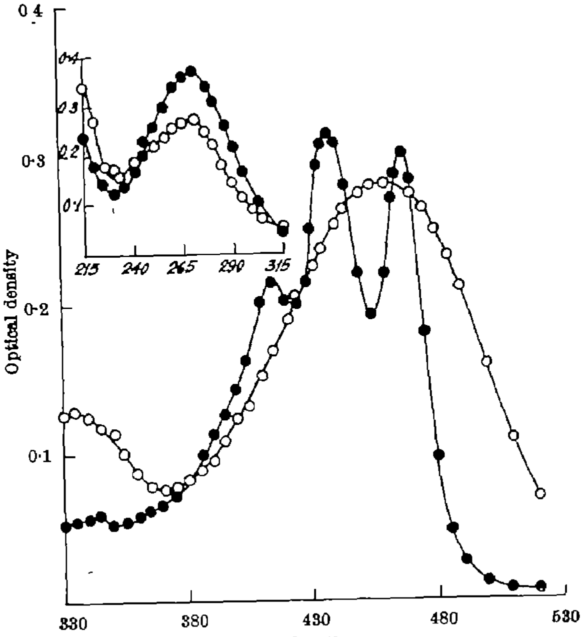

Fig. 1. Absorption spectra of the pigments extracted from normal and pyrithiamine-adapted *S. aureus*. ○, Normal; ●, pyrithiamine adapted. 50 ml. of methanol were used for the extraction of 1 g cell (wet wt., 24 h growth at 37° C), diluted twice for visible spectrum and five times for ultra-violet spectrum studies

to sulphathiazoles[3] or fluoropyrimidines[4]. The absorption spectrum studies of the methanol extracts of both the normal as well as of the pyrithiamine-adapted *S. aureus* have been made in a Beckman spectrophotometer model *DU*. Maximum optical densities of the methanolic solutions of pigments have been observed at 337 mμ and at 460 mμ for the normal organism and at 414 mμ, 437 mμ and at 468 mμ for the adapted organism. In the ultra-violet region, the absorption maxima for either of the strains were at 267 mμ (Fig. 1). The changes in the nature of the pigment as reported here is due to some alteration in the biosynthetic pathways leading to the formation of carotenoids, besides some significant changes as observed with respect to the utilization of carbohydrates by *S. aureus* after adaptation to pyrithiamine.

We thank Prof. B. C. Guha for his interest in this investigation.

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Isolation of Antigenic Fractions from *Bordetella pertussis* by means of Lysozyme

A considerable number of Gram-negative bacteria have been broken up in recent years by means of lysozyme[1–4]. The results of lysozyme treatment of *Bordetella pertussis* and the analysis of the resulting antigenic fractions form the subject of this communication. Suspensions (phase I), obtained from 24-h-old liquid shaken cultures, were used. After washing them with distilled water, we adjusted their germ count to 4 to 6 × 10^{10}/ml. with reference to the International Standard for Opacity. We determined the conditions under which lysis would least impair antigenicity and found that our purpose would be best served by using a combined treatment with crystalline lysozyme (100 μg/ml.) and versene (200 μg/ml.) in a 0·03 M *tris* buffer of *p*H 8·0 under continuous stirring in a water bath of 37° C for 2–3 h.

Fig. 1 shows the effect of the *p*H and the buffer on lysis as measured by the diminution of opacity. A one-cell photometer was used for the determination of opacity, and its decrease was expressed as a percentage of turbidity. Unpublished experiments showed that a rise of temperature (especially one within the range between room temperature and 37° C) promoted the decrease in opacity. Still higher temperatures, for example, 45 and 56° C, promoted lysis to a considerably lesser extent. We therefore performed our experiments at 37° C, and this the more so because treatment at 45° or 56° C had been found definitely harmful to the antigens.

A very cautious acidification of the viscous substance obtained by treatment with lysozyme yields a precipitate between *p*H 6·7 and 7·05; this precipita contains the undissolved bacterial components. I centrifuging we obtained a pellucid, and but ve slightly viscous, supernate (fraction 1). If fraction 1 further acidified, a floccular precipitate is prompt obtained which is readily soluble in a phospha buffer of *p*H 7·2 (fraction 2). Table 1 shows, in cor parison with the initial suspension, the immunogenic properties of fraction 2 obtained at *p*H 4·1 and adju ted to the volume of the original substance. W treated the control suspension with 'Thiomersa (0·02 per cent) and kept it in a water bath at 37° C f 3 h. Fraction 2 contained 0·01 per cent of 'Thiomersal'. Table 1 indicates mouse protective potenc (ED_{50}), histamine-sensitizing activity (HSD_{50}), aggl tinin-producing ability in mice (AgD_{50}), leth (LD_{50}), and dermonecrotic toxicity (DNT). A ascertained by the micro-Kjeldahl method, the tot nitrogen contained in the initial suspension amounte to 0·28 mg nitrogen/ml., against 0·04 mg nitrogen/m in fraction 2. Figures in Table 1 express the antigen value of the substance per 1 mg nitrogen. It evident that the purified fraction 2 contained the mo important antigens of *B. pertussis*.

Table 1. Antigenic Properties of *Bordetella pertussis* Fractions

Material	ED_{50}*	HSD_{50}*	AgD_{50}*	LD_{50}*	*DNT*
Control	1,052	48·5	153·8	11·7	nil†
Fraction 2	1,786	490 0	147·0	847·4	16 × 16 *N*‡

* ED_{50}, HSD_{50}, AdD_{50}, LD_{50}/mg nitrogen.
† Diluted 1 to 50.
‡ In mm; *N*, necrosis; diluted 1 to 100.

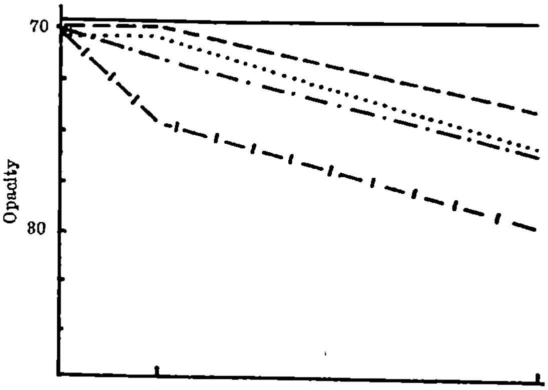

Fig. 1. ——, Control; – – –, phosphate buffer *p*H 7·2; . . . phosphate buffer *p*H 8·0; —·—, *tris* buffer *p*H 7·2; —ı—, *tri* buffer 8·0

The concentration of the protective antigen ro slightly, that of the histamine-sensitizing factor (HSF rose about ten-fold, and the amount of agglutinoge remained unchanged in the course of purification. Th behaviour of both the lethal and the dermonecroti toxins was noteworthy. The LD_{50}/mg nitrogen c fraction 2 was 77 times that of the initial suspensio This must have been due to the treatment wit lysozyme facilitating the passage of a great mass c toxins from the bacterial protoplasm into the solutio while the liberation of toxins from intact bacteri cells is a slow process.

The lethal toxic action of fraction 2 can be stoppe in 30 min at 56° C and in 3 h at 37° C. Its DNT-effec does not diminish within 3 h at 37° C. It should b noted that the HSF-content of fraction 2 disappear in 30 min at 56° C, while that of the whole suspensio is not affected[5,6]. The total nitrogen-content of th purified substance becomes 6–8 times less in th course of fractionations. As ascertained by th method of Ogur-Rosen, the level of nucleic acid (referred to as protein nitrogen) was about twice a much in fraction 2 as in the initial suspension.

By varying the *p*H values, applied for the produc tion of fraction 2, between 6·0 and 3·5, we obtaine fractions which contained the antigenic properties i fferent proportions. Experiments are now in ogress with the view of isolating the different tigens of *B. pertussis* in a possibly pure form by eans of the method described here, and also with e aid of other biochemical methods.

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VIROLOGY

Erythrocyte Receptors for Type A_2 Influenza Viruses

The close similarity in chemical structure between -type inhibitors of influenza virus hæmagglutination nd erythrocyte receptors for myxoviruses is now well known[1,2]. The affinity of some A_2 influenza viruses for a new type of serum hæmagglutination nhibitor with distinctive properties[3-5] therefore uggested the possibility of a new type of cell receptor or these viruses. Evidence for this has, in fact, been btained by some authors[6,7], who have shown that rythrocytes treated with neuraminidase remain gglutinable by inhibitor sensitive A_2 strains but not y insensitive ones or by type *B* virus.

Treatment of erythrocytes with graded doses of euraminidase destroys sequentially their capacity to e agglutinated by various strains of influenza virus[8,9]. On this basis viruses can be arranged in a sequence called the receptor gradient. The possibility that agglutination of neuraminidase-treated erythrocytes by inhibitor-sensitive A_2 strains is a manifestation of this phenomenon was, therefore, considered and the experiments reported here support the suggestion.

The effect of neuraminidase treatment on the agglutinability of human, fowl and guinea pig erythrocytes by two influenza virus strains Lee, Type *B*, and an inhibitor-sensitive variant of *A*/Singapore/1/57, Type A_2, was first investigated. A 5 per cent suspension of erythrocytes was incubated at 37° C with neuraminidase suitably diluted in calcium acetate saline *p*H 6·2. Aliquots were removed at intervals, centrifuged, washed and resuspended in physiological saline to a concentration of 1 per cent. Erythrocytes suspended in calcium acetate saline without neuraminidase were treated similarly and served as controls. Hæmagglutination of treated and control cells by the two prototype strains was then assessed by the standard pattern method of titration. The results summarized in Table 1 show that receptors for the inhibitor-sensitive *A*/Singapore variant on cells from the three species tested were destroyed by neuraminidase. It is noteworthy that receptors for the Lee strain were destroyed more rapidly than those for *A*/Singapore and that receptors for the latter strain on guinea pig erythrocytes were the most resistant to destruction by neuraminidase; indeed, in one experiment there was incomplete destruction even after 7 hours treatment.

These results do not support the conclusions of other authors that inhibitor-sensitive A_2 viruses attach to specific cell receptors qualitatively different from those utilized by other myxoviruses[6,7]. Rather, they imply that these viruses attach to the same receptors but occupy a position in the receptor gradient which allows them to agglutinate enzyme-treated cells which are inagglutinable by other strains.

In order to determine if there is a direct relationship between the inhibitor sensitivity of A_2 strains and their position on the receptor gradient a number of sensitive and insensitive variants were investigated. In these experiments 2·5 per cent suspensions of human erythrocytes in serial two-fold dilutions of neuraminidase were held at 37° C for 3 h, centrifuged, washed and resuspended in physiological saline to a concentration of 1 per cent. Each suspension was now tested for hæmagglutination by the addition of 0·25 ml. to an equal volume of a number of test viruses, containing 4 agglutinating doses. The results of three replicate experiments are summarized in Table 2, where the highest dilution of neuraminidase sufficient to prevent hæmagglutination reflects the ease with which the receptors for each test virus are destroyed. No precise correlation between this and inhibitor sensitivity is apparent. Nevertheless, the

Table 1. Hæmagglutination Titres before and after Treatment of Red Cells with Neuraminidase

	Human cells				Guinea pig cells				Fowl cells			
Duration of treatment (h)	Lee		*A*/Singapore		Lee		*A*/Singapore		Lee		*A*/Singapore	
	Control	RDE-treated	Control	RDE-treated	Control	RDE-treated	Control	RDE-treated	Control	RDE-treated	Control	RDE-treated
1	1,280	26	640	640	320	<10	1,280	1,280	640	214	640	640
4	640	<10	640	<10	120	<10	480	480	426	<10	640	<10
7	*	*	*	*	640	<10	640	<10	*	*	*	*

* Not tested

Table 2. Neuraminidase Destruction of Receptors for A_2 Virus Variants

Strain	Inhibitor sensitivity of variants	Highest dilution of neuraminidase preventing hæmagglutination*		
			Exp.	
		1	2	3
A/SING/1/57	*R*	320	320	1,066
	R	320	40	266
	R + *S*	80	32	67
	S	40	30	30
	R	N.t.	40	60
	S	N.t.	40	60

* Duration of neuraminidase treatment, 3 h.
S, inhibitor sensitive.
R, inhibitor insensitive.
N.t, not tested.

results obtained with our inhibitor-sensitive strains do indicate that the difficulty of removing their erythrocyte receptors with neuraminidase is due to their position in the receptor gradient. If so, the rate at which they are destroyed by neuraminidase will depend on the concentration of the enzyme as well as the duration of treatment. Variations in either of these factors would explain the discrepancy between our results and those of other authors.

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Latent Ectromelia

Ectromelia, a highly infectious disease of mice, is caused by a pox virus closely related to the causal agents of variola and vaccinia. The disease is frequently found in laboratory mice, and its existence poses a difficult problem for those responsible for the supply of laboratory animals. It therefore seems worth while to record certain preliminary observations which may bear on the epidemiology of the disease.

Fenner[1] showed that a large amount of virus was excreted from the skin of mice which developed a rash, and he believed the chief mode of infection was contact of virus with abraded skin[2]. Virus ceased to be excreted 3 weeks after the onset of the rash, and recovered mice were immune to re-infection. The epidemic behaviour and antibody response resembled the pattern associated with smallpox and seemed incompatible with persistent infectiousness of recovered mice. However, the carriage of ectromelia virus in colonies of apparently healthy mice has long been suspected, and accounts have been published of the appearance of acute ectromelia following the inoculation of mice with various biological substances[3-5]. The authors interpreted their observations in terms of the hypothesis that the virus may be carried in latent form in apparently normal mice.

In the hope of resolving these differences, attempts were made to isolate the virus from mice which had survived infection with small doses inoculated intraperitoneally. Although the virus was not isolated from cultured spleens later than 37 days after inoculation and most recovered mice appeared healthy, a few developed regressive lesions on the tails quite insignificant compared with those produced by the inoculation of the virus into the tails of normal mice. This observation and the observation of Dr. Janet Niven that apparently healthy mice may manifest microscopic lesions characteristic of ectromelia in the intestine led me to look for virus in the tail skin and fæces of recovered mice. Virus was isolated both from the intestinal contents and tail skin of each of six mice 84 days after infection and from the tail skin of 2 of 5 mice 99 days after infection[6]. Th results indicated that virus is excreted into intestine and from the tail skin of recovered m long after the development of immunity and clini recovery. Such mice could presumably infect t next generation of mice and infection of each succ sive generation might follow.

Experiments are being undertaken to investig whether ectromelia can be transmitted from gene tion to generation in this way, and some facts interest have come to light, although these expe ments are still incomplete. It appears that mice c be regularly infected *per os* with large doses of vir (10^6 LD_{50} or more, as measured by intraperitone inoculation of mice) and that even with much small doses of virus *per os* ($<10^2$ LD_{50}) about 1 : 5 m are infected. Oral infections, unlike infections follo ing intraperitoneal and footpad inoculation, are oft inapparent with large doses of virus and practical always so with small doses. Such inapparent infectio may be recognized by the development within 3 weeks of immunity to withstand intraperitone inoculation of 10^6 LD_{50} of virus and by isolation virus from the tails and intestinal contents. Thu two mice died of 120 (2 per cent) infected *per os* wi doses ranging from 10 to 10^4 LD_{50}, while 20/88 (23 p cent) of these became immune. Twenty-seven mi died of 59 (46 per cent) after 10^6 or more LD_{50} of vir *per os* and 27/31 (87 per cent) of the survivors we immune. Virus was isolated in mice from the ta skin and fæces of one mouse of seven given *per* 10^2 LD_{50} of virus 81 days before. Virus was similar isolated from the tail skin and/or fæces of mi infected *per os* with large doses (10^6 LD_{50} or mor as shown in Table 1.

Table 1

Days after oral infection	Ratio $\frac{\text{Mice which yielded virus}}{\text{Mice examined for virus}}$
32–35	2/9
69	3/8
81	2/4
116	1/4
	8/25 (32%)

These results show that ectromelia can be an in apparent infection and that mice may harbour th virus in the intestinal tract and tail skin for a lon time. The possibility of a similar situation with othe diseases of the pox group deserves consideratio Virus was isolated more frequently from the tail ski than the fæces. Nevertheless, it seems likely tha fæces are the principal source of virus and that th tail skin becomes infected by fæcal contamination The presence of antibody in the fæces would explai the fewer virus isolations from this source. Isolation of virus have been attempted only from individua mice, but experiments are now being undertaken t determine whether virus can be isolated from tail ski or fæcal suspensions of groups of mice. This migh provide a simple screening test for ectromelia i colonies of mice, to supplement the usual test fo hæmagglutination-inhibiting antibodies.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 22

nstitution of Electrical Engineers (at Savoy Place, London, .2), at 5.30 p.m.—Discussion on "Earthing Arrangements for ctrical Equipment, with particular reference to Double Insulation".

nstitution of Electrical Engineers, Electronics Division Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. F. G. Heath: ne Theory and Practical Value of Ternary Systems".

oyal Geographical Society (at 1 Kensington Gore, London, .7), at 8.30 p.m.—Mr. Brian J. Moser and Mr. Donald Taylor: ost Tribes of the Piraparana".

Tuesday, October 23

ociety of Chemical Industry, Agriculture Group (at 14 Belve Square, London, S.W.1), at 10.30 a.m.—Poultry Meeting.

niversity of London (in the Anatomy Theatre, University lege, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. P. R. Bell. ne Mystery of the Plant Egg".*

araday Society (in the Chemistry Lecture Theatre, The Univer-, Nottingham), at 5 p.m.—Dr J. H. van der Waals (Amsterdam). udy of the Phosphorescent State of Organic Molecules by Electron sonance" (Bourke Lecture).*

nstitution of Electrical Engineers, Science and General vision (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. Denis ylor: "Radioactivity and Process Measurements" (Chairman's dress).

niversity of London (at the London School of Hygiene and opical Medicine, Keppel Street, Gower Street, London, W.C.1), at 0 p.m.—Dr. H. A. Sissons: "Bone Structure and Metabolic Bone sease".* (Third of eighteen lectures on "The Scientific Basis of dicine" organized by the British Postgraduate Medical Federation.)

nstitution of Mechanical Engineers, Industrial Administran and Engineering Production Group (at 1 Birdcage Walk, estminster, London, S.W.1), at 6 p.m.—Discussion on "Planned intenance".

ociety of Chemical Industry, Plastics and Polymer Group 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. C. H. mford: "Solid State Polymerization".

Wednesday, October 24

nstitute of Fuel (at the Institution of Civil Engineers, Great orge Street, London, S.W.1), at 5.30 p.m.—Dr. R. S. Silver and e Hon. W. K. J. Weir: "Fuel, Heat and Energy Aspects of Seater Distillation".*

University of London (at the Warburg Institute, Woburn Square, ndon, W.C.1), at 5.30 p.m.—Prof. H. Bondi, F.R.S.: "Time and smology".*

British Institution of Radio Engineers (at the London School Hygiene and Tropical Medicine, Keppel Street, Gower Street, ndon, W.C.1), at 6 p.m.—Annual General Meeting of Corporate embers. 7 p.m.—Dr. A. Von Engel: "An Introduction to Plasma ysics".

nstitution of Mechanical Engineers (at 1 Birdcage Walk, estminster, London, S.W.1), at 6 p.m.—Mr. J. H. Pitchford esidential Address.

Society of Chemical Industry, Food Group—Nutrition Panel 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Miss D. F. llingsworth: "Changes in the Nation's Food Habits".

Society for Analytical Chemistry, Microchemistry Group "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—scussion Meeting on "Difference Between Continental and British actice in the Determination of Elements in Organic Compounds" troduced by Dr. W. Schoniger.

Royal Institute of Chemistry, Manchester and District ction (in the Manchester Town Hall), at 7 p.m.—Sir Lawrence agg, F.R.S.: "Living Molecules" (Twelfth Dalton Lecture).

Wednesday, October 24—Friday, October 26

Institution of Electrical Engineers, Electronics Division Savoy Place, London, W.C.2)—Second Symposium on "Electronics uipment Reliability".

Thursday, October 25

University of London (in the Anatomy Theatre, University llege Gower Street, London, W C.1), at 1.15 p.m.—Prof. Sir William olford: "The Life and Death of the Big City".*

Chemical Society (in the Main Lecture Theatre, Chemistry Dertment, University College, Gower Street, London, W.C.1), at 45 p.m.—Symposium on "The Chemistry of the Early Transition ements".

University of London (in the Physiology Theatre, University llege, Gower Street, London, W.C.1), at 5 p.m.—Prof. H. L Kornrg: "The Metabolism of Two-Carbon Compounds in Microganisms".* (Further lectures on November 1 and 8.)

University of London (at Westfield College, Kidderpore Avenue, ndon, N.W.3), at 5.15 p m.—Prof. J. E. Webb: "Tropical Zoology" naugural Lecture).*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. B. E. C. Nordin: "Calcium and Phosphorus Metabolism".* (Fourth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Thursday, October 25—Friday, October 26

Royal Geographical Society (jointly with the Medical Research Council, and the three Services, at the Royal Army Medical College, Millbank, London, S.W.1)—Symposium on "Exploration Medicine".

Friday, October 26

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. P. J. Lawther: "The Clinical Consequences of Air Pollution".*

Faraday Society (in the Main Lecture Theatre, Chemistry Department, The University, Sheffield), 4.30 p.m.—Dr. J. D. van der Waals (Amsterdam): "Study of the Phosphorescent State of Organic Molecules by Electron Resonance" (Bourke Lecture).*

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. W. T. Williams: "Computers as Botanists".

Saturday, October 27

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Felix Mann: "Origin and History of Chinese Acupuncture".*

Monday, October 29

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. C. E. Everard, Mr. R. J. Chorley and Mr. B. T. Bunting: "Slope Profiles".

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Prof. E. J. Richards: "Noise Control".

Institution of Electrical Engineers, Electronics Division (joint meeting with the British Institution of Radio Engineers Medical Electronics Group, at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Data Processing in Medicine".

Institution of Mechanical Engineers, Process Engineering, Refrigeration, Ventilation and Vacuum Plant Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Reducing the Cost of Maintenance".

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. B. F. Skinner (Harvard): "The Future of Teaching Machines".*

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. G. Sykes: "Horizons in Disinfection and Antisepsis" (Jubilee Memorial Lecture).

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer (well qualified in physics with suitable teaching, research or industrial experience) in Physics—The Principal, Brunel College. Woodlands Avenue, Acton, London, W.3 (October 27).

Scientific Officer (with a first- or second-class honours degree in chemistry, with or without postgraduate experience) in the Chemistry Section, Forensic Science Laboratory (Home Office), which deals mainly with toxicological problems—The Director, N.R. Forensic Science Laboratory, 32 Rutland Drive, Harrogate (October 27).

Scientific Officer (Unestablished) (with a first- or second-class honours degree in a scientific or engineering subject), to help to undertake, or promote investigations into selected aspects of scientific and technical communication which will assist in guiding the future information policy of the Department—The Secretary, Department of Scientific and Industrial Research, H.Q. Information Division, State House, High Holborn, London, W.C.1, quoting Ref. E/AH/040 (October 29).

Assistant Lecturer (with medical qualifications registrable in this country) in Anatomy—The Registrar, The University, Manchester 13 (October 30).

Research Fellows in Metallurgy, Chemistry and Physics, and Research Assistants in Metallurgy and Physics—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref. N/17D (October 30).

Lecturer (with special interest in reactor physics or reactor kinetics and control), and a Lecturer (with special interest in reactor design) in the Nuclear Power Group—Dr. P. J. Grant, Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7 (October 31).

Senior Scientific Officer (with a first- or second-class honours degree in mechanical engineering and at least three years postgraduate experience), to join a group concerned largely with research in ergonomics—The Director, Warren Spring Laboratory, D.S.I.R., Stevenage, Herts, quoting Ref. WSL 24 W (October 31).

Lecturer or Assistant Lecturer (man or woman with a degree in physiology, and preferably with experience in teaching histology) in the Department of Physiology—The Assistant Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (November 5).

Reader (with considerable research experience in the chemistry (including biochemistry) of proteins, particularly of enzyme proteins) in Protein Chemistry in the Department of Agricultural Chemistry at the Waite Agricultural Research Institute—The Registrar, University of Adelaide, or the Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, November 15).

Senior Lecturer or Lecturer in Electrical Engineering at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 15).

Lecturer in Organic Chemistry—The Secretary of University Court, The University, Glasgow (November 17).

Professor of Archæology in the Department of History—The Registrar, The University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C 1 (November 18).

Professor of Geography at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C.1 (November 18).

Associate Professor in Educational Psychology or in Educational Sociology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 19)

Graduate Entomologist (preferably with a knowledge of genetics), to work in the resistance of insects to insecticides—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (November 23).

Chair of Applied Mathematics in the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (Australia and London, December 1).

Junior Technician or Technician (male) in the Department of Physiology—The Assistant Secretary, Royal Veterinary College, Royal College Street, London, N.W.1

Medical Laboratory Technician (A.I.M L.T. or equivalent)—The Administrative Officer, North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex.

Microbiologist (with a good honours degree, and preferably previous experience of relevant subjects), for work on the microbiology of irradiated food—The Secretary, Low Temperature Research Station, Downing Street, Cambridge.

Physicist (with experience in dosimetric techniques)—The Director, Experimental Radiopathology Research Unit, M.R.C., Hammersmith Hospital, Ducane Road, London, W.12.

Reader (highly qualified in physics with suitable academic and/or industrial experience) in Physics—The Academic Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

Reader (with evidence of research ability in the academic and industrial fields, and preferably experience in the food industry) in Food Science—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

Research Assistant in the Department of Physics, to carry out work on the propagation of ultrasonic vibrations in semiconductors under the direction of the Head of the Department—The Academic Registrar, Brunel College, Woodlands Avenue, London, W.3.

Research Assistant (organic chemist or biochemist), to undertake work on polysaccharides of invertebral disks and connective tissues—The Secretary, The Royal Infirmary, Bradford 9, and The Registrar, Bradford Institute of Technology, Bradford 7.

Senior Research Assistant (preferably with experience in the field of high pressure research), to join a team doing research in high pressure chemistry, with some applications to geochemistry—Dr R. S. Bradley, Department of Inorganic and Structural Chemistry, The University, Leeds 2.

Statistician or Ecologist, to develop statistical techniques in ecological studies of plankton and herring, in hydrography and in the analysis of sampling methods (fifteen years data and computer facilities available)—The Officer-in-Charge, Oceanography Laboratory, Scottish Marine Biological Association, 78 Craighall Road, Edinburgh 6.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Freshwater Biological Association. Thirtieth Annual Report for the year ended 31 March 1962, including Reports of the Council and the Director, and Accounts for 1961–62, with lists of Officers, Council Staff and Members Pp. 100. (Ambleside· Freshwater Biological Association, 1962.) 4*s*. [238

White Fish Authority. Progress Report on the Trawl Gear Development Project. Pp. 14. (London: White Fish Authority, 1962.) [238

Department of Scientific and Industrial Research. D.S I.R., Universities and Colleges 1956–60: a Report on D.S.I.R. Suport for Research and Training in Universities and Colleges, 1956–60 Pp. viii + 232 + 15 plates. (London H.M. Stationery Office, 1962) 15*s* net. [129

Year Book of the Royal Society of Edinburgh, 1962, (Session 1960–1961.) Pp. 158. (Edinburgh. Royal Society of Edinburgh, 1962.) 20*s*.; 3.50 dollars. [129

Proceedings of the Royal Irish Academy. Vol. 62, Section A, No. 4 Combination Coefficients of Ions and Nuclei. By D. Keefe and P. J. Nolan. Pp. 43–53. 1*s*. 6*d*. Vol. 62, Section B, No. 7: Some Aspects of the Problem of the Distribution of *Bifurcaria bifurcata* (Velley) Ross on the Shores of Ireland, North of the Shannon Estuary. By Mairin de Valéra. Pp. 77–100 + plate 7. 3*s*. Vol. 68, Section B, No. 8 · The Influence of Insulin on the Gaseous Exchange of Isolated Tissues. By J. M. O'Connor. Pp. 101–116. 2*s*. 6*d*. (Dublin. Hodges, Figgis and Co., Ltd., 1962.) [129

Bulletin of the British Museum (Natural History). Entomology. Vol. 12, No. 7: Notes on *Tunga caecigena* (Siphonaptera: Tungidae). By Karl Jordan. *Neotunga euloidea* gen. n., sp. n (Siphonap Pulicidae). By F. G. A. M. Smit. Pp. 351–377 + plate 1. 10*s*. Geo Vol. 7, No. 3. The Trilobites of the Caradoc Series in the Cross Inlier of Northern England. By W T. Dean. Pp. 65–134 + plates 42*s*. Vol. 7, No. 4: Fossil Flora of the Drybrook Sandstone i Forest of Dean, Gloucestershire. By K. M. Lele and J Wa Pp. 135–152 + plates 19–23. 12*s* 6*d*. Vol. 7, No. 5 Fossil In from the Lower Lias of Charmouth, Dorset By F E. Zeuner. 153–171 + plates 24–27. 12*s*. (London· British Museum (Na History), 1962)

Library Association Pamphlet No. 23. The Hospital Librar Sweden. Translated into English by Mr. and Mrs. Frykman. P (London. Library Association, 1962.) 10*s*. (L. A. members 7*s*. 6*d*.)

Frank Mason and Co , Ltd. Directors' Report and Stateme Accounts for the year ended 31st March 1962. Pp. 8. (London: F Mason and Co , Ltd., 1962.)

Jacksons' Millboard and Fibre Co., Ltd. Directors' Report Accounts, 1962. Pp 8. (Bourne End, Bucks. Jacksons' Millboar Fibre Co , Ltd.. 1962.)

Other Countries

Universidade de Sao Paulo: Instituto Oceanografico. Contribu Avulsas—Technologia No. 2: Estudo Sôbre Pesqueiros de Arrasto a Costa do Brasil. Por Yapery Tupiassu de Britto Guerra. Pp. i (Sao Paulo: Instituto Oceanografico, Universidade de Sao P 1962.)

Instituto Antartico Argentino, Buenos Aires. Contribuc No. 48· Contribucion de la Armada Argentina en la Investig Cientifica. Por Rodolof N. Panzarini. Pp. 26. No. 55: La Auroral del Hemisferio Sur. Por Dr. Otto Schneider. Pp. 19. No Actividades Aeronavales en la Estacion Cientifica Ellsworth en E de 1961. Por Luis Iriart y Carlos J. Sagastume Pp. 16. No. 62 Primera Fanerogama Adventicia Hallada en el Continente Anta Por Alfredo Corte. Pp. 14. No. 64: Estudios y Resultados Prelim es sobre Trobajos Oceanograficos en el Area del Mar del Weddell. Ruben N. Paccagnini y Alberto O. Casellas. Pp. 12. No. 68: Pri Cita de la Familia de Hepaticas "Marchantiaceae" para la Anta Por Alfredo Corte. Pp. 12. (Buenos Aires: Instituto Antartico A tino, 1960, 1961 and 1962)

Metropolitan Life Insurance Company. Statistical Bulletin, Vo (July, 1962): Marriages in the United States and Canada. Slight Inc in Mortality. Changing Mortality in the Type of Heart Di Surgical Treatment of Retired Women. Pp. 12. (New York. M politan Life Insurance Company, 1962.)

Japanese Journal of Applied Physics. Vol. 1, No. 1, (July, 19 (Published monthly with the co-operation of the Physical Socie Japan and the Japan Society of Applied Physics.) Pp. 1–70. scription rate: Annual 10 dollars; Single copy 1 dollar. (Bunky Tokyo Faculty of Science, University of Tokyo, 1962.)

Centro de Investigaciones Pesqueras, Habana. Del Departmen Pesca del Instituto Nacional de Reforma Agraria. Contribucion No Datos Oceanograficos de la Playa Habana III. Por Jose A. Sori. Pp. 27. Nota sobre Investigaciones, No. 4 Estudios Estadi y Biologicos sobre la Biajaiba (*Lutianus synagris*). Por Zeida Rodr Pino. Pp. 92. (Playa Habana, Bauta, Habana Centro de In gaciones Pesqueras, 1962)

Bulletin of the Astronomical Institute of the Netherlands Vo No. 518, (1962 July 6). Polarization of the Galactic 75–CM Radi By Gart Westerhout, Ch. L. Seeger, W. N. Brouw and J. Tinbe Pp. 187–212. Further Polarization Measurements at 75–CM. By Brouw, C. A. Muller and J. Tinbergen. Pp. 213–224. Vol. 16, No. (1962 July 9)· The Continuous Absorption Coefficient in the and Ultra-violet Region. By C. Zwaan. Pp. 225–233. (Leiden Utrecht. Astronomical Institutes of the Netherlands, 1962.)

Duke University: School of Forestry. Bulletin No. 16: Param of Site for Certain Growth Components of Slash Pine (*Pinus el* Engelm). By Dr. D. S. Jackson. Pp. vii + 118. (Durham, N. Car Duke University, 1962.) 2 dollars.

Food and Agricultural Organization of the United Nations. Pacific Fisheries Council Proceedings—9th Session, Karachi, Pak 6-23 January, 1961, Sections II and III. Pp. iii + 136. (Ban IPFC Secretariat, FAO Regional Office for Asia and the Far 1962)

Cenco International News, Vol. 1, No. 1, (July 1962). Pp. 4. (Ch Cenco Instruments Corporation, 6450 W. Cortland Street, 1962.)

Population Reference Bureau. *Population Bulletin*, Vol. 18, (August, 1962): The Story of Mauritius—From the Dodo to the Pp. 93–116. (Washington, D.C.. Population Reference Bu 1962.)

The Research Institute of Applied Electricity, Hokkaido Unive Monograph Series, No. 10. Platinum Blood Flowmeter. Edite Prof. K. Higasi. Pp. v + 78. (Sapporo, Japan: Research Institu Applied Electricity, Hokkaido University, 1962.)

International Council of Scientific Unions Year Book, 1962. Pp Financial Statement for the year ended 31 December, 1961. P (Rome· International Council of Scientific Unions, 2 Via Seb 1962.)

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SCIENCE AND THE NATURE OF POLITICS

IN moving the second reading of the Finance Bill in the House of Lords on July 30, Lord Hailsham made an appeal for unity and realism which expresses an attitude frequently found among scientists. Continuing the plea he had made in reference to broadcasting policy on July 18, he urged that at this juncture what was needed was integrity and resolution, common sense and clarity of thought—Britain was tired of obsolete and unrealistic political theories and looked for leadership and originality on both sides. It was not simply the Government which was on trial, but the whole nation—Parliament, Britain's belief in freedom, her experiment in democracy, her religions, her philosophies, her civilization, and all that went to make up her way of life. He did not believe there was anything radically wrong with the British society, but it was important to eliminate the hypercritical attack so fashionable on things and people who had succeeded. Further, although the element of chance entered into almost every human enterprise, it was essential to reduce this element so far as possible by conscious and methodical forethought and realistic appraisal of the facts.

There were, Lord Hailsham suggested, three great issues before the world. First, there was the fact that its political and social institutions, whether in the Communist or the Free World, no longer matched the requirements of the human race or the technical facts of life. This was true both of internal and external relations. Secondly, there was the moral crisis: the West had failed to live up to its moral ideas, and the East, rejecting those moral ideas, now found it could not live without them. Thirdly, there was the inability of the West to generate sufficient economic power to support the volume of activity which would enable it to win the 'cold war'. This was an international problem which could only be solved at international level, but Lord Hailsham pointed out that there were moral and social realities which were not altered by shifts in technology or the balance of power. What was required was an absolute determination that Britain's distinctive national society should thrive and prosper in the changed conditions of the twentieth century—but for this purpose it was essential that Britain should be radical and flexible in much else. Relations between the State and the individual, between the State and the trading organization, between the State and the voluntary associations needed to be radically recast, and many noted concepts which had served her forefathers required constant re-examination if Britain were to pursue realistic courses in the changing currents of a revolutionary world. This placed an immense strain on the flexibility of mind and consistency of purpose of public men.

Britain was, Lord Hailsham continued, suffering from rigidity and a degree of inefficiency, and was trying to operate a system of essentially nineteenth-century customs and technologies. Inefficiency of management and restrictive practices must be eliminated and bring Britain's traditional and inadequate system of apprenticeship into line with economic and educational reality. Old and obsolescent craft industries needed a new scientific basis, and reluctance to adopt modern methods of machining in the engineering industry, slowness to apply scientific ideas in shipping operation and shipbuilding, the clinging to batch rather than continuous methods of processing, the condition of the docks—all these, as well as comparative neglect of social studies, required consideration in Britain to-day, and this was much more than a task for the Government: it was a task for the whole people and called for the rejection of the national hypocrisy which refused to face realities in Government expenditure and taxation.

While there is much truth in what Lord Hailsham said, his plea for unity needs to be considered in the light of some such analysis of the nature of politics as Prof. J. D. B. Miller gives us in his recent book*. Moreover, special considerations are required of his insistence that politics is both a rational and a non-rational activity, that it consists essentially of conflict, in varying degrees, over the use of Government, engendered by many forms of diversity of condition, status and opportunity, and that there are serious limitations to what politics can accomplish. These limitations, he had pointed out, were built into the fabric of any society, including those imposed by natural resources, by the lack of omnipotence of Governments, and by the plain fact of incompatibility in its widest sense, to which indeed Lord Hailsham referred. With a penetrating discussion of the existence of varying interests in any developed society and of the assumption that there is a general interest in society, Prof. Miller provides the basis for a realistic approach to the problem of Government and science which should correct a good deal of loose thinking on such themes. Furthermore, it should stimulate a practical approach to some of the most pressing problems of Government to which Lord Hailsham and Sir Charles Snow, to mention only two, have recently directed attention.

Prof. Miller, in fact, devotes some attention to what Sir Charles Snow described as "closed" politics and on which Sir Charles has since written a *Postscript*†, reiterating the importance of the decisions which are taken in this way, and of the implications if they are taken by men who have no first-hand knowledge of what the cardinal choices available depend on or of what their results may be, and, in particular, urging the danger of ever depending on a single scientific judgment. Prof. Miller is, of course, concerned with the more general issue, and points out, indeed, that the closed politics described by Sir Charles, and which he himself discusses in terms of Government bureaucracy, are equally characteristic of the politics of institutions like a university, a cathedral chapter or a club. Nevertheless, his more detailed analysis, following Sir Charles Snow's distinguishing of the three categories of committee politics, hierarchical politics and court politics, is one that deserves the careful attention of any scientist who is concerned with the way in which effective and wise action can be secured on matters in which scientific or technical factors can be of decisive importance. Incidentally, he notes, though without discussion, the increasing importance in the modern state of tension between scientific and non-scientific departments.

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* *The Nature of Politics.* By Prof. J. D. B. Miller. Pp ix+296. (London: Gerald Duckworth and Co., Ltd., 1962.) 30*s*.

† *A Postscript to Science and Government.* By Sir Charles Snow. Pp. ii+87. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1962.) 3*s*. 6*d*. net.

Prof. Miller makes two points which are frequently ignored in discussions of the relations between science and Government—for example, in J. S. Dupré and S. A. Lakoff's study *Science and the Nation: Policy and Politics*‡, and in D. K. Price's article *The Scientific Establishment*§—though they have a decisive influence on the means that are adopted for resolving differences. General interests, he maintains, are found only occasionally and do not last long. Secondly, conflict lies at the heart of politics, which is a matter of the expression, advocacy, settlement and modification of disagreements. It also involves agreement, though not necessarily more permanent than the disagreements.

As this view is worked out by Prof. Miller it bears striking resemblance to the scientific method, with its emphasis on diversity, its opposition to regimentation, its denial that all wisdom resides in a few people whose right to rule must not be questioned, its recognition of individual and human interests, and its search for voluntary, not compulsory, agreement for what—though Prof. Miller does not use the term—might be described as something akin to the idea of 'the sense of the meeting'. It is this changing, dynamic conception of the recognition of changing and diverse interests and their reconciliation and adjustment under the rules regulating interests and individuals made by the appropriate body which also provides the opportunities for position, privilege, security and advantage out of which the interests arise, that the scientist will recognize as having affinity to the way in which he works. It should provide both the safeguard which Sir Charles is seeking against the dominance of a single judgment and the flexibility which Sir Bernard Lovell desiderated in a comment on Sir Charles's *Postscript*.

If scarcely as profound as Sir Ernest Barker's *Reflections on Government*, Prof. Miller's book should appeal to the scientist for its pragmatic approach to the practical tasks of Government and its restraint in discussing political theory and systems, which are given their due weight but no more. He has comments on the education of the politician which have implications for education for management and dispose just as effectively of excessive claims or loose thinking. He has no use for narrowly professional education, but dogma is kept firmly in its place as a servant or instrument of thought and denied all claim to be guide let alone master.

In suggesting that existence of a general interest is exceptional and transient rather than normal, Prof. Miller judges that a multiplicity of interests is the normal and healthy condition and that political movements, whether in the nation or society as a whole or in groups or institutions (which are enemies of such plurality), should be viewed with suspicion. "There cannot be any discussion, or any system of government by discussion," wrote Sir Ernest Barker in 1942, "except upon the assumption that truth dwells in more than one habitation, and that its elements have to be collected, and not only collected but reconciled, before it can be enthroned." He saw the real issue as one between two different tempers of mind—the spirit and temper of accommodation and the spirit and temper of intransigence. The soundness of that view is thoroughly attested by post-war experience, and Prof. Miller's concern with the element of conflict and continuous adjustment seems to lead him to a very similar position.

This is apparent in the scope he sees for the individual in the interplay of interests which constitute the essence of politics, although the changes which individuals can effect cannot run completely counter to the general social inclinations of the community. The alleged dichotomy between administration and policy Prof. Miller rejects as false, and he stresses the existence between these two extremes of a vast no-man's-land where the departmental view, the advice of the expert, the considerations of the Cabinet, the personal view of the Minister, and the convictions of the political party may function according to the circumstances. Here, pragmatism, regard to what is possible in practice, rather than what is theoretically desirable, is the decisive factor, and while Prof. Miller never quotes the first Earl of Lytton, much in this book recalls the latter's insistence that expediency alone is the basis of all Government, that circumstances are the only things with which Government has to deal and that political action is good or bad, right or wrong according to its consistency, not with theory, but with circumstance, in all its human, fluctuating variety.

This is particularly noteworthy when Prof. Miller deals with the sovereign state at the international level. Here he deals soberly with the assumption that a vague generalized interest in peace is likely to lead to a voluntary agreed creation of a world Government. The sovereign State must still be recognized as the prime fact of political life, and his comments in this connexion are as pertinent in reference to Britain's proposed entry into the Common Market as they are in relation to the functions of the United Nations. The limits of national action in the field of foreign policy are set by the lines of force prevailing in the world at large, and even more than domestic policy, foreign policy is the slave of possibility.

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‡ *Science and the Nation: Policy and Politics*. By J. S. Dupré and S. A. Lakoff. Pp. x+181. (Prentice Hall, Englewood Cliff, New Jersey, 1962.) 16s. or 1.95 dollars net.

§ *Proceedings of the American Philosophical Society*, No. 3, 106 235 (1962).

This welcome note of sanity does not, of course, dispose of the awkward, disturbing issues either in internal or external politics which are set by technical or scientific advance. Nevertheless, the field within which the expert may seek to function is clearly delineated, and also the points where critical and constructive thought is required about the whole apparatus of advice and its limitations. In this connexion there is much wisdom in what Prof. Miller writes about ideas in politics and their effect. Each country, he suggests, develops its own ideas in accordance with its own practice, and, while ideas assist in the creation of a climate of opinion in which selfish and altruistic interests can blend, the existence of climates of opinion is usually a national phenomenon.

This is not to deny that movements such as the Pugwash Conferences are entirely without effect, but rather to suggest that the tempo is slow and their limits of influence strictly circumscribed. It is for discussion how the process could be accelerated, but the discussion must be realistic and guided by the nature of politics. Here, perhaps especially, Prof. Miller's reason for studying politics—that it is better to know the truth than not to know it—applies. He makes no large claims for the value of political study, but he sets out soberly some potentialities of utility that the scientist who is concerned with the relations of Government and science could examine with profit. His book should at least help scientists to avoid the over-simplifications which are a major weakness of Dupré and Lakoff's study of the changes in institutional relations between science and the Federal Government during the past thirty years, in which science has become the major Establishment in the American political system: the only set of institutions, as D. K. Price observes, for which Federal funds are appropriated almost on faith, and under concordats which protect the autonomy, if not the cloistered calm, of the laboratory.

Dean Price's analysis is worth considering in the British context, for he claims that the major difference between the two systems is the persistent dominance in Britain of the Administrative Class of the Civil Service, staffed by men mainly without a scientific or technical education, whereas in the United States scientists move freely into the highest administrative positions and in and out of Government. This system, he admits, maintains a continuous interchange of men and ideas between the Government and universities, but it also makes it impossible to maintain an institutional distinction between ends and means, between policy decisions on one hand and on the other scientific research or administration. Party responsibility in the parliamentary sense becomes impossible and the task of co-ordinating either policy or administration is greatly complicated.

The value of this continuous interchange is undeniable though it should not be limited to the universities, and the Zuckerman Committee fully recognized that it was something to be encouraged, though Britain need not copy the American system to do so. The blurring of the distinction between ends and means is, however, questionable, even if Dean Price is right in suggesting it is part of the scientific approach. On the other hand, though universities and private foundations in the United States have a hand in the initiation of new public policies to an exceptional extent, policy decisions remain the responsibility of Government and cannot be delegated. This, however, is merely a change in the character of the interests and of their conflicts rather than a new system. For all that, the development and its political and administrative patterns may have been stimulated by a demand that the organization and procedures of Government, as well as its policies, should be open to scientific inquiry and independent criticism, and may to some extent reflect the way scientists themselves behave.

Unlike Sir Charles Snow, Dean Price, however, sees little reason to fear that major Government decisions affecting science will be secret but rather fears that they will be popular. In either way could lie disaster, for neither is consistent with the existence of well-informed discussion. As G. M. Young pointed out*, the compactness of party organization necessitated by the vast size of a modern electorate and the technical intricacies of modern administration are gradually contracting the area of such discussions. He suggested that to re-engage the interest of those best qualified, by education or experience, to occupy it, restoring the weight they should carry in a well-balanced constitution, was a task to which Britain's more enlightened statesmanship might well lend itself. The emphasis placed on free and informed discussion by the American writers, no less than by Sir Ernest Barker, Sir Charles Snow, Prof. Miller, or again by Prof. K. C. Wheare, who has examined the whole system of *Government by Committee* in his book under that title, is notable. It should be a first objective in any modification of the organization by which the participation of scientists in the task of Government is promoted; this being so, the dangers which Sir Charles Snow and Sir Bernard Lovell fear should largely be avoided.

With this, however, must go the other things. First, a wide understanding of the nature of politics such as Prof. Miller seeks to encourage. Secondly, a fuller public understanding of what science is about, of its limitations as well as its potentialities. Thirdly, educational programmes and institutions which will ensure the provision of scientists and of administrators capable of understanding the problems of each other and the significance of the factors they are called on to assess in political decisions. It is largely in the measure in which these problems of communication are handled satisfactorily that the conflicts and tensions between the diverse, changing interests of society will occur under conditions and in ways that permit decisions to be made which do not involve technical disaster or catastrophe.

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* Introduction to *Last Essays*. By G. M. Young. Pp. 288. (London: Harper Davis, 1950.)

ROTHSCHILD COLLECTION OF FLEAS

An Illustrated Catalogue of the Rothschild Collection of Fleas (Siphonaptera) in the British Museum (Natural History)

By G. H. E. Hopkins and Miriam Rothschild. Vol. 3: Hystrichopsyllidae (Acedestiinae, Anomiopsyllinae, Hystrichopsyllinae, Neopsyllinae, Rhadinopsyllinae, Stenoponiinae). Pp. viii+560+10 plates. (London: British Museum (Natural History), 1962.) 168*s*.

LIKE its predecessors, this volume is essentially monographic in nature, since the authors have made every effort to include all relevant information which could be obtained about six of the subfamilies of Hystrichopsyllidae in order amply to meet the stated criterion of facilitating the rapid identification of fleas. Inasmuch as the Rothschild Collection contains 1,478 of the known 1,740 species and sub-species of fleas, and includes type-specimens of 826 forms, even strict adherence to the title of the volume would have been of inestimable value to scientists in this field. However, in Volume 3 alone, Hopkins and Rothschild discuss and illustrate 42 forms not represented in the collection, largely species little known heretofore to scientists of the Western World.

There are approximately 350 names of species or sub-species discussed (of which 73 are cited as synonyms or misidentifications), accompanied by 1,049 figures, of which 308 were especially prepared by Arthur Smith and F. G. A. M. Smit for this volume. There are 34 particularly useful illustrations depicting the skeletal anatomy of fleas, not restricted to the Hystrichopsyllidae, and these include details of the male and female genitalia which have been inadequately used in the past studies on Siphonaptera. There are ten plates of photographs of representative genera and species, illustrating the general configurations of these fleas. The glossary supplements the terms used in Volumes 1 and 3, and will do much to standardize the terminology used by siphonapterologists, an end that will be furthered by the table which synonymizes and compares the terms used by Hopkins and Rothschild with those recently proposed by Peus. The family, sub-family and tribal names and references for the fleas considered in Volumes 1 and 2 are presented in this book now that the question of priority for family group names for insects has been settled.

There are terse, dichotomous keys to all the superfamilies and families of fleas, and similar but illustrated keys to the sub-families, genera, sub-genera, species and sub-species of fleas. Under each name there is a citation of all pertinent references of a taxonomic nature. The text consists of diagnoses and short descriptions designed to include characters sufficient to categorize the species or sub-species and pared of extraneous details. The discussions of the various species are followed by detailed data on material represented in the Rothschild Collection, including careful acknowledgment of the source of specimens donated to the Collection.

The figures are all clear, and well illustrate the pertinent morphological features used in modern systematics. The keys are simple, accurate and easy to use, especially since so many of the critical characters are illustrated. The diagnoses and descriptions are lucid, and deal with well-chosen anatomical structures. The list of records contribute much data of value regarding distribution and even affinities of both the fleas and their hosts.

The system of classification used, based on that of Karl Jordan, but modified, especially concerning taxa and little-known forms, is fully accepted by modern siphonapterologists. The logic and soundness of this scheme of systematics are demonstrated by the relative ease by which unidentified fleas can be determined when using any of the volumes in this series. This facility in accurate identification, and in correctly and promptly learning the affinities of specimens, is also an indication of the scientific and intrinsic value of the book, as well as a tribute to the authors' ability to transfer a thorough knowledge of their subject to the printed page in assimilable form.

Despite the complexity of the subject, the technicality of the terms and multiplicity of references and citations, no errors have been noted by me. The high standards of preparation and editing utilized throughout are illustrated by the fact that the corrigenda for the previous volumes, although based on comments from critical specialists, consist mainly of errors in geographical names due to misspelled labels, clarification about the names used for localities and hosts, and changes in status.

The series of volumes by Hopkins and Rothschild constitute the definitive works on Siphonaptera, and Volume 3 is a fit companion to its predecessors in being completely authoritative and indispensable to anyone whose duties or interests in preventive medicine, mammalogy or entomology entail the identification of fleas or information about their affinities, host-range and geographical distribution.

Publication of the next volume in the series is awaited with keen anticipation. ROBERT TRAUB

ORGANIC CHEMICAL METHODS

Methoden der Organischen Chemie

Von J. Houben und Th. Weyl. Vierte, völlig neu gestaltete auflage. Herausgegeben von Eugen Müller. Unter besonderer mitwirkung von O. Bayer, H. Meerwein und K. Ziegler. Band 14, Teil 1: Makromolekulare Stoffe. Pp. lxiv+1360. (Stuttgart: Georg Thieme Verlag, 1961.) 287 D.M.

ORGANIC chemists sometimes tend to regard synthetic macromolecules as dull, structurally indefinite species, and essentially the concern of their physico-chemical or industrial colleagues. The editors of the fourth edition of the Houben–Weyl encyclopædia of organic chemical methods have challenged this view in deciding to devote two massive Volumes to the subject. Part 1 deals with the polyzation of vinyl and divinyl compounds, while its companion, when available, will provide a similar treatment of polycondensation and of polymerizations resulting from ring-opening in heterocyclic molecules, such as epoxides.

Librarians of teaching and research institutes who have purchased other, indispensable volumes of the Houben–Weyl series should have no hesitation in adding this one to their shelves. It appears opportunely, when four decades of development of the science and art of polymerization can be surveyed, when naturally occurring polymers have a central place in biochemical thought, and when chemists in general find themselves increasingly concerned with macromolecular structures. The book will have most direct interest, however, to those engaged in polymerization research, and such prospective purchasers will wish to know what principles have guided editors and authors in their selection of topics. The vast extent of the available literature is indicated by the 19 pages which are devoted in this volume to lists of monographs, and by the 158 pages required for the author- and subject-index.

It is the general practice, in compiling Houben–Weyl volumes, to classify chemical reactions primarily as methods of preparation, and to give subordinate space to the chemical properties of products. This treatment suits an account of synthetic macromolecules, since variation in preparative techniques, in accordance with the nature of the monomer, with properties desired in the polymer, and with the economics of manufacture, is the dominant theme in polymerization literature.

The volume inevitably required multiple authorship, and has been divided nearly equally into general and specialized parts. This arrangement leads to some duplication of subject-matter. The general section begins with a short discussion of terminology, followed by 108 pages devoted to polymerization in bulk and in solution (from Prof. W. Kern and Dr. R. C. Schulz, of the University of Mainz), and then by an account, nearly four times as long, of polymerizations in emulsion and in other heterogeneous systems (from Dr. H. Logemann of the Farbenfabriken Bayer *A.G.*, and other industrial chemists). Kern and Schulz's section is an excellent concise survey, with several hundred references; it includes tabulated data on polymerizable compounds, initiators, copolymerization parameters, and solvents for polymers; it describes apparatus and other practical matters; and it deals briefly with some large themes, such as polymer characterization, branched and block polymers, and (in a sub-section by Dr. B. Braun) stereochemical features of polymerization.

The section on emulsion methods blends chemistry with some technology, and comes from veteran and expert practitioners of the art. All practical aspects are thoroughly considered, with abundant references, and discussion of properties of polymer latices is included. There are many tables; those dealing with peroxides and other initiators, and with components of redox catalyst systems are particularly welcome.

The specialized part of the book is effectively a series of monographs on individual monomers. Kern, Schulz and Logemann are again the authors, supplemented by several other chemists, including Dr. E. Trommsdorf and his colleagues of the Rohm and Haas Company. This latter group deals with acrylic and methacrylic polymers in a little more than 100 pages, and similar space is given by other authors to butadiene and isoprene. About 90 pages are devoted to styrene, about 50 each to ethylene and to vinyl ethers, and smaller sections (10–30 pages each) to other olefines, 2-chlorobutadiene, vinyl halides, vinyl esters, olefinic aldehydes and ketones, vinylsulphonic acid, *N*-vinyl compounds, allyl compounds, and copolymers containing carbon monoxide or sulphur dioxide. Discussions of methods of preparing stereoregular polymers appear in various places. The following topics, discussed in the case of acrylonitrile, exemplify the contents of a typical section: the monomer; its polymerization in bulk, in solution, in emulsion, with ionic catalysts, and with redox catalysts; its use in copolymerizations, summarized in 12 pages of tabulated data.

The book is rich in experimental details of laboratory preparative methods and in references to the patent literature of the polymer-producing industries, but the treatment of physico-chemical aspects of polymerization and of polymer properties was not the intention of its authors. Some readers might prefer less discussion of particular topics and more of others. A book dealing with so large a subject is bound to encounter minor criticisms, but its undoubted achievement is to present, in a most authoritative way, and between the same covers, a vast amount of information that is either scattered through many smaller books or is unobtainable except by laborious searches through chemical abstracts. R. G. R. BACON

CHEMISTRY OF LITHIUM AND SODIUM

Supplement to Mellor's Comprehensive Treatise on Inorganic and Theoretical Chemistry

Prepared under the direction of an Editorial Board. Vol. 2, Supplement 2: The Alkali Metals, Part 1. Pp. xxxix + 1458. (London: Longmans, Green and Co., Ltd., 1961.) 360*s*. net.

THE original second volume of Mellor's *Treatise* dealt with both the alkali metals and the halogens in 894 pages. The supplementary volume on the halogens, published in 1956, contained 1,152 pages and the present one, dealing only with lithium and sodium, has 1,458 pages. Some idea of the growth of knowledge since 1922 is conveyed by these figures. The older volume was the work of one author: the supplements of editorial boards. A good proportion of Mellor's work was done with original sources; the supplements are stated to be based on abstract literature, but the originals must have been used in many cases to provide the numerical information given. Reliance on abstract literature, which seems to have been the origin of most of the work, has its drawbacks, since information on particular topics may be contained in papers of which the titles give no clue to this. As an example, it may be mentioned that no method for the preparation of pure lithium iodide is to be found either in the original volume or the supplement, since it is contained in a paper on electromotive forces. The statement that lithium hydride has no large-scale uses is surely misleading for the deuteride. Such omissions are inevitable when work is divided among many persons, each responsible only for a small field and relying on abstract titles.

The arrangement of literature references at the end of each sub-section is a great improvement on that of the original volumes. The meaning of the figures in brackets after each reference should have been repeated from the first supplement. The collection of literature abbreviations shows what a very wide field of publications, including Russian, has been covered.

The book is not intended for elementary students but for practising chemists and works managers, who in designing plant costing many thousands of pounds require factual information. The surface tension of lithium, we are told, has been calculated by wave mechanics as 400, 639, and 890 dynes cm^{-1}, and empirical estimates are 430 and 473. If the value had not recently been determined experimentally it would have been economical to make such a determination. In chemical industry, again, processes and plants described in students' text-books as 'obsolete' may be of considerable importance.

Every student is taught that the Deacon chlorine process is obsolete, and his teacher has probably never heard of an older and even more obsolete Dunlop process, yet both these are to-day of considerable technical interest. In the case of sodium production, it is by no means improbable that the old thermoreduction with carbon may assume importance, and something is rightly said in the present volume of such processes.

Disquisitions of purely theoretical interest are not very prominent, but enough is said to indicate where further information can be found. In some cases they could with advantage have been further reduced, as in the discussion of alloys in the section on lithium alloys. There is really a good deal of theory in the book, and the treatment is commendably well balanced. The numerical information is very detailed and there are many clear graphs and phase diagrams, and some illustrations of technical apparatus. The type was set up on a 'Varitype' machine and the work reproduced and printed by offset lithography. The result is clear and free from flaws, although some may prefer conventional typography. The price is reasonable in view of the enormous amount of factual information provided, much of it available only in large libraries and there to be acquired only by expenditure of costly time and effort. The book is one which every practical chemist must have available, and all concerned in its production deserve congratulation for the result of their careful and skilful work.

J. R. PARTINGTON

SCIENTIFIC METHOD

The Development of Scientific Method

By W. S. Fowler. (The Commonwealth and International Library of Science, Technology and Engineering. History of Science and Technology Division, Vol. 1.) Pp. xiii + 116. (London and New York: Pergamon Press, 1962.) 10*s*. net.; 1.75 dollars.

SOME fifty volumes of the Commonwealth and International Library of Science, Technology and Engineering are planned for this year, and by 1967 it is hoped that the total will reach one thousand (see *Nature* of May 19, p. 631). The vision behind such an enterprise in scientific literature is matched by the experience and standing of those associated with it. Sir Robert Robinson is chairman of the Honorary Editorial Advisory Board and Prof. W. H. G. Armytage is general editor of the History of Science and Technology Division.

The table of contents of the book under review covers five pages and may well act as an index: it has the advantage of presenting a summary of the material contained in each of the eight chapters. As the title suggests, emphasis is on the method of the sciences, though this is not divorced from the history of science on one hand, or from contemporary philosophy on the other. The book should therefore appeal to a wide circle of general readers as well as to the man of science; it is specially to be recommended for its numerous quotations from sources.

Chapter 1, aptly headed "The Heroic Age of Science", covers the Greek period from Thales to Plato. The importance of Aristotle in relation to method is discussed in Chapter 2. The unusual title, in a book on science, "Theology the Queen of the Sciences", is justified as Mr. Fowler unfolds the influence which Neoplatonism had on scientific method. After reference to the practical aspect of Islamic science, the author returns to the main theme as illustrated by the Schoolmen. This naturally leads to a survey of the Copernican revolution and its results. Another chapter is devoted to "Method and Metaphysics", beginning with Descartes and extending to Kant. Chapter 6, "Revolution and Experiment", surveys the practical approach, in physics, chemistry and biology, of the eighteenth and nineteenth centuries. In a chapter devoted to the exploration of Nature, Mr. Fowler takes the reader into the heart of twentieth-century theory, whether of the atom or of the universe. The book closes with a stimulating approach to the "Nature of Method".

The thoughtful reader cannot fail to be impressed by the way the author presents a comprehensive survey within the confines of a relatively small book. To assist those members of the public who would like to read further, Mr. Fowler has added a book-list arranged under the three headings: "History of Science", "Method of Science", "Philosophy and Science". This bibliography has the merit of comparative brevity. There is, in addition, a valuable "Glossary of Principal Names". The book is illustrated and well produced.

H. D. ANTHONY

SPELÆOLOGY IN BRITAIN

British Caving

An Introduction to Speleology. Edited by C. H. D. Cullingford. Second edition (revised). Pp. ix + 592 + 48 plates. (London: Routledge and Kegan Paul, Ltd., 1962.) 75*s*. net.

THE publishing of *British Caving* in 1953 marked a great advance in the development of scientific spelæology in Britain. This much-enlarged second edition of the book, compiled by members of the Cave-Research Group, shows the amount of work which has taken place in the past nine years. The revision is somewhat uneven. Two chapters, Chapter 4 ("Cave Formations and Deposits"), and Chapter 9 ("Cave Fauna and Flora"), have been both rewritten and enlarged. All the other chapters have been simply reprinted, but many of the more important ones are followed by extensive new supplements and bibliographies indicating the scope of the work that has taken place since 1953. Thus more than 30 pages are added to the chapter on the caving regions of the British Isles and the length of accompanying bibliography is increased by 13 pages. A similar long supplement occurs at the end of the section on archæology and palæontology. Many of the other chapters have short supplements but one or two are completely unchanged. This method of revision means that the new material is not well integrated into the text, but it was probably adopted to keep down the price of this second edition.

The value of the book has been increased by more extensive reference to present-day European and American developments. This is indicated in the comprehensive bibliographical collections, but I still maintain, as I did in 1954, that the value of these would be even greater if brief notes were added. The good format of the book and the high standard of the plates and diagrams have been maintained. The index has been thoroughly revised; but the lists of "Caves, Mines and Resurgences" and of British caving organizations given in Appendix *B* have not been revised.

M. M. SWEETING

NUCLEAR EXPLOSIONS IN SPACE

By Prof. S. F. SINGER
University of Maryland, College Park, Maryland

CONCERN has been voiced[1] about the effects of nuclear explosions in space. The present article deals with these matters. The discussion will be first on a relatively simple level suitable for a non-specialist, followed by a more detailed quantitative treatment. I shall discuss in turn: (1) the injection of electrons from the β-decay of fission fragments; (2) injection of electrons from the β-decay of slow neutrons, from nuclear reactors as well as from bombs; (3) injection of ~ 10 MeV protons from fusion neutrons; (4) removal of naturally trapped particles by 'space sweeping' and by hydromagnetic waves.

Fundamental Problems and Inputs

From a scientific point of view, the major questions raised are whether such explosions can tell us more about the origin of the natural radiation belts around the Earth; from other points of view one wishes to know whether such explosions can affect or possibly do away with the natural radiation belts; or whether they increase significantly the level of radiation in the space around the Earth. These questions lead naturally to the possibility that there may be other methods, apart from nuclear explosions, for affecting the natural radiation belts or for producing an artificial one.

As we shall see, practically all these questions can be answered in a direct way. The only prerequisite is an understanding of the properties of the Earth's magnetic field and atmosphere at high altitudes, and the interactions of the products of a nuclear explosion with the field and the atmosphere.

Most of the relevant facts and theories have already appeared in the open scientific literature. For example, a theory for the trapping of charged particles in the Earth's magnetic field was put forth as long as six years ago[2]. It, in turn, was based on basic theories developed by the Swedish astrophysicist Alfvén[3]. Even earlier, Ross Gunn in the United States and a Norwegian astrophysicist, Störmer, made many fundamental mathematical investigations of the motion of particles in a magnetic field.

The only item not released is a description of the nature of particles and radiations coming from a nuclear explosion. Here, however, a basic knowledge of nuclear physics, and in particular of the fission process of uranium, can supply much of the needed information. Thus, a physicist can work out the consequences of atomic explosions in space. It may seem strange to the non-specialist that such assessment is possible without recourse to classified data or even actual experiments. It turns out, however, that order-of-magnitude calculations are in many cases able to give a clear-cut yes-or-no answer. In most practical cases, it is sufficient to know that an effect is either many times too large or many times too small.

Trapping in Earth's Field

Basic to all the discussion to follow is an understanding of the motion of charged particles in a magnetic field.

A proton or electron will simply spiral about a line of force. The spiral can be quite flat with pitch angle of 90° just as in a cyclotron. Or it can have a smaller pitch angle, in which case it will progress along the line of force and trace out a helix or screw thread. The pitch angle depends essentially on how the particle is started out in its motion in the magnetic field.

The situation becomes of great interest when the magnetic field changes in intensity from point to point, as it does in the case of the Earth. As the particle moves into a region where the magnetic field increases (as shown by the fact that the lines of force crowd together), the pitch angle steepens, the spiral becomes flat, and the particle is reflected at the point where the pitch angle reaches 90°. The Earth's magnetic field is constructed in such a way that its strength increases as one approaches the Earth's surface. Therefore, a particle released in space will spiral about a line of force until it reaches a point where the magnetic field is strong enough to reflect it. From then on it will bounce back and forth between the mirror points and be trapped (Fig. 1).

The two major problems now are: (1) How do particles get into the magnetic trap, how many, and where? (2) How long are particles stored in the magnetic trap, and what happens to them to remove them?

Most of the trapped particles, and practically all beyond a radial distance of 2 Earth radii (altitude of 4,000 miles), appear to derive their energy from the Sun by a process of local acceleration. A similar process seems to furnish the many low-energy particles necessary to produce the aurora borealis and australis.

The inner belt of trapped particles found by Van Allen[4] in 1958 and identified by Freden and White a year later[5] is probably not of solar origin. I believe that it is produced by high-energy neutrons

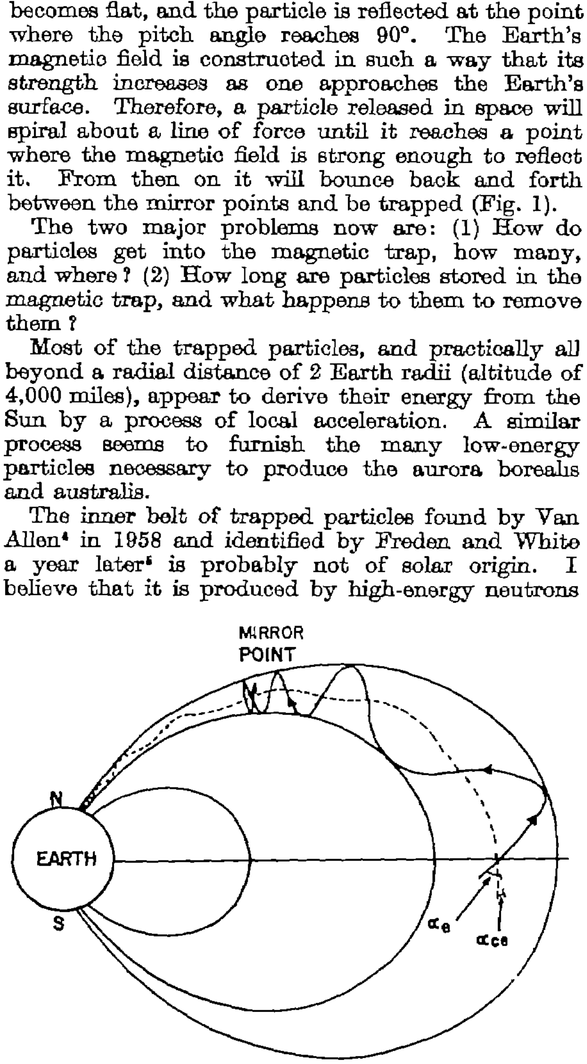

Fig. 1. Schematic diagram showing the helical paths of two particles in a dipole magnetic field. Solid line corresponds to a pitch angle greater than critical; the particle survives for many north-to-south orbits and is properly termed 'trapped'. Dashed line corresponds to critical pitch angle; the particle enters the 'loss cone' and disappears quickly into the dense atmosphere

arising from the impact of cosmic rays on the Earth's upper atmosphere. On the way out into space some of these neutrons decay radioactively into protons and electrons which are then trapped by the Earth's magnetic field. This theory predicts a maximum intensity for high-energy protons close to the Earth, and at low latitudes near the equator[6].

Particle Motion and Injection

In addition to bouncing back and forth between the mirror points, which are located on about the same magnetic meridian, it has been shown that the particles will also drift in longitude. Positive particles will drift to the west, and negative particles, such as electrons, will drift to the east.

It is of great practical importance in discussing the effects of high-altitude explosions to know the speed with which the particles transfer from the southern to northern hemisphere and the speed with which they drift around the Earth. Calculations made in connexion with the trapped particle theory of magnetic storms and auroræ are reproduced in Table 1.

Table 1. CHARACTERISTIC TIMES FOR TRAPPED PARTICLES IN THE GEOMAGNETIC FIELD (FROM REF. 18)

(1) Gyro period = $2\pi\, m/qB$ (sec; B in gauss)
Protons: $6·5 \times 10^{-4}/B$
Electrons: $3·55 \times 10^{-7}/B$

(2) Bounce period = $4\, s r_e/\beta c$
with $s = 1·38 - 0·32\,(\sin \alpha_e + \sin^{\frac{1}{2}} \alpha_e)$
For $\beta = 1$ (relativistic): $\sim 8·5 \times 10^{-2}\,(r_e/R)$ sec

(3) Azimuthal drift period $= 2\pi q B_e R^2\,[mc^2\gamma\beta^2\,(r_e/R)]^{-1}$
Non-relativistic—in equatorial plane.
$= 2·65 \times 10^9\,[E(r_e/R)]^{-1}$ in sec; E in eV
Relativistic—in equatorial plane:
$= 5·3 \times 10^9\,[mc^2\gamma\beta^2(r_e/R)]^{-1}$ sec, mc^2 in eV

For particles which move out of the equatorial plane (that is, for $\alpha_e \neq 90°$) the drift period is increased somewhat, namely, by a factor $1·5\,(1 + 0·5 \sin \alpha_e)^{-1}$.

Here R is the Earth's radius, $r_e = LR$ the distance to the crest of the line of force in the equatorial plane, with L the so-called magnetic shell parameter, s is the dimensionless length of the spiral path from equatorial plane to mirror point.

An important consideration in discussing high-altitude nuclear tests is to know what fraction of the particles released is immediately dumped into the atmosphere and what fraction is stored in the Earth's field. This result depends on the exact location at which the explosion is carried out.

If we release a particle 'above the equator' (that is, in the plane of the magnetic equator) with a certain initial (directed) velocity and therefore a certain initial pitch angle, then as it proceeds along the line of force it will either be reflected before it hits the atmosphere, or it will hit the atmosphere first, in which case it is absorbed and therefore removed from storage. We can define a critical pitch angle α_{ce} at the equator: particles that have a smaller angle hit the atmosphere, while those that have a larger angle are reflected and stored (see Fig. 1). Table 2 gives this information for a number of lines of force. (A line of force is defined by the latitude at which it intersects the Earth's surface. It then sweeps out to a very high altitude LR above the equator and re-enters the Earth in the other hemisphere at approximately the same magnetic latitude.)

If we assume an isotropic burst above the equator, then the last column in Table 2 gives the fraction of particles stores. However, if we move the burst point along the same line of force off the equator, the fraction stored becomes less and less.

Finally, if the explosion takes place just above the atmosphere, practically all the particles will be released into the atmosphere and only a few will be stored. This fact can be clearly seen from Fig. 1. Only particles which start out with a pitch angle of nearly 90° will again be reflected above the atmosphere at the other hemisphere, but as we shall see later, their lifetime will not be very long.

Table 2. FRACTION OF PARTICLES WHICH REMAIN TRAPPED*

It is assumed that particles are released isotropically at a point near the plane of the magnetic equator. Particles having a pitch angle less than α_{ce} are immediately absorbed into the atmosphere. The rest remain trapped with a lifetime which increases with the particle's pitch angle α_e (always referred to when the particle crosses the equatorial plane). Particles with $\alpha_e = 90°$ stay in the equatorial plane and live longest

Line of force defined by L	λ	Critical pitch angle α_{ce}	Fraction trapped† (per cent)
1·1	17°	55°	57
1·5	35°	27°	89
3·0	54°	12°	97
5·0	63°	9°	99
7·0	68°	2°	99·9

* Ref. 6.

† This fraction is greatly reduced when the burst point is off the equatorial plane, and becomes very small when it is at the limit of the atmosphere.

The point of bomb burst therefore controls what happens. If one wants to dump the particles into the atmosphere in the opposite hemisphere, the burst altitude should be as low as possible. (For added effect, the burst should take place where the magnetic field is higher than usual[7]. Then the particles going to the opposite hemisphere will enter the atmosphere much deeper and produce more pronounced effects, such as artificial aurora.)

On the other hand, if one wants to lose as little of the radiation as possible into the atmosphere and store it in space for a long time, then the burst point should be high above the equator.

Particle Lifetime

A crucial problem is the lifetime of the particles trapped in the field. In the absence of any atmosphere and in a perfectly quiet magnetic field, the particles could stay trapped for ever (except where the field is too weak). But in fact there exists a very tenuous atmosphere around the Earth which becomes more and more rarefied the higher we go. As the trapped particles collide with atmospheric atoms or ions or atmospheric electrons, the particles are scattered, their kinetic energy decreases, and their pitch angle changes slightly. If, by chance, the pitch angle should drop below the critical value, then the trapped particle is released into the atmosphere and of course disappears.

It is almost immediately obvious that those particles which penetrate deepest into the atmosphere, where the density is highest, will lose energy fastest and will have the shortest lifetime, while the particles which stay close to the equator, and therefore at high altitudes, will be preserved much longer.

This calculation of the length of time for which a particle remains trapped in the Earth's magnetic field is a complex theoretical problem.

The lifetime T depends on the nature, energy and trajectory of the particle, on the density of neutral atoms and of ions in the outer atmosphere, and on the degree of 'noisiness' of the magnetic field which in turn is related to 'activity' on the Sun and therefore quite variable.

A very detailed treatment has recently been given of lifetime for electrons[8] and for protons[9], with the following results:

(1) Up to about 2 Earth radii, the atmosphere determines the lifetime of trapped particles except for very high energy protons ($\gtrsim$ 100 MeV).

(2) In any event, the atmosphere sets an upper limit; magnetic field fluctuations would decrease this lifetime.

(3) For protons generally, and for relativistic ($> 0{\cdot}5$ MeV) electrons, energy-loss sets the lifetime, and pitch angle scattering can be neglected.

(4) For non-relativistic electrons, scattering must be considered, but this will not concern us here since we will count the electron as 'dead' when its final energy E_f drops below 0·5 MeV (a typical detector threshold).

(5) A useful approximate formula for relativistic electron lifetime is[10] (in sec):

$$T = 3{\cdot}33 \times 10^{13} (\bar{\rho}\, mc^2 \ln\Lambda)^{-1} (E_0 - E_f) \qquad (1)$$

where $\bar{\rho}$ is the averaged atmospheric density in 'effective' electrons per cm^3 (ref. 8), mc^2 is the electron rest energy in MeV, $\ln\Lambda \sim 23$; E_0 and E_f are measured in MeV.

For electrons (with $E_0 = 2$ MeV and $E_f = 0{\cdot}5$ MeV) which do not travel too far from the plane of the magnetic equator, we derive approximate lifetimes (Table 3). These values are substantially less for lower-energy electrons and for particles the trajectories of which extend far along the line of force into the denser atmosphere.

Table 3. LIFETIME FOR A 2-MeV ELECTRON TO SLOW TO 0·5 MeV

Altitude (km)	500	1,000	2,000
Air density (gm cm^{-3})	$4{\cdot}5 \times 10^{-15}$*	3×10^{-17}*	8×10^{-20}†
$\bar{\rho}_E$ electrons cm^{-3})	6×10^{8}	4×10^{6}	1×10^{4}
Lifetime	2 hours	2 weeks	14 years

* Predominantly oxygen (ref. 18).
† Predominantly hydrogen (ref. 9).

Thus, it is shown that the lifetime increases as we deal with radiation released at higher altitudes or higher latitudes, that is, along a line of force which sweeps out to a greater altitude above the equator. Beyond 3 Earth radii, the atmosphere is probably no longer the determining factor for lifetime. Instead, it is believed that the 'noisiness' of the rather weak magnetic field causes scattering of the particles and eventually removes them from the trapped condition.

Artificial Radiation Belt

Now, in fact, a direct measurement of particle lifetime is of fundamental importance for studying the origin of the natural radiation belts, for studying interactions of magnetic waves with trapped particles, and for studying the density of the outer atmosphere; many other applications could be mentioned.

The best way to measure lifetime is to inject more trapped particles suddenly and look at their decay. For scientific purposes, an atomic explosion is a very crude injection device. So, for that matter, is the natural injection which takes place, for example, after solar flares. A much more refined measurement is possible if one uses an electron accelerator for injection, an experiment suggested independently by N. C. Christofilos[11] and by me[12]. Then the energy of the electrons can be precisely controlled, the direction of injection can be controlled if desired, and the number of electrons released is definitely known. Also, it can be turned on or turned off at will.

The Uranium Fission Process

We now summarize some of the properties of the fission process[13]. A uranium nucleus which has absorbed a neutron will undergo fission and break up into two smaller fragments. Most frequently the fragments are krypton of atomic weight 93 and barium-143. The velocity of the fragments is of the order of 10^8 cm/sec corresponding to a total kinetic energy of more than 160 MeV (million electron volts).

In the fission also an average of 2·5 neutrons are released having a most probable energy of 0·8 MeV and a mean energy of 2 MeV; this multiplication of neutrons makes a chain reaction possible. A small fraction of neutrons, about 1 per cent, are delayed and released after about 0·01 sec (this fact makes the operation of a controlled nuclear reactor possible). Prompt γ-rays are released having an energy of about 5 MeV.

The two fission fragments are of particular interest. They are highly unstable and will undergo several radioactive decays before they reach a stable nucleus condition. In each decay they emit an electron. The energy of the β-rays can be calculated from nuclear theory and averages about 2 MeV.

Now let us take as our reference a fission bomb of 1 megaton TNT (equivalent) yield. A simple calculation tells us that this release of energy corresponds to $4{\cdot}2 \times 10^{22}$ ergs or $2{\cdot}5 \times 10^{28}$ MeV. If we make use of the fact that each fission releases about 200 MeV then we derive the total number of fissions as $1{\cdot}3 \times 10^{26}$. (The weight of the fissioned atoms is only 10 lb., but the amount of fissionable material in the bomb is of course much greater.)

Knowing the number of fissions, we can calculate the output of neutrons, γ-rays, and β-rays (electrons). The latter are emitted with a wide range of energies, but it seems reasonable to assume about one high-energy electron (about 2 MeV) per fission with the rest at much lower energies where their effects are of little importance because of their much smaller lifetime.

Only the highest energy electrons emitted from the fission fragments are of any significance for trapped radiation. The fission fragments themselves are unimportant, the γ-rays escape from the Earth's field since they are uncharged, and most of the neutrons will escape. In any event, the decay of a low-energy neutron only gives a low-energy proton and an electron of about 0·5 MeV; both have very short lifetimes.

Limits to Trapped Particle Flux

It would seem that effects of successive bomb explosions could be superimposed indefinitely to build up extremely large trapped particle fluxes. This, however, is not the case. There is a theoretical limit to what can be trapped in the Earth's magnetic field; it can store only a certain maximum concentration of particles set by their reaction on the magnetic field itself. The particle energy density can be at most a fraction, about 50 per cent, of the magnetic field energy density. Since the latter varies as the inverse cube of distance, the energy density, which is $B^2/8\pi$, varies as $1/r^6$ (Table 4).

Electron Injection in the Pacific Test

Case I: Injection of electrons from fission fragments. We are now ready to consider the injection of fission electrons from the forthcoming test shots in the Pacific. According to the *New York Times*, a nominal 1 megaton bomb will be exploded 500 miles over Johnston Island (longitude 191° E., latitude 17° N.). Two sub-cases will be considered: (*a*) injection at the point of explosion; (*b*) injection along the line of force.

Case I (a): What will happen to electrons which are released at the point of explosion with an isotropic

Table 4. UPPER LIMIT TO PARTICLE FLUX WHICH CAN BE TRAPPED

Distance from centre of Earth (in Earth radii)	1 2	1·5	3·0	5 0
Maximum particle concentration cm^{-3} (2-MeV electrons)	400	110	1·7	$7·8 \times 10^{-2}$
Maximum particle flux cm^{-2} sec^{-1}	$1·2 \times 10^{13}$	$3·3 \times 10^{12}$	5×10^{10}	$2·3 \times 10^{9}$

These limits are based on the principle that the energy density of the trapped particles may not exceed 50 per cent of the energy density $B^2/8\pi$ of the unperturbed magnetic field.

distribution in the directions and with an assumed unique energy of 2 MeV ? (This case would be realistic only if the fission fragments had a β-decay half-life very much shorter than the bounce period.) In order to solve this problem we have to examine first of all the properties of the magnetic line of force which passes through this point. From published tables[14], it is seen that at the 'crest' of the line of force the magnetic intensity will be 0·20 gauss; the altitude will be 1,200 km.

It would seem, from Table 3, that these particles could survive for many bounce periods above 800 km. But now we must consider the peculiar construction of the geomagnetic field. To a reasonable approximation the field can be represented by a dipole which is placed eccentrically about 436 km from the centre of the Earth. (Therefore particles trapped in the magnetic equatorial plane do not maintain a constant altitude as a function of longitude but describe an eccentric orbit which is displaced with respect to the Earth's centre.) Adding to this the effects of local anomalies as determined by the actual magnetic field causes the particles to dip down very deeply in the South Atlantic (Fig. 2). In fact, all the particles which are released at the explosion point 800 km above Johnston Island, even those with 90° pitch angle, will be removed on their first trip around the Earth.

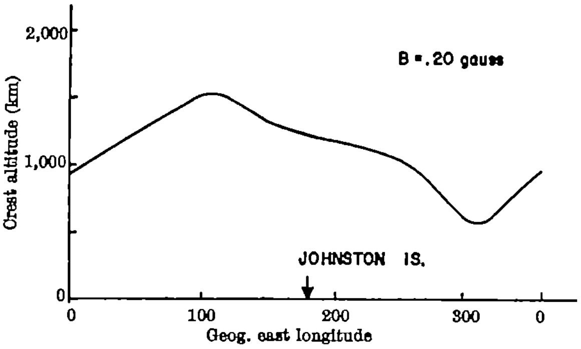

Fig. 2. Altitude of the crest of a magnetic shell of the actual terrestrial field corresponding to a particular value of B. The dip corresponding to the South Atlantic anomaly shows up clearly at 310° longitude east. This curve was obtained from the Tables of ref. 15 and from ref. 14

The calculation is carried out in the following way. We note first of all that the magnetic moment μ, the so-called first adiabatic invariant, is given by:

$$\mu = E_{\perp}/B = \text{const.}; \therefore \sin^2\alpha/B = 1/B_m \qquad (2)$$

where $E_{\perp}$ is the kinetic energy perpendicular to the magnetic field, B the local value of magnetic field and B_m the value at the mirror point.

There exists also a second adiabatic invariant, the so-called longitudinal invariant, given by:

$$I = mv \oint (1 - B/B_m)^{\frac{1}{2}}\, dz = \text{constant} \qquad (3)$$

Together, conservation of the two invariants determines the orbit of the particle completely and locates the magnetic shell in which it travels. Tables of the second invariant have been computed[15] on the basis of Vestine's spherical harmonic expansion of the actual magnetic field.

We obtain first the minimum mirror altitude corresponding to complete energy-loss of a particle, as it passes the South American anomaly.

For orbits which go to a low enough altitude where the scale height is small compared with R, the Earth's radius, the average density $\bar{\rho}$ over the orbit is related to the density at the mirror point ρ_m by[16]:

$$\bar{\rho} \sim \rho_m \frac{1}{s}\left[\frac{\pi}{6}\,\frac{H}{r_e}\,\frac{x_m \quad (4 - 3x_m)^2}{(8 - 5x_m)\,(1 - x_m)}\right]^{\frac{1}{2}} \qquad (4)$$

where $x_m = r_m/LR$; r_m is the geocentric distance to the mirror point, LR is the distance to the line of force in the equatorial plane for an equivalent dipole field; s is the dimensionless length of the spiral path from equatorial plane to mirror point.

The thickness of atmosphere traversed from equatorial plane to mirror point is (for low mirror points $r_m \sim R$):

$$d = \bar{\rho}sLR \sim \rho_m\left[\frac{\pi\ HR\ (4 - 3L^{-1})}{6(8-5L^{-1})(1 - L^{-1})}\right]^{\frac{1}{2}}\ \text{g/cm}^2 \qquad (5)$$

Now L is defined as (sea-level field/crest field)$^{\frac{1}{3}}$; here $L \sim (0·31/0·20)^{\frac{1}{3}} \sim 1·20$; hence:

$$d \sim 2·8 \times 10^4 H^{\frac{1}{2}}\rho_m \qquad (6)$$

The South Atlantic anomaly is ~ 30° wide. Calculating a drift time of 30 min and a bounce time 0·1 sec, a particle makes 30 × 60 (30/360)/0·1 = 1,500 reflexions in the anomaly.

In each penetration it passes through d g cm^{-2}. Now a 2-MeV electron has a range ~ 1 g cm^{-2}. Therefore, if we set:

$$d = 2·8 \times 10^4 H^{\frac{1}{2}}\rho_m = (1{,}500)^{-1}\ \text{g cm}^{-2} \qquad (7)$$

we find the maximum permissible value of $H^{\frac{1}{2}}\rho_m$ to be $\sim 2·38 \times 10^{-8}$ g cm^{-3} $cm^{\frac{1}{2}}$. Taking $H \sim 10$ km (near 100 km altitude) we have $\rho_m \sim 2·4 \times 10^{-11}$ g cm^{-3}. This density occurs near 110 km; and this is the minimum mirror altitude for just one pass.

On the other hand, Tables in ref. 15 give an estimate of the mirror altitudes from 35 to 110 km for Case (*a*). Therefore, it is quite likely that most electrons will not survive the first passage of the anomaly.

Case I (*b*): We now consider a more realistic case in which the electrons are not released at the explosion point but all along the line of force which passes through this explosion point. It may be assumed that the fission fragments after being created will stay tied to the line of force, bouncing back and forth but moving very slowly in longitude. Therefore, some β-decay of fission fragments, and release of electrons, will take place near the peak of trajectory, that is, in the immediate vicinity of the equatorial plane. These electrons have the best chance for survival.

Let us consider an extreme case of an electron which is released with a 90° pitch angle in the equatorial plane; its lifetime gives an upper limit to the decay of the trapped electrons. The procedure is to determine again the magnetic shell in which it moves, integrate the density within the equatorial plane (that is, along the 'crest' of the shell), and compare with the range of the electron.

A plot of the crest line (Fig. 2) (obtained from the Tables in ref. 15 for $B = 0·20$) gives a curve which has an altitude of 1,200 km at longitude 191° E. (Johnston Island); a maximum altitude of 1,540 km at 106° E., and a minimum of 560 km at 310° E.

The width of the minimum is 30°, measured at an altitude one scale height ($H \sim 100$ km) above 560 km. The average density therefore is $2 \times 10^{-16} \times (30/360)$ g/cm³ and the lifetime, for a range of 0·9 g/cm², is:

$$0{\cdot}9 \left(\frac{360}{30}\right) \frac{1}{2 \times 10^{-16}} \; \frac{1}{3 \times 10^{10}} = 2 \times 10^{6}$$

or about one month.

As a very rough approximation, a change of the firing altitude by $\pm$ H km will change this maximum lifetime by $\pm$ a factor e.

We next must calculate the fraction of fission electrons which may survive for this maximum length of time. This number is obtained in three steps:

(i) We assume that at the explosion point the fission fragments are emitted isotropically. We then have an immediate loss of all fission fragments except those which are emitted in a small solid angle around a local pitch angle of 90°. This solid angle is calculated by finding the pitch angle α_e at the emission point which leads to a mirror point at an altitude which is lower by 1 scale height.

Denoting the emission point by:

$$r_1 = x_1 r_e \tag{8}$$

and the lower mirror point by:

$$r_2 = x_2 r_e = r_1 - H = (x_1 - h) r_e \tag{9}$$

and using the relation (2) as well as:

$$B(x) = B_e \frac{(4 - 3x)^{\frac{1}{2}}}{x^3} \tag{10}$$

we have:

$$\sin^2\alpha_e = B_1/B_2 = (x_2/x_1)^3[(4 - 3x_1)/(4 - x_2)]^{\frac{1}{2}} \tag{11}$$

For $H \ll R$, or $h \equiv H/R \ll 1$

$$\cos \alpha_e \sim h^{\frac{1}{2}} \, [(3/x) + 3/(8 - 6x)]^{\frac{1}{2}} \tag{12}$$

A typical value for $\cos \alpha_e$ is 0·24; it corresponds to an emission point at 800 km altitude, and to $H \sim 100$ km. (Actually the remaining 76 per cent of the fission fragments are not really all lost; a good fraction, however, is slowed down so appreciably that the chances of decaying near the crest line are negligible.)

(ii) Only a fraction of the fission fragments will actually decay near the peak of trajectory—this fraction must be estimated by looking at the particular magnetic line of force, and calculating the fraction of time which the particle spends near the crest.

The time taken to spiral from the crest (in the equatorial plane) to the mirror point is:

$$sRL/v \tag{13}$$

where $s \sim 1{\cdot}0$ for a wide range of equatorial pitch angles and v is the particle's speed.

The neighbourhood of the crest is defined by letting the particle descend one scale height along the line of force to a latitude λ. Then $\cos^2\lambda = 1 - h$, and for small values of h, one finds $\lambda \sim h^{\frac{1}{2}}$. Therefore, the time spent near the crest is $\sim RLh^{\frac{1}{2}}/v$ (assuming a small equatorial pitch angle), and the fractional time becomes $h^{\frac{1}{2}}$.

(iii) Finally, in the β-decay the electron is also emitted isotropically, and again only those survive which are emitted in a small solid angle around 90°. This fraction is given by an expression like (12).

If we now multiply these three fractions together, we see that of the initial fission fragments only about $4h^{3/2}$ will give electrons which have the right orbit to survive for the maximum length of time.

For our particular example, this time was ~ one month, and the fraction turns out to be $\sim 5 \times 10^{-3}$ for a scale height of 90 km. It should be noted, however, that because of the non-dipole character of the magnetic field, the altitude of the trapped particle, and the relevant scale height, will vary with longitude. Just as in the foregoing lifetime calculations where the peak density encountered had a significant effect, so it is necessary to use the smallest scale height encountered to calculate the fraction having this lifetime. Since H can be as low as ~ 8 km in the E-layer, the fraction may be as small as 10^{-4}.

Numerical Estimates

In the foregoing discussion, the assumption was made that the β-decay lifetime of a fission fragment is long compared with the bounce time; this leads to an upper limit for the fraction of $\sim 10^{-3}$–10^{-4}.

Also it was assumed implicitly that all the explosive power is due to uranium fission. In fact, an unspecified fraction, depending on the cleanness of the bomb, will be due to hydrogen fusion, which leads to a great reduction in the number of fission fragments. However, to get upper limits, we will ascribe all the explosion to fission.

The number of 2-MeV electrons injected is therefore $\sim 10^{23}$. The volume into which they diffuse after a few drift periods around the Earth is $\sim 2 \times 10^{24}$ cm³; here the thickness of the shell is adopted as ~ 40 km, similar to that of the *Argus* experiment. The concentration of electrons thus becomes 5×10^{-2} per cm³, and the flux $\sim 1{\cdot}5 \times 10^{9}$ per cm² sec. It should be emphasized again very strongly that this flux is an upper limit for the various reasons already mentioned. In addition, a greater shell thickness, as might be expected from the larger explosion, increases the volume in proportion, and thus decreases the flux further.

Adverse effects produced by such large radiation fluxes on unshielded components in satellites are not in the least surprising. Semiconductors, for example, solar cells, transistors, etc., are most sensitive to large radiation doses; a value of $\sim 10^{6}$ r. can result in a deterioration in performance, or produce failure. Now a dose of 10^{6} r. corresponds to 6×10^{13} MeV of energy deposited in 1 gm of tissue. Using a minimum energy loss rate of 1·6 MeV per gm-cm⁻², the flux of $1{\cdot}5 \times 10^{9}$ per cm²-sec gives the damage dose in about 1 h. This time may be more like 100 h, considering that this flux is an extreme upper limit, and that the solar cells spend only a fraction of each orbital period in the artificial radiation belt.

Case II. Injection of electrons from the β-decay of Fission Neutrons. As described earlier, neutrons of (most probable) energy 0·8 MeV are emitted in the fission process. In a bomb explosion a certain fraction, partly moderated, will escape. Their effects in producing trapped particles are quite inconsequential (compared, for example, with the fission electrons just discussed); hence, order-of-magnitude estimates will suffice. The maximum energy of electrons from neutron decay is 780 keV, with a mean near 300 keV. Hence the lifetime, and the intensities, are greatly reduced compared with 2-MeV electrons (by more than a factor 10).

The β-decay of slow neutrons from natural cosmic ray albedo contributes a portion of the trapped electrons in the Earth's radiation belts[8]. At the present time we do not know just what this fraction is; but in any event it is likely to be small in number as compared with the electrons which are produced by other natural sources. Particularly, farther out in the radiation belt, at lines of force connecting to high latitudes, the production by a process of local acceleration must be quite important since electrons are often precipitated into the atmosphere at a very heavy rate.

It will be useful to compare the neutrons emitted in a fission explosion directly with the cosmic ray neutron albedo; taking the latter to have an intensity of about 1 per cm^2 per sec, we arrive at a figure of $1 \cdot 6 \times 10^{26}$ emitted over the whole Earth each year. This is just about the number of fissions of a 1 megaton bomb, and if we assume that the number of escaping fission neutrons equals the number of fissions we can then state that an explosion will contribute about one year's worth of neutrons.

In addition, with a velocity of $\sim 1 \cdot 5 \times 10^9$ cm per sec, and a decay half-life of $\sim 10^3$ sec, only a small fraction ($\sim 10^{-3}$), of the neutrons actually decay within 2 or 3 Earth radii. We have therefore $\sim 10^{23}$ electrons diffused in a volume of $\sim \frac{4}{3}\pi\,(1 \cdot 5 \times 10^9)^3 \sim 10^{28}$ cm^3, leading to a flux of $\sim 10^5$ per cm^2 sec. (A more accurate estimate could be made; see, for example, Case III.)

It is interesting to consider a case of continuous injection, for example, the artificial radiation belt produced by operating a nuclear reactor, for example, in a communication satellite[7]. If the reactor's power-level is 100 kW, and its orbit 1,000 miles above the equator, then the following estimates apply: number of uranium fissions about 6×10^{15} per sec, which also gives roughly the number of escaping neutrons.

Assuming these to be of thermal energy, they travel an average distance of 200 miles before decaying radioactively into an electron and proton. The electrons are released with an energy of about 0·5 MeV into a volume around the Earth of about 6×10^{25} cm^3. Taking a lifetime of a few months, their concentration build up to 10^{-3} per cm^3; but their flux will be something like 10^7 per cm^2 per sec.

Fortunately, it is easy to shield against 0·5-MeV electrons, which have a range of $1 \cdot 7 \times 10^{-1}$ g/cm^2 of aluminium; the reactor therefore produces no hazard. However, the phenomenon of trapped radiation can be used to detect the existence of an orbiting nuclear reactor.

Case III. Injection of protons from fast neutrons. In the case of explosion of a fusion bomb, in which isotopes of hydrogen are involved, the reactions between deuterons and tritons give rise to 14-MeV neutrons. Let us assume as an extreme upper limit that everyone of these neutrons escapes. (Obviously this cannot happen unless the bomb has essentially no mass, but this assumption does give an extreme upper limit for the resulting trapped protons.) For 1 megaton, their extreme maximum number N is 2×10^{27}.

These neutrons may lose some energy in traversing the bomb material; therefore, we shall assume a mean energy of 10 MeV. These neutrons will travel with a speed of 4×10^9 cm per sec and will therefore move ahead of the bomb debris, fission fragments, etc. While travelling through the Earth's magnetic field, a certain fraction of them will decay and the resulting protons will carry forward the kinetic energy.

In a sense this calculation is very similar to that already performed[9] for neutrons which are produced by solar 'cosmic rays' impacting on the Earth's polar regions, but with two important differences: (i) the solar cosmic rays produce many neutrons with energies higher than 10 MeV which lead to highly penetrating protons; in the present case, the neutron energy is 10 MeV which means that we arrive at a proton that cannot penetrate more than $1 \cdot 7 \times 10^{-1}$ g/cm^2 of aluminium. (A thickness of $\sim$ 0·6 mm.) (ii) But on the other hand, solar cosmic rays have a very small injection efficiency for trapped protons, $\sim 10^{-3}$ on intermediate lines of force, zero on low latitude lines of force[9]; for a fusion bomb explosion near the equator the injection efficiency is near unity.

From a scientific point of view, it is worth while to do a detailed calculation on the distribution of the injected protons, and on their lifetimes. This is not the case for the electrons (cf. Case II). The reasons are as follows:

(i) Protons are essentially unscattered in atmospheric collisions; their magnetic moment is conserved. This makes the lifetime analysis much simpler.

(ii) There seem to be other sources for $\sim$ 0·5 MeV electrons, unaccountable quantitatively on an *a priori* basis. For protons the only other source appears to be cosmic-ray neutron albedo which can be described quantitatively[8]. Therefore, the fate of the newly injected protons can be traced with little ambiguity.

(iii) Protons can be easily detected, down to about 1 MeV. Very low energy electrons ($\lesssim$ 50 keV) are difficult to measure precisely.

It is first necessary to calculate the decay density, that is, the number of decays per cm^3 as a function of distance from the explosion. The flux of neutrons will vary according to the inverse square law, with the neutrons streaming out radially from the explosion point.

The number of decays is quite small; it is given as a probability per cm by the expression:

$$\lambda = \Lambda(\beta\gamma c)^{-1} \tag{14}$$

where Λ is the decay constant, 9×10^{-4} per sec, βc the velocity, and γ the relativistic dilation factor $(1 - \beta^2)^{-\frac{1}{2}}$.

This decay probability turns out to be 2×10^{-13} per cm, which indicates that within the Earth's magnetic field the neutrons will be scarcely attenuated by decay; therefore, the assumption of the inverse square law is well justified.

For purposes of rough estimates, we will assume that only about 10 per cent of the decays, those within an angle of 30° of zenith, are significant. The reason is that neutrons coming off at larger angles release protons in a fairly dense atmosphere; these therefore have a relatively shorter lifetime.

Consider now the number of neutron decays Q within a volume which is determined by the intersection of a solid angle cone Ω and a spherical shell of radius h (approximately the altitude above sea-level if the explosion is at not too high an altitude) and thickness d. Because Ω is small this portion of a spherical shell can be considered as a portion of a shell of magnetic lines of force, and therefore the protons injected are spread around in longitude into a much larger volume V.

$$Q = N(\Omega/4\pi)\lambda\, d \tag{15}$$

$$V = 2\pi(R + h)(\sim h)\, d \tag{16}$$

The resulting concentration of trapped protons is then:

$$n = Q/V = N(\Omega/4\pi)\lambda\left(\frac{1}{2\pi}\right)(Rh + h^2)^{-1} \text{ per cm}^3 \tag{17}$$

Numerically

$$n = 2 \times 10^{27}\ (0{\cdot}05)(2 \times 10^{-13})\left(\frac{1}{2\pi}\right)(Rh + h^2)^{-1}$$
$$\sim 4 \times 10^{12}\ (Rh + h^2)^{-1}$$

Table 5 gives the initial flux nv as a function of altitude for an assumed burst at 530 km over the equator; in equation (17) $R = 6{,}370 + 530$ km; h = altitude − 530 km.

Table 5. 10 MeV Proton Fluxes and Lifetimes

Altitude (km)	730	1,030	1,530	2,530
Flux (cm² sec)$^{-1}$	$1{\cdot}1 \times 10^6$	$4{\cdot}3 \times 10^5$	2×10^5	9×10^4
Lifetime (sec)	4×10^3	2×10^4	2×10^6	4×10^9

The lifetime is given quite accurately as (range/atmospheric density). Some caution must be exercised in obtaining the relevant density $\bar{\rho}$. Because of the non-dipole structure of the geomagnetic field, the value of $\bar{\rho}$ depends on altitude as well as on longitude; an average must be obtained over the crest of the magnetic shell (cf. the analogous treatment for the fission electrons, Case I). For a Johnston Island firing (longitude 191° E.) $\bar{\rho}$ corresponds to ρ at longitude 290° E. and 330° E. where the crest altitude is ~470 km less than at 191° E. Applying the same correction at all altitudes we obtain the lifetimes of Table 5.

Case IV. Removal of naturally trapped radiation. One of the most intriguing aspects of a nuclear explosion is to determine whether it will dump some of the naturally trapped protons. The opinion has been expressed that some dumping will take place in a restricted region[17]. In the explosion, the material of a bomb is ionized and expands, its pressure being sufficient to move magnetic lines of force. The expansion of this bubble gives rise also to a hydromagnetic wave which may scatter a fraction of the trapped radiation into the loss cone.

I am of the opinion that the effect on the naturally trapped protons will be even smaller than that surmised by Kellogg and Ney, if the explosion occurs away from the magnetic equator. First, as the bubble expands it excludes a magnetic field within it. The resulting compression of the lines of force will tend to produce mirror points ahead of the bubble so that protons will be reflected before they approach the bubble and are scattered by it. Next, the bubble expansion velocity, even assuming all the energy is kinetic, cannot exceed 10^8 cm per sec, and may be as low as 10^7 cm per sec. Therefore, Fermi effects from a single collision off the moving mirror point are negligible. Further, the bubble effect is likely to be adiabatic: the time to traverse a proton gyroradius is ~ few seconds while the proton gyro period is in the millisecond range (Table 1).

To some extent this fits in well with what is observed for natural disturbances of the field. The most careful analyses seem to show that in spite of the existence of strong hydromagnetic waves produced by natural events, the trapped protons are scarcely affected—at least up to about 4,000 miles[9,18]. The observations can so far be explained in terms of removal of protons chiefly by the atmosphere, plus an effect whereby the dipole field eventually loses its trapping ability when the radius of curvature of protons becomes comparable with the scale of the magnetic field.

It is puzzling indeed to realize that the naturally trapped protons have such long lifetimes (up to 100 years) in spite of the existence of large amplitude hydromagnetic waves above the ionosphere. It is therefore quite important to establish whether a man-made hydromagnetic wave of large amplitude can displace any of the trapped protons. The effect, if it exists, is likely to be small but its actual magnitude appears to be an open question.

Particle 'Sweeping'

On the other hand, high-energy protons are constantly being removed by other human activities in near space. The operation of artificial Earth satellites results in the absorption of protons which hit them. An idea of the effectiveness of this process may be gained by analysing the operation of a 'space sweeper' satellite[12]. This scheme may work, because the injection-rate for the hard protons is so small. It requires, however, satellites of very large area (and therefore mass) distributed throughout the radiation belt.

The effective removal-rate of the particles is given by the cross-sectional area of the satellite; that is, the number of particles removed depends on the product of satellite area, A_s, and operating time, T_D. The fractional reduction in intensity, $\delta J/J$, is given very roughly by the number of particles absorbed divided by the total number of particles in the volume affected. The former is simply $JA_sT_D = nvA_sT_D$, and the latter is $n(2\pi rA_c)$. The cross-sectional area A_c is determined by the radius of curvature, a, of the protons in the Earth's magnetic field, B, and is therefore quite large. At a distance r from the Earth's centre we find[12]:

$$A_c = (r\,.\,\Delta\lambda)a = r\,\frac{H}{2r}\;\frac{Mc^2\beta}{300B} \qquad (18)$$

where $\Delta\lambda$ (the effective latitude interval over which the concentration is $\sim n$) is proportional to the scale height, H; and $Mc^2\beta$ is the proton momentum in electron volts (Mc^2 = 931 MeV).

Hence:

$$\frac{\delta J}{J} = \frac{v}{2\pi rA_c}\,A_sT_D \qquad (19)$$

In our specific example, assuming an intensity reduction of 50 per cent, the product A_sT_D turns out to be about 10^{15} cm²-sec. Thus the operating time would be about one year if the sweeper satellite had a radius of about 30 m, or about one month if twelve such sweeper satellites were used.

It is an interesting consequence of this theory that the sweeping time is independent of the proton energy to a first approximation, but that the satellite in a given time sweeps up a larger volume of the higher energy particles, which is, of course, very desirable.

Conclusions

Nuclear explosions in space produce negligible effects on the Earth, for example, a distinctly decreased intensity of fall-out; they produce more significant perturbations of the upper atmosphere, but these are very temporary (measured in hours). It is in near-space itself where very noticeable effects can be produced which can last for many months because of the presence of the Earth's magnetic field.

It is evident that weapons tests in space can have important scientific by-products. The most interesting are the creation of a thin shell of 2-MeV fission electrons the decay and diffusion of which can be studied, as well as the creation of a thick belt of low-energy protons from fusion neutrons. It will probably be desirable to monitor these artificial radiation belts with quite sophisticated energy-resolving detectors, to suppress the naturally trapped radiation, as well as the much larger changes (of factors of 10–100 and even higher) produced by such natural events as solar flares and magnetic storms.

I thank Mr. A. M. Lenchek for assistance in the calculations.

Note added in proof. The present paper was originally prepared in mimeographed form (May 28, 1962) while I was at Jet Propulsion Laboratory of the California Institute of Technology, and was given private distribution. The paragraph concerning the effects of the radiation on solar cells was added at the time of submission when it was learned that solar cells on some satellites had been adversely affected. It would appear, however, that in cases where the solar power supply had an adequate power margin, as, for example, in the case of the weather satellite *Tiros V*, no effect on overall performance occurred.

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SUBSTITUTION OF STONE TOOLS FOR BONE TOOLS AT MAKAPANSGAT

By PROF. RAYMOND A. DART

University of the Witwatersrand, Johannesburg

TOWARDS the end of 1961 a block of the basal grey breccia from Makapansgat, while undergoing laboratory development, exhibited a structure roughly resembling a deciduous proboscidean molar. The opposite side of the block showed antelope bones (Fig. 1) and other curious forms. Some of these were large and rather amorphous, but others were smaller and rod-like (? tusk), and some of these round objects were pointed at one or both ends. Others again were plate-like but triangular or partially rounded.

The block was photographed and its constituents carefully isolated (see Fig. 2, which exhibits forty of its forty-one elements). The supposed 'molar tooth and tusk' proved on section and examination by Dr. H. B. S. Cooke, now assistant professor of geology in Dalhousie University, to be "only stalactite". Indeed, seventeen of the objects—most of which are seen in the upper three rows of our second illustration—are stalactitic, but they were used as a source of tools. The third article in the second row from the top on the left side (Fig. 2) is an actual artefact not of stalactite but in chert and has a rostrocarinate form.

On the right side of this chert tool (Fig. 2) is the broken end of a slender stalactite, the point of which fits neatly into the punctate depression found in the spirally broken shaft of the antelope humeral distal end, seen just below it (Fig. 2). These two objects lay right alongside one another in the centre of the compact block (Fig. 1). It is justifiable therefore to assume that this was the actual stalactite used to do the puncturing and cracking of this right humerus and its fellow left humerus (seemingly from the same animal) alongside it (Fig. 2), before their having been spirally broken by twisting. In any event, the lower three rows of the second illustration exhibit twenty-three objects, which are chiefly skeletal, some of which were definitely used as tools before their fossilization.

The essential purpose of this article, however, is to point out that the greatest proportion of this mixed assortment of brecciated material consists of debitage and artefacts which, apart from the chert rostrocarinate tool, are stalactitic in origin. Secondly, despite the deliberate damage these stalactites suffered prior to fossilization, their derivation, whether from long and slender or from thick and massive stalactites, is self-evident. The plate-like forms, whether triangular or rounded, show that they too were produced, like the rostrocarinate tool, by a deliberate process of splitting and trimming stalactites.

The most unexpected artefacts in this fantastic mixture of stalactitic and osteodontokeratic articles are the second object from the left and the second

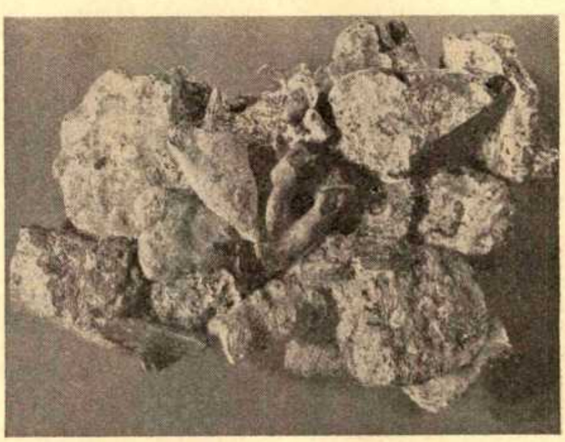

Fig. 1. Block of grey breccia *M.G.B.7* from Makapansgat Limeworks containing many bone fragments and numerous cylindrical, rounded and plate-like, or even triangular, pieces of stalactite. Note the pointed stalactite alongside the distal end of the antelope humerus in the centre of the block, the rod-like stalactite below it and the rounded edge of the chert rostrocarinate tool in the bottom right-hand corner

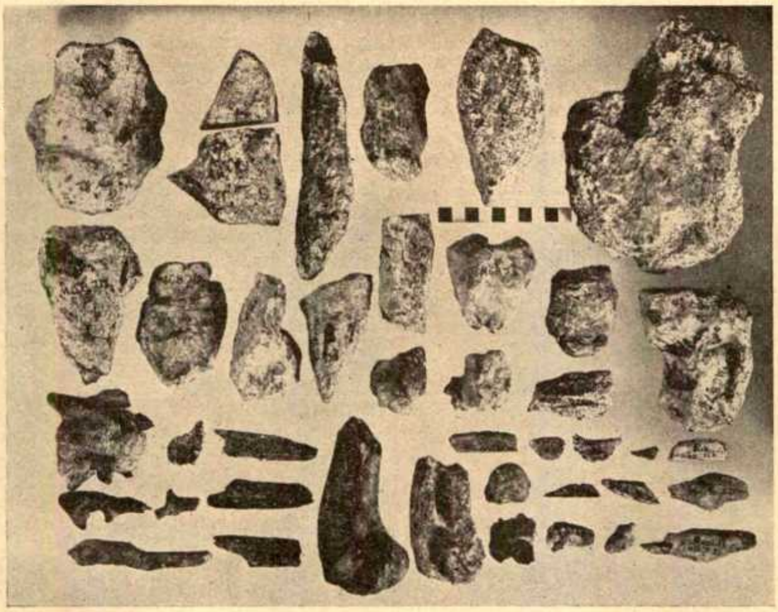

Fig. 2. Forty of the forty-one objects isolated from the compact grey breccia block *M.G.B.7*. The three upper rows are mainly stalactitic; the three lower rows are chiefly osteodontokeratic. Note the chert rostrocarinate (third from left in second row from top), the pointed stalactite on its right and the distal humeral end (with punctate depression) below the pointed stalactite, along with its fellow distal humeral end and other skeletal objects. The two stalactitic hand axes are in the top row; the second from the left (with its very blunted edge facing upwards) is the transected 'elephant molar'; the second from the right has its sharp edge facing downwards

from the right respectively, in the top row of Fig. 2. The second from the left has a spur of colophonite attached to it and is actually the supposed 'molar tooth' after its transection to display its own stalactitic origin. Its counterpart, the second from the right, is also part of a similar thick stalactite and has been oriented to show the implemental purpose of both of them. They are the simplest possible sort of stalactitic hand-axes imaginable (Figs. 2 and 3).

The more lacunar centre and the obviously concentric lamination of their artificially sectioned surfaces show that the stalactites, from which these two hand-axes came, were originally about five inches thick. The contrast between their rounded external surfaces and their flat planes of cleavage display with diagrammatic clarity the three direct, consecutive steps of the unsophisticated, yet ingenious and deliberate, technique involved in their manufacture.

Short cylindrical blocks about 4 in. high were first detached from suitably thick stalactites. This could only have been done by powerful lateral blows with a block of dolomite or some other weighty stone furnished with a sharp enough edge, probably in the same way as Australian Aborigines still use natural eoliths to chop through trees and to fashion wooden implements[1].

The resultant short cylindrical stalactite blocks were then placed on the dripstone floor or some other suitable anvil and each split into two half-cylinders by a vertical blow down through the middle of the block with a shoulder blade or some other blade-like tool. Thereby each half-cylinder automatically acquired two sharp borders or chopping edges.

The third step was to select the edge which would make the keener blade and to provide this chosen cutting edge with a suitable blunt back. This was done by merely removing the blunter edge with another blow. With the half-cylinder's vertical cleavage face flat on the floor or anvil the detaching blow was delivered about the middle of the half-cylinder alongside the edge to be removed. Judging from the two specimens available, the same three steps were adopted for making both hand-axes. They could both have come from the same thick stalactite.

The concurrence of chert, stalactitic and osteodontokeratic tools in one and the self-same block shows that it formed part of a living floor that was also a factory site. The intimate association of the chert artefact with these two hand-axes made from split stalactites is a clear proof that the many other stalactitic and chert, as well as the few foreign quartzitic and shale objects encountered in other blocks of the grey breccia, were not, as previously imagined, accidental occurrences. They provide authentic, objective evidence of the natural manner in which inorganic substances were becoming involved in the manufacture of implements by bone-tool-making australopithecines.

It was in *Nature*[2] that the late Prof. C. van Riet Lowe first announced the original discovery by Dr. C. K. Brain of the earliest stone implements recognized at the Makapansgat Limeworks site. They came from the upper, or pink breccia, and were then believed to be post-australopithecine; but later, in the same year, I reported[3] the discovery by Mr. Alun R. Hughes of an australopithecine maxillary fragment from the same

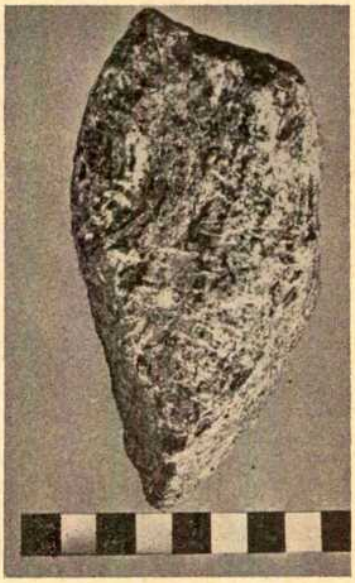

Fig. 3. An enlarged view of the stalactitic hand-axe (seen in Fig. 2, second from the right in the top row) to show: its right rounded peripheral stalactitic surface; its left plane of cleavage, that passed through the more lacunar structure of the centre of the stalactite; and how a flat back or hand grip was provided by removing the less preferable cutting edge of the split stalactitic half-cylinder

locality. Since that time the conjunction of stone implements with australopithecine remains has been demonstrated both at Sterkfontein[4] and at Olduvai[5].

The importance of the present discovery lies first in its corroborating Prof. C. van Riet Lowe's prescience in recognizing that at Makapansgat men used the local dolomite cavern materials such as dolomite and chert for toolmaking by extending his observations to other unexpected, but patently serviceable, dolomitic stony materials such as stalactites. Secondly, it demonstrates that, despite the preponderance of their osteodontokeratic tools and utensils, the earliest Makapansgat australopithecines were intelligent enough to substitute stalactites for teeth as penetrant tools, and for bones as pounding utensils from the beginning of their occupation of the Limeworks Cavern.

Thirdly, and most importantly, this block has furnished an essential and dramatic illustration of the manner in which cave home-life in general, and perhaps dolomitic cavern home-life in particular, provided from the outset natural multi-millenial environmental settings for evolving mankind such as rendered the ultimate substitution of stone tools for some of the piercing, chopping and cutting as well as pounding and crushing operations, previously discharged by osteodontokeratic artefacts, virtually automatic or inevitable among our small-brained but intelligent tool-manufacturing ancestors.

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FIFTIETH ANNIVERSARY OF THE STRANGEWAYS RESEARCH LABORATORY, CAMBRIDGE

1912–62

By Dr. HONOR B. FELL, F.R.S.
Director of the Laboratory

THIS year is an important one for the Strangeways Research Laboratory, because it marks the fiftieth anniversary of the opening of the original building on the present site. To celebrate the occasion the Trustees have published a short *History** of the Laboratory.

Our founder, Thomas Strangeways Pigg Strangeways, qualified at St. Bartholomew's Hospital. There he worked under Prof. Kanthack; when Kanthack moved to Cambridge to take the chair of pathology at the University, Strangeways went with him and later became Huddersfield lecturer in pathology under Sims Woodhead. In 1902 Dr. Strangeways married Dorothy Beck, eldest daughter of Dr. Beck, Master of Trinity Hall. To her he imparted an idea that he had had in mind for some years, of making "a systematic investigation of some more important diseases the pathology and treatment of which are as yet undetermined". She received what became known to them as the 'Scheme' with enthusiasm, and throughout his life gave it all the help and encouragement in her power. We are greatly indebted to Mrs. Strangeways for writing the first chapter in the *History* covering the period 1905–26, in which she vividly describes their adventures in bringing the 'Scheme' to life.

To carry out Strangeways's idea, "it would be necessary to have the patient in a small hospital under a qualified nurse. The patients would be told that they would be there for observation and research". The disease selected for study was rheumatoid arthritis and allied disorders. Money was raised from various sources, including Dr. Strangeways himself. Mrs. Strangeways writes: "to launch the Scheme we were spending one-third of our income of £150 per annum upon it, and we had two children". A fund was established of which the Trustees were Sir W. Selby Church, Sir Thomas Clifford Allbutt, Prof. Sims Woodhead and Dr. Strangeways. In 1905 a small house in Hartington Grove, Cambridge, was taken and converted into a six-bed hospital, the coal-shed being turned into a laboratory. A matron and nurse (both unpaid) looked after the patients who came in relays for clinical study. In addition, Strangeways visited the London infirmaries, where he was allowed to examine patients and from which he received much pathological material.

In 1907 the annual cost of the whole project was about £500, including £256 for the upkeep of the hospital. Small though this sum was, in 1908 funds were exhausted and the little hospital had to be closed; however, it was reopened the following year. At about this time, a friend persuaded Strangeways that he must build a larger hospital if the work was to continue. So a public appeal was launched with considerable success. A freehold site was found and bought with a gift of £300; later, through the generosity of the late Dr. G. P. Bidder, another acre of ground was purchased. A building was erected at a cost of £1,600 and opened, free of debt, on May 24, 1912. Visitors to the Laboratory often suppose, from its appearance, that this original block was a converted house, but this is not so. In the report for 1910 the plan of the future building is described, and the architect (who, incidentally, gave his services without fee) is congratulated "on the skilful manner in which he has carried out the difficult task of designing a building which can be used either as a private residence, a hospital or a hospital and laboratory combined".

During the First World War the building was used as an officers' hospital under the War Office, but was still directed by Dr. Strangeways. Throughout this period all the running expenses were paid by the late Sir Otto Beit, who had been a generous supporter of the hospital from its foundation. In 1917 the last officers left, and the study of rheumatoid arthritis was resumed.

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* *History of the Strangeways Research Laboratory (formerly Cambridge Research Hospital)*, 1912–1962. Pp. 38+5 plates. (Cambridge: Strangeways Research Laboratory, 1962.) See also p. 320 of this issue of *Nature*.

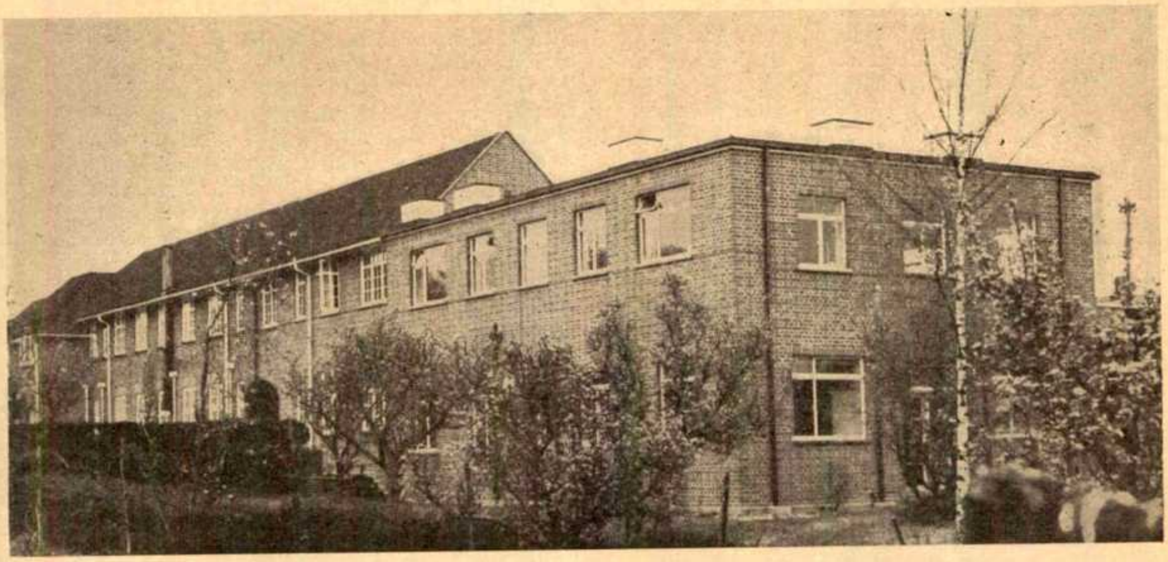

Fig. 1. The Strangeways Research Laboratory, Cambridge: new wing

Strangeways made many valuable contributions to our knowledge of rheumatic diseases, as a result of his investigations at the Research Hospital, but he came to the conclusion that research on rheumatoid arthritis was being seriously hampered by ignorance of the physiology of the cells of the joints. For this and other reasons, in 1923 he decided to close the wards, transfer the clinical work to St. Bartholomew's Hospital, and to devote himself mainly to the study of living cells by means of tissue culture *in vitro* and *in vivo*, a field in which he was one of the chief pioneers. In 1923 I joined him as his assistant in this work. So in this rather curious way, cell biology first became established in the Research Hospital, and it is interesting that investigations now in progress in the Laboratory "show signs of illuminating some of the obscurities of arthritic disease" (to quote from Sir Rudolph Peters's foreword to the *History*).

Strangeways died on December 23, 1926, on the eve of his sixtieth birthday. Owing to severe deafness, he never liked to have more than a few people working with him, so that with his death the Research Hospital faced sudden extinction. At a crucial meeting in January 1927 two of the three surviving Trustees at first favoured closing the hospital, but the third (Dr. Malcolm Donaldson) fought stubbornly for its continued existence. The situation was saved for the time being, when the Medical Research Council, under the chairmanship of the late Sir Walter Fletcher, agreed to continue and even to increase the annual grant that they had made to the establishment during Strangeways's lifetime.

Dr. Andrews, a retired doctor from Scotland, was put in charge (without salary) and the hospital was kept going on a temporary basis throughout 1927. The following year Dr. Andrews resigned, and I was appointed in his place. In the task that lay ahead, I had the close and invaluable co-operation of my colleague, Dr. F. G. Spear, whose unfailing help, advice and moral support in times of crisis I acknowledge with the deepest gratitude. Dr. Spear, who had joined the Laboratory in 1924, was later given the title of deputy director, an office that he held until 1958, when he retired after a distinguished career in radiobiological research. His devoted service to the Laboratory will never be forgotten.

Since the 'hospital' no longer contained patients, the Trustees decided to rename it, and it was called "The Strangeways Research Laboratory" in honour of its founder. But whether our hopes of making it a permanent memorial would be realized seemed doubtful at that time. For some years we lived a hand-to-mouth existence, never knowing whether our efforts would be in vain and the Laboratory closed. Nevertheless, progress was made. Our financial position was materially improved by the receipt of a legacy, and in 1929 the Medical Research Council provided a capital grant of £800 to erect a suitable building to house an X-ray set that had been given to us by the late Dr. Meyrick Jones. A condition of the grant was that the new laboratory should be so designed that it could be converted into a garage should the main building ever be sold as a private residence. There was even talk of incorporating a steel girder in one wall to hold the garage doors in days to come, but, to my lasting regret (it would have been a pleasing historical relic to show to visitors), the girder was omitted on the grounds of expense.

From now on our fortunes began to rise. More people came to work in the Laboratory; in 1931 a bequest of £5,000 from Sir Otto Beit enabled another storey to be built on the X-ray laboratory and talk of selling the place gradually faded away. The first major extension was completed in 1938, thanks to a generous grant from the Rockefeller Foundation.

During the Second World War, the population of the Laboratory naturally dwindled and further development was arrested; the remaining research staff turned their attention mainly to problems of wound-healing and the biological action of chemical warfare agents. As soon as life returned to normal, however, our research revived and began once more to expand. The year 1949 was marked by the receipt of a grant of £12,000 from the Nuffield Foundation, spread over a five-year period, to enable us to develop our work on cell physiology. Other support was forthcoming. The Medical Research Council continued its grants on an ever-increasing scale, as also did the British Empire Cancer Campaign, which for many years has borne most of the cost of our cancer research programme. In 1951 the Royal Society gave the Laboratory a grant of £3,500, which was particularly welcome because, being unrestricted, it could be used at the Trustees' discretion.

During the past decade, the biological interests of the Laboratory have shifted more and more from purely morphogenetic to physiological problems; in consequence the lack of an adequate biochemical section became a severe handicap. The Wellcome Trust, however, came to our rescue with a magnificent grant of £60,000 with which to build and equip a new wing (Fig. 1). This extension not only provided modern biochemical laboratories but also suitable accommodation for the Siemens electronmicroscope, presented to us in 1954 by the Rockefeller Foundation, and for the new X-ray set supplied for our use by the British Empire Cancer Campaign from money collected by members of the Inner Wheel. The new wing was opened by Sir Henry Dale in 1959, and has extended the scope of our research to a remarkable degree. Recently, in honour of our fiftieth anniversary, the Fleming Memorial Trust has made a splendid gift of £10,000 to buy equipment for fundamental research on cell physiology.

Legally, the Strangeways Research Laboratory remains what it was in its research hospital days, namely, an independent charitable institution controlled by a board of Trustees. The present Trustees are: Sir Rudolph Peters (chairman), Dr. Malcolm Donaldson, Lord Adrian, Sir Henry Dale and Dr. P. B. Medawar. At the present time it usually accommodates 25–30 research workers. The staff includes a varied assortment of biologists, two pathologists, four biochemists, three physicists and a biophysicist; in addition, there are always a number of guest workers from other institutions in the United Kingdom and abroad, and usually two research students working for the Cambridge Ph.D. degree.

An unusual feature of the organization is the fact that no member of the research or technical staff is paid out of the Laboratory's own funds. Three members of the research staff hold Sir Halley Stewart fellowships which were generously established by the Sir Halley Stewart Trustees to enable us to provide some permanent key appointments. Other members of the Laboratory belong to the Medical Research Council's external staff, others are supported by the British Empire Cancer Campaign, and I myself hold a Foulerton Research Fellowship under the Royal Society. No account of the Laboratory's history would be complete without a tribute to the loyal service of the technical staff, some of whom have been with us for 25–50 years. The wide variety of scientific disciplines represented by the research staff is a great advantage, because it enables us to approach a given problem from several different directions; in addition, we collaborate with a number of specialists in other departments. The methods in general use include animal experiments, organ culture, cell culture, microchemical and cytochemical procedures, and electronmicroscopy.

Among the purely biological problems now being investigated are: the action of vitamins and hormones on the structure and metabolism of tissues and cells *in vivo* and *in vitro*, and isolated cell organelles; the properties of the cell surface; intercellular relationships; the formation of keratin; and the synthesis of intercellular materials. Radiobiological research dates back to 1921, to the pioneer experiments on tissue cultures by Strangeways and Oakeley; radiation work was continued under the supervision first of Dr. F. G. Spear and then of the present deputy director, Dr. A. Glucksmann, who has extended it to the clinico-pathological analysis of irradiated human tumours supplied by collaborating hospitals. Other cancer research comprises studies on human and animal leukæmia, and experiments on chemical carcinogenesis *in vivo* and in culture. In microbiology, work is being done on bacterial metabolism and fine structure, and on the host : parasite relationship between cells and pathogenic mycobacteria.

Looking back over our Laboratory's long and eventful career, I realize how extraordinarily fortunate the place has been in its friends. I think gratefully of that long line of disinterested people who have worked for it, given it money, and—on occasion—strenuously fought for it during the past fifty years. It seemed fitting to dedicate the *History* to our first benefactor, E. Dorothy Strangeways, without whose courage and unselfishness the little Research Hospital of 1912 would not have lived to grow into the Strangeways Research Laboratory of 1962.

OBITUARIES

Dr. Harry Wexler

THE news of the sudden death of Dr. Harry Wexler, director of meteorological research in the U.S. Weather Bureau, at the early age of fifty-one came as a blow to his many friends in the world of international meteorology. He graduated in mathematics at Harvard University in 1932, followed by postgraduate work at the Massachusetts Institute of Technology which gained him the degree of Sc.D. in 1939. From 1940 until 1941 he was assistant professor of meteorology at the University of Chicago, but most of his scientific life was spent in the U.S. Weather Bureau, in which he built up an impressive research division.

Wexler wrote profusely on atmospheric science, contributing nearly eighty papers dealing with such varied topics as the radiative cooling of the air, polar anticyclones, atmospheric turbidity, the structure of hurricanes and the study of the high atmosphere. He also published papers on glaciology and oceanography. Much of his work dealt with possible large-scale climatic effects of terrestrial and extra-terrestrial events, such as the production of dust by volcanic eruptions and variations in the solar output, but recently he became absorbed in two major topics. One was the study of antarctic meteorology, especially as regards the consequences for global meteorology of the existence near the hub of atmospheric circulation in the Southern Hemisphere of "the world's most efficient cold air factory". During the International Geophysical Year he did much to organize meteorological observations at the South Pole, which he visited several times by aircraft. Afterwards, he turned his attention to the potentialities of weather satellites, in which he had an almost fanatical belief. His enthusiasm for this matter resulted in a long stay in Geneva earlier this year, where, with Prof. Bugaev of the U.S.S.R., he conducted an intensive and far-reaching survey, culminating in the publication by the World Meteorological Organization of a lengthy report on the advancement of atmospheric sciences and their application in the light of developments in outer space. The essence of this report is a comprehensive scheme for a 'world weather watch' which, if constituted, might well revolutionize meteorology. Wexler was undoubtedly the driving force behind these ambitious proposals.

The lasting impression left by Wexler is that of an enthusiast whose cheerfulness, colourful speech and general friendliness remain as a cherished memory for his friends. In his own country his achievements were widely recognized. In 1945 he received the Robert M. Losey Award of the Institute of Aeronautical Sciences, in 1956 the U.S. Air Force Award for Exceptional Services, and his work for the International Geophysical Year and antarctic meteorology gained him the Department of Commerce Exceptional Service Medal and the U.S. Navy Public Service Award. He leaves a widow and two daughters.

GRAHAM SUTTON

Dr. F. R. Irvine

DR. FREDERICK ROBERT IRVINE, who died suddenly on August 20 in Ghana, at the age of sixty-four, was well known in West African agricultural and botanical circles. His long association, from 1924, with Achimota College in the Gold Coast and later with the University, at Legon, Ghana, earned him many African friends. He taught botany and agriculture at Achimota for sixteen years and his *West African Botany* (1931) met a great need as it was the first text-book on the subject.

Irvine was an assiduous collector and compiler of information on plants, especially as to their uses and vernacular names. His agricultural training at the then Armstrong College, the Newcastle Division of the University of Durham, naturally gave him a bias towards crops and food plants. His *Plants of the Gold Coast* (1930) dealt principally with their uses, and in 1934 he published *West African Agriculture* which he was revising at the time of his death. However, he regretted that he was no taxonomist and this lack of a background in plant systematics was something of a handicap to him, especially during the compilation of his recent volume on the *Woody Plants of Ghana* (1961). He had recently accepted a research fellowship in Ghana to complete the companion volume on the herbs, but this remains unfinished. A further work, his *Vocabularies of Plant Names in the Nigerian Languages*, also remains unpublished.

His tall, slim, rather stooping figure was a familiar sight in the Kew Herbarium, where he would seek some obscure publication on food plants or ask advice on the taxonomy of the group in which he was then interested. Any further information was usually added to an already copiously annotated manuscript or inserted in a black pocket-book with the comment that he would now have to change half a dozen indexes. His interest in food plants ranged far and wide, especially those of the more primitive peoples such as the Australian Aborigines and the North American Indians. Besides his botanical activities, he also collected zoological information. Thus, he collaborated with J. R. Norman in *The Fishes and Fisheries of the Gold Coast* (1947).

He was keenly interested in the welfare of overseas students in the United Kingdom, and his work with the Society of Friends, notably as warden of their International Centre at Tavistock Square after the Second World War, brought him into contact with a large number of them.

While staying for a year in the United States, in 1959, under Quaker auspices, he became seriously ill, but he made a remarkable recovery on his return to Britain, though he quickly aged and did not enjoy good health thereafter. He is survived by his wife, a son and two daughters.

F. N. HEPPER

NEWS and VIEWS

Nobel Laureates in Medicine: Drs. F. H. C. Crick, F.R.S., M. H. F. Wilkins, F.R.S., and Prof. W. Watson

READERS of *Nature* will be pleased to learn of the award jointly of the Nobel Prize for Medicine to Dr. F. H. C. Crick, research worker for the Medical Research Council in the Council's Laboratory for Molecular Biology, Cambridge; Dr. M. H. F. Wilkins, deputy director of the Medical Research Council Biophysics Research Unit, Department of Biophysics, King's College, University of London; and Prof. James Watson, professor of biology in Harvard University, especially since the work of these three scientists has become well known through the columns of *Nature* and elsewhere. The official announcement cites the recipients for their achievement of a "most fundamental biological problem", the discovery of the molecular structure of deoxyribonucleic acid. The establishment of the 'biological code' especially for the formation of enzymes is now well known, and is exerting a profound influence on biochemical and biological research, especially in the fields of molecular biology and heredity. Recently, Dr. Crick was awarded the Gairdner Foundation Prize, and, among other awards, he was the first to receive the Charles-Léopold Mayer Prize of the Paris Academy of Sciences (*Nature*, 193, 223; 1962).

History of Science and Technology at Imperial College: Prof. A. R. and Mrs. Hall

SIMULTANEOUSLY with the announcement by the University of London of the appointment of Prof. A. Rupert Hall to the chair of the history of science and technology in the Imperial College of Science and Technology, the College announces the appointment of Prof. Marie Boas Hall, his wife, to a senior lectureship in the same Department. Prof. Hall and his wife are at present professors of history and logic of science in the University of Indiana. They take up their new duties in the summer of 1963. The study of the history of science and technology in its own right presents both a challenge and an opportunity. The new Department will be headed by distinguished scholars who, both individually and in collaboration, have made an important contribution in this field. *Unpublished Scientific Papers of Isaac Newton* appeared under their joint authorship as did various articles on the man himself. Prof. Rupert Hall's major publications are *Ballistics in the Seventeenth Century* and *The Scientific Revolution, 1500–1800*. He was one of the co-editors of *A History of Technology* published in five volumes during the 1950's. Among Prof. Marie Boas Hall's works are *Robert Boyle and Seventeenth Century Chemistry* and the recent *The Scientific Renaissance 1450–1630*, Vol. 2 of *The Rise of Modern Science*.

The new department will at first be chiefly concerned with research, and postgraduate education in research methods. Its primary objectives will be the training of future historians of science and technology, and the introduction of scientists and engineers to these studies. A generous contribution towards the cost of maintaining the new department has been made by Imperial Chemical Industries, Ltd., which recently supported the study of the history of technology by subsidizing some valuable publications, including the major *History of Technology* already mentioned.

Materials Science and Engineering at M.I.T. : Prof. R. A. Smith, C.B.E., F.R.S.

PROF. ROBERT ALLAN SMITH, known for his work on semiconductor and infra-red physics, has been appointed the first director of the new Center for Materials Science and Engineering at the Massachusetts Institute of Technology. Dr. Smith will also hold a simultaneous appointment as professor of physics in the Institute. He carried out pioneer radar research for the British Government during 1939–46, and from 1947 until 1961 was head of the Physics Department in the Royal Radar Establishment, Malvern. He spent the year 1961–62 as professor of physics in the University of Sheffield (see *Nature*, 190, 960; 1961).

Director-General of the Indian C.I.S.R. : Dr. Syed Husain Zaheer

DR. SYED HUSAIN ZAHEER, director, Regional Research Laboratory, Hyderabad, has been appointed director-general of the Council of Scientific and Industrial Research. He took charge of his office on September 1. Born on November 7, 1901, Dr. Zaheer was educated at Lucknow, Oxford and Heidelberg. From 1930 until 1946 he was reader in organic chemistry, Lucknow University, and two years later be became professor of organic chemistry. In 1948 he became director of the Central Laboratories for Scientific and Industrial Research, Hyderabad, which were later to become the Regional Research Laboratory, Hyderabad. During the fourteen years of his directorship he built up the Regional Research Laboratory as one of the foremost centres for pilot plant and developmental research in India. Dr. Zaheer has been responsible for many important industrial development projects in India, and also for the preparation of project reports and blueprints for starting many new industries in India. In 1954 he undertook a study of post-war German industry as a Senior United Nations Fellow and visited West Germany and Finland in 1955 to study plants and techniques for the utilization of low-grade coals at the invitation of the Government of the Federal Republic of Germany. Dr. Zaheer is a member or president of numerous learned societies.

CERN Research Directorate : Prof. G. Puppi

PROF. G. PUPPI recently joined CERN as a member of the directorate for research. Early next spring he will replace Prof. Gilberto Bernardini, who returns to his chair of physics at the University of Rome. Prof. Giampietre Puppi was born on November 20, 1917, in Bologna, Italy. He studied at the University of Padova under Bruno Rossi and Gian Carlo Wick. After the War, he was lecturer at the Universities of Bari, Bologna, Rome and Padova. In 1950 he was appointed to the chair of theoretical physics in the University of Naples. Since 1953, Prof. Puppi has been professor of higher physics and director of the Physics Institute in the University of Bologna. He will resume these responsibilities in October 1963. Prof. Puppi's research work has been mainly in the fields of cosmic rays and sub-nuclear particles.

The Strangeways Research Laboratory

THE Strangeways Research Laboratory, founded originally as a research hospital and now famous all over the world as a centre of tissue culture and cell biology, has recently celebrated its fiftieth anniversary by the publication of a short history of its development (*History of the Strangeways Research Laboratory (formerly Cambridge Research Hospital)*, 1912–1962. Pp. 38+5 plates. Cambridge: Strangeways Research Laboratory, 1962). The part of the history from 1905–26 has been written by Mrs. Strangeways, the widow of the founder and still a guiding spirit, well known to the workers in the Laboratory. It is a remarkable document and might be read with profit by all young doctors and research workers of to-day. The devotion and perseverance displayed by Dr. T. S. P. Strangeways and his wife in the pursuit of their goal of establishing a research hospital is almost incredible. Perhaps it is best illustrated by the following extract from the history. "Indeed, to launch the Scheme, we were spending one-third of our income of £150 per annum upon it, and we had two children. He [T. S. P. Strangeways] approached his Class and asked if they would back the project to the extent of £5 each; this they agreed to do, but I am thankful to say that they were never called upon." Needless to say, the scheme was launched, and how the institution has evolved and progressed is well told in the two other chapters of this slim but fascinating volume [see also p. 316 of this issue of *Nature*].

British Participation in the Indian Ocean Expedition

THE Royal Society and the British National Committee for Oceanic Research have announced proposals for United Kingdom participation in the International Indian Ocean Expedition which is supported by Unesco and the Intergovernmental Oceanographic Commission. Altogether, forty ships carrying scientists and specialized equipment from more than twenty countries are expected to take part. Most of the United Kingdom effort will be made in the Arabian Sea, between Africa, India and Ceylon, and will be based principally on two of H.M. Survey Vessels, H.M.S. *Owen* and H.M.S. *Dalrymple*, together with the National Institute of Oceanography's new research vessel R.R.S. *Discovery*. This extension of international co-operative research to oceanography will cover all aspects of marine science. The Indian Ocean is particularly suited to such an investigation on account of the nature of its current systems, and the information to be gained concerning the interaction between winds, climate and general circulation. The economic importance of its potential fishing grounds is already apparent, and chemical and biological measurements will increase basic knowledge of those factors influencing productivity and fertility of food fishes, and the general ecology of the region. Geophysical observations will include studies of bottom topography and sedimentology together with magnetic and seismic surveys. Physical measurements, in addition to the study of particular current systems, will include the operation of additional tide gauges, radiosonde/wind stations, and synoptic observations by all survey vessels and H.M. ships in the area (The Royal Society. British National Committee for Oceanic Research. *United Kingdom Scientific Programmes during the International Indian Ocean Expedition 1961–64.* Pp. 22. London: The Royal Society, 1962).

The Engineering Institutions Joint Council

BRITAIN's leading bodies of professional engineers, with a combined membership of more than 200,000, have set up the Engineering Institutions Joint Council under the chairmanship of Sir Kenneth Hague. The purpose of the Council is to enable representatives of the bodies concerned to meet together to consider and take action on matters of common interest relating to the advancement of engineering and the dissemination of knowledge in that field. It also aims at establishing a sound and useful channel of communication and understanding between the professional engineers, the Government, the public, and national and international bodies concerned with engineering. The bodies represented on the Council are: Royal Aeronautical Society, Institution of Chemical Engineers, Institution of Civil Engineers, Institution of Electrical Engineers, Institution of Gas Engineers, Institution of Marine Engineers, Institution of Mechanical Engineers, Institution of Mining Engineers, Institution of Mining and Metallurgy, Institution of Municipal Engineers, Institution of Production Engineers, British Institution of Radio Engineers, Institution of Structural Engineers. Mr. K. H. Platt has been appointed honorary secretary of the Council, the office of which is in the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, S.W.1.

Automatic Weather Station

ON August 17, 1961, the world's first isotope-powered automatic weather station began transmitting weather observations to the U.S. Department of Commerce Weather Bureau from Axel Heiburg Island in Canada's Northwest Territories. Throughout its first year of operation, the unattended station has continued to report on the weather in this bleak, uninhabited region about 700 miles from the North Pole. The station was developed by the Martin Company for the United States Atomic Energy Commission and the Weather Bureau. Wind direction and speed, barometric pressure and temperature are continuously measured by the station's instruments. The measurements are fed into a data-processing system and transmitted every three hours to the Joint Canadian–United States Weather Station at Resolute, Canada. Ultimately, the Weather Bureau hopes that hundreds of these automatic stations will be placed in mountainous and polar regions, oceans and deserts, where it is impossible to establish permanent, manned weather stations.

Biorheology

AFTER a new name has been coined for a new division of science it does not take long for the appropriate specialist journal to appear. The name 'rheology' was first given to the science of the deformation and flow of matter in 1929. This has covered an extremely wide field, including such diverse subjects as metals, plastics, solutions and suspensions, building materials and biological substances, in which workers may have little in common except the need for rheological techniques. As biologists have increased their interest in rheological problems, the name 'biorheology' was first used in 1948, and now a journal appears to cater for this branch of science. The reputation of its editors-in-chief, Prof. A. L. Copley, of New York, and Dr. G. W. Scott Blair, of Reading, will guarantee the standing of the new journal. They are assisted by a Board of Editors from five other countries and by an Honorary Editorial Advisory Board even more widely distributed. The journal will only accept papers within the strict limits of its subject. Hydrodynamics or the classical theory of elasticity are excluded. Papers must deal with the rheology of "processes in the living organism (*in vivo*) and with material originating from the organism (extracorporeal)". The inter-relationship between rheological properties and structural aspects of the systems will be stressed. Papers will be in English, French and German. In addition to papers on original work (limited to 15 pages in general), there will be letters to the editor, book reviews, reports of meetings and news of interest to rheologists. The first number is well produced and clearly printed (1, No. 1; July 1962. Pp. 82. Annual subscription-rates: (*A*) For libraries, Government establishments, research laboratories, etc., £10 (30 dollars); (*B*) for individuals who place their orders directly with the publisher and certify that the journal is for their personal use, £5 (15 dollars). Oxford and New York: Pergamon Press, 1962). The new venture can be warmly welcomed and confidently expected to be successful in its purpose.

A Directory of British Scientists

ERNEST BENN, LTD., who are to publish the first edition of the *Directory of British Scientists* in April 1963, hope to include details of some 30,000 British science graduates engaged in scientific work at the present time. This has been made possible by the co-operation of the registrars of all the British universities. While the dispatch of questionnaires to all graduates has inevitably resulted in a number of duplications, there have also been a number of forms returned owing to changes of address, etc. Any scientists who consider themselves eligible for inclusion in the *Directory* (which does not cover dentistry, engineering, medicine or veterinary studies), and who have not yet received a form, are requested to write as soon as possible to: the Editorial Secretary, *Directory of British Scientists*, Orchard House, Caldecott Road, Abingdon, Berkshire.

A Bibliography of Plastics

A BIBLIOGRAPHY of plastics, compiled by E. R. Yescombe, with a preface by M. Kaufman, of the National College of Rubber Technology, has been issued by the Library Association as *Special Subject List* No. 38 (*Plastics*. Pp. 40. London: Library Association, 1962. 8*s*.; L.A. members, 6*s*.). Besides a select list of periodicals, it includes some 280 entries. These are arranged under guides to the literature; abstracts and indexes; surveys and reviews; introductory—elementary treatises; general—more advanced ; nomenclature ; trade names ; classification; high-polymer chemistry; high-polymer physics; properties, testing and quality control; materials; raw and ancillary materials; plant and processes; special applications. There is an author and title index and the pamphlet should prove a useful guide to librarians and others.

Lalor Grants and Awards for 1963

THE Lalor Foundation has announced the programme of awards for 1963, which it is offering for support of research on the fundamental biochemical and physiological mechanisms concerned with fertility and the early stages of reproduction in various forms of life. The objectives are to further the knowledge and understanding of the basic phenomena involved and to extend and develop the possibilities for effective regulation and control. The awards are open to all nationalities. The awards may range up to 8,000 dollars a year, depending on the scope and duration of the projects approved. Preference will be given to younger members of university and college faculties and staff with an upper age limit of forty-one years. The work may be carried on at the applicant's own institution or elsewhere. The Foundation will also grant post-doctorate summer or short-term research awards at the Marine Biological Laboratory at Woods Hole, Mass., or elsewhere, for appropriate projects in the fields specified. For these awards, the stipends will normally not exceed 1,000 dollars for a single man or woman, 1,200 dollars for a married man working at his home institution, and 1,350 dollars for a married man with principal programme at another institution. Applications and inquiries should be sent to the Lalor Foundation, 4400 Lancaster Pike, Wilmington 5, Delaware, and applications must be received by January 15, 1963.

The Perkin Centenary Trust: Awards

APPLICATIONS for the awards of the Perkin Centenary fellowship and scholarships are invited by the Trustees. *The Perkin Centenary Fellowship* is offered for one or two years for the purpose of higher study of any subject approved by the Trustees. Candidates will be required to show either that they have had experience in an industrial firm or other institution concerned in the manufacture or the application of colouring matters, or that their intended field of study has a direct bearing on these subjects. The value of the fellowship is £750 per annum with an additional grant of up to £100 per annum towards certain designated expenses. It is tenable, from October 1963, at any university, technical college or other institution approved by the Trustees. *The Perkin Centenary Scholarship:* two such awards are offered, each for one or two years, to enable candidates employed in an industrial firm or other institution concerned with the manufacture or the application of colouring matters to receive an education at a university or technical college. Each award will have a value of £350 per annum. This may be increased, at the discretion of the Trustees, to £400 per annum if the candidate appointed is required to live away from his normal place of residence. Applications and inquiries should be addressed to the Secretary, Perkin Centenary Trust, c/o Chemical Society, Burlington House, London, W.1. Applications must be made by May 1, 1963.

Research Assistantships and Fellowships in Forestry

THE College of Forestry of the State University of New York is offering twenty-six assistantships for the college year 1963–64, each worth between 1,800 and 2,400 dollars. Research fellowships, sponsored by industry, research foundations and government agencies, are also offered to qualified applicants for work on assigned research projects in several fields related to forestry. Each fellowship is worth from 2,100 dollars annually. Applications for assistantships or fellowships should be received by the College before March 1, 1963. Forms of applications and further information can be obtained from the Associate Dean for Graduate Studies, State University College of Forestry at Syracuse University, Syracuse 10, New York.

Announcements

PROF. DUDLEY STAMP has been awarded the honorary degree of Doctor of Natural Science by the University of Warsaw in recognition of his international work in applied geography.

PROF. E. B. CHAIN, professor of biochemistry in the Imperial College of Science and Technology, London, has returned from a lecture tour of Chile, Argentina, Uruguay and Brazil. He lectured in the universities of those countries on subjects of his research, including the new penicillins, the mode of action of insulin, and sugar–amino-acid relationships in brain. During his tour he was awarded honorary degrees from the Universities of Cordoba and La Plata (Argentina), Montevideo (Uruguay) and Brazil. In Argentina he was declared official guest of honour of the Republic of Argentina by special decree of the President.

THE fourteenth annual Ciba Foundation Lecture on "The Future of Man: Evolutionary Aspects" will be given by Sir Julian Huxley at the Royal Institute of British Architects on November 26. Further information can be obtained from the Conference Assistant, The Ciba Foundation, 41 Portland Place, London, W.1.

THE autumn meeting of the British Society for Immunology will be held at the Wellcome Building, London, during November 16–17. The programme will include a symposium on "Pharmacology of Allergic Reactions and Related Inflammatory States". Further information can be obtained from Dr. R. G. White, Bacteriology Department, London Hospital, London, E.1.

THE eleventh convention of the Scientific Instrument Manufacturers' Association of Great Britain Ltd. on "Looking Ahead—the Next Five Years" will be held in Eastbourne during November 7–9. The programme will include talks on: the economic outlook; future trends in engineering and production; instrumentation in space research; science in everyday life. Further information can be obtained from the Press Relations Officer, SIMA House, 20 Queen Anne Street, London, W.1.

A SYMPOSIUM on "Quality in Photographic Reproduction", arranged by the Royal Photographic Society of Great Britain, will be held at the Royal Society of Arts, London, during December 5–7. The topics under discussion will include: colour photography; aspects of tone reproduction in the fields of graphic arts, professional photography, photogrammetry and micro-reproduction; sharpness of photographic and electronic imaging systems; modulation transfer functions. Further information can be obtained from Dr. C. Roberts, Research Laboratory, Kodak Ltd., Middlesex.

SCIENTISTS IN THE PUBLIC SERVICE

PROMOTION OF INDIVIDUAL RESEARCH WORKERS

EACH year a special committee reviews the work of scientists carrying out research work in Government establishments and in similar establishments of other public bodies and recommends, under the provisions of the White Paper on the Scientific Civil Service (Cmd. 6679 of 1945), the promotion of individual research workers of exceptional merit. The promotions were effective from July 1 and include the following:

Deputy Chief Scientific Officers

Mr. G. K. Adams joined the then Armament Research Establishment in 1942 after graduating at the University of Bristol with first-class honours in chemistry. He moved to the Explosives Research and Development Establishment, now a part of the Ministry of Aviation, in 1946 and worked on the mechanism of combustion of liquid propellents. In 1955 he was awarded a special merit promotion to senior principal scientific officer and since then has had responsibility for combustion and sensitiveness research, ballistic assessment, and theoretical investigation of propellent burning and initiation of detonation of shock waves. Mr. Adams's best-known contribution is probably that to the theory of laminar flame propagation, where he has analysed the mechanism of the hydrazine decomposition flame and calculated the flame properties from isothermal kinetic data, a system more complex than any previously subjected to theoretical treatment. His calculations of the spatial distribution of radicals and the resulting hydrazine decomposition-rate as a function of flame temperature and pressure confirmed his theory of a chain reaction scheme. They led to the experimentally observed coefficient of flame speed and revealed a possible explanation of the anomalous pressure coefficient. An assessment of the explosive hazards of some of the higher energy propellent systems led Mr. Adams to study, in collaboration with Prof. D. C. Pack, the problem of the development of the shock waves in accelerating combustion and the initiation of detonation by shock waves.

Dr. O. Simpson first joined the scientific civil service in 1944 and served in the Admiralty Research Laboratory until 1946, when he returned as a research scholar to Trinity College, Cambridge. He was elected a Fellow of that College in 1949 and for the next three years he was assistant professor of physics in the University of Michigan. In 1953 he joined the Services Electronics Research Laboratory and gained a special merit promotion to senior principal scientific officer in 1957. Dr. Simpson initiated a new branch of solid-state physics by making the first comprehensive study of the fluorescence and conductivity of the crystalline aromatic hydrocarbons. In addition to his own research work, Dr. Simpson has since 1956 been head of the solid-state physics group at the Services Electronics Research Laboratory, which has acquired an international reputation for its contributions to research on super-conductivity in metals, compound semi-conductors and organic semi-conductors, and has pioneered in new applications of these solids. Dr. Simpson is active on various inter-service committees covering a wide range of electronic subjects.

Mr. J. H. Wilkinson joined the Scientific Civil Service in 1940 and was transferred from the Ministry of Supply to the National Physical Laboratory in 1946, where he worked in the Mathematics Division on the *ACE* electronic computer project under Dr. A. M. Turing. In 1948, when Dr. Turing left, he assumed charge of the *ACE* project. Mr. Wilkinson specializes in numerical methods and the exploitation of high-speed computers. The field that he has made particularly his own is that of linear algebra, a subject the importance of which has been enormously increased by the advent of the high-speed electronic computer. For some years he devoted himself to the development of new algorithms for the solution of matrix problems, and the testing and improvement of existing ones, with the view of developing as powerful and practical methods as possible. For many years, work had been seriously hampered by an inability to assess the accuracy of solutions of large-scale matrix problems, but in 1955 Mr. Wilkinson developed a new method of error analysis which proved to be applicable to a wide range of algebraic problems including the more difficult eigen-value problems; this also applied equally well to both fixed-point and floating-point calculations. His successful application of this method in the succeeding years led to a real understanding of the factors that govern the accuracy of solutions obtained by numerical methods. It has also led to the possibility of obtaining, for the error of the solutions, theoretical bounds that are close to those actually obtained in practice. Mr. Wilkinson, who was awarded a special merit promotion to senior principal scientific officer in 1954, is continuing to work in this field and hopes to extend his studies to cover non-linear problems.

Senior Principal Scientific Officers

Mr. R. L. Bickerdike, of the Royal Aircraft Establishment, is at present working on the development of new methods of preparing shaped pieces of graphite by impregnating loose or lightly bonded aggregates of particles with carbon deposited from the gas phase.

Dr. H. A. Gebbie, of the Basics Physics Division, National Physical Laboratory, has made outstanding contributions to the study of infra-red spectroscopy and has pioneered the use of interferometers in the far infra-red region and their application both in the laboratory and in astro-physics. He has led the team of the National Physical Laboratory which has been studying the transformation of non-metals under the highest obtainable pressures to the metallic state.

Dr. C. Hilsum, of the Services Electronics Research Laboratory, has been engaged since 1950 on various researches, some of which have eventually been developed for commercial use, among them being a new infra-red detector, the photo-electromagnetic cell and the Hall effect susceptibility meter. He is at present responsible for an intensive research programme on a compound semi-conductor, gallium arsenide, and is already well known for his work on this increasingly important material.

Dr. A. E. Johnson, of the National Engineering Laboratory, East Kilbride, is internationally known for his work on the creep properties of metallic alloys subjected to complex stress systems at elevated temperatures and the application of his results to the analysis of the stress/strain relations in engineering structures operating under such conditions.

Mr. N. B. Marshall, an ichthyologist at the British Museum (Natural History), has special interests in the taxonomy of fishes, the environmental effects of depths on the structure of the fish skeleton, and comparative and functional anatomy of the swim bladders in fishes.

Dr. R. J. Murgatroyd has for the past ten years been in charge of the Meteorological Research Flight of the Meteorological Office. He specializes in high-altitude meteorology, and has been relieved of administrative duties in order to undertake further research work.

Dr. R. A. Pattle, of the Microbiological Research Establishment, is in charge of a team carrying out research in the Munitions Research Division.

Dr. P. H. Thomas, of the Fire Research Station, is engaged in the study of the behaviour of fires in buildings, in particular the characteristics of fires in single compartments. He has also developed small-scale techniques which fully evaluate the venting of fires by openings in the roof and has produced a theoretical background to this work. His recent publications include new dimensionless correlations of the height of flames from natural fires and an extension of the theory of thermal explosion.

Similar promotions have been made by:

(1) United Kingdom Atomic Energy Authority: Senior Principal Scientific Officer

Mr. A. E. Glennie joined the Atomic Energy Authority in 1949 and worked with the Atomic Weapons Research Establishment, first at Fort Halstead and then at Aldermaston. Since 1957 he has been leader of an analysis group engaged in numerical solution of physical problems. This group was responsible for the systems programming for '*IBM* 704' and subsequent computers; it is at present concerned with the preparations for the installation of the new fast computer *STRETCH* to be installed at Aldermaston. Mr. Glennie is also interested in research work leading to the more effective use of fast computers, including an automatic programming language which could be used on large computers.

(2) Agricultural Research Council: Deputy Chief Scientific Officer

Dr. H. L. Penman joined the staff of Rothamsted Experimental Station in 1937 and became head of the Physics Department in 1954. He has worked on a wide range of agricultural and botanical problems and has made outstanding contributions to the knowledge of soil aeration, the availability to plants of water in soil, the role of stomata in controlling transpiration and the dependence of total transpiration on current weather. His investigations of the use of water by crops are the basis for modern irrigation practice. He has shown that routine weather records could be used to assess the likelihood that irrigation would increase yield, when crops needed irrigating, and how much water should be given. Dr. Penman has shown that irrigation often unexpectedly produces substantial increases in yield and has provided farmers with information that defines need for water objectively, so taking irrigation out of the realms of 'guess-work'. He has also studied the effects of microclimate on pests and diseases. Dr. Penman recently became an officer of the Order of the British Empire and is president of the Royal Meteorological Society. Earlier this year he was elected a Fellow of the Royal Society.

Senior Principal Scientific Officers

Dr. J. J. Bullen is the head of the Pathology Department, Rowett Research Institute, Bucksburn, Aberdeen. His research programme has been particularly concerned with host–parasite relationships in bacterial infections and he has made a number of notable contributions in this field.

Dr. A. T. Cowie, of the Physiology Department, National Institute for Research in Dairying, Reading, carries out all the major surgical procedures required by the Institute. He has made great contributions to endocrinological studies and has a special interest in the field of hormonal regulation of mammary growth and lactation.

Dr. G. A. Garton is a member of the Protein and Lipid Chemistry Section of the Rowett Research Institute and has done much original work on various aspects of lipid metabolism in animals, with particular reference to the pig, ox and sheep, in the course of which he has developed a number of new methods of isolating and analysing lipids.

Dr. J. S. Kennedy is a member of the Agricultural Research Council's Unit of Insect Physiology at Cambridge. His research work has had a great influence on present-day ideas about the behaviour of mosquitoes, the behaviour and nutrition of aphids and the behaviour and polymorphism of locusts.

(3) Development Commission: Senior Principal Scientific Officer

Dr. Mary Parke, senior algologist at the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, is distinguished for her research work on the systematics and life-histories of marine unicellular algae.

SPECTROSCOPY IN THE METALLURGICAL INDUSTRY

THE trend towards more automatic methods for production control analysis was shown at a recent symposium held at Buxton, Derbyshire, arranged by the Spectroscopy Group of the Institute of Physics and the Physical Society in collaboration with the British Iron and Steel Research Association and the British Non-Ferrous Metals Research Association. An audience of about 130, including some overseas visitors, heard a range of eight papers in three half-day sessions (July 5–6) covering X-ray, optical and mass spectroscopic methods.

Analytical procedures depending on X-ray fluorescence are based on the simple and characteristic radiation emitted when X-rays fall on an element while those relying on optical spectroscopy involve the line spectra emitted when the element is excited by an electrical discharge. Mass spectroscopic methods involve measurement of the mass of the ion produced from similar discharges. All three methods have contributed to the knowledge of the electronic configuration of atoms, but it is only in the past decade that developments in instrumental techniques have allowed the extensive analytical application, particularly in the two former fields, where electronic detection can replace photographic methods. Commercially produced instruments are now available on which a sample can be spectroscopically analysed on a routine basis for some twenty to thirty elements.

The talk given by Mr. J. F. Duke (National Physical Laboratory, Teddington) on "Mass Spectroscopy" showed how a double focusing mass spectrometer fitted with a vacuum spark source could be used to detect impurities at, and below, the part-per-million level. The small sample size (μg) and the possibility of detecting upwards of seventy elements in a single analysis offset the high cost of equipment. Future possibilities of the method were discussed. In contrast to some of the more industrial developments for automated and repetitive analysis, Mr. J. S. Forbes (London Assay Office) gave an account of the application of emission spectroscopic methods to: (*a*) determine impurities in fine gold and silver (and incidentally show the changes in alloy and impurity elements in silver over the past few centuries); (*b*) aid in the examination of antique silver articles for detection of fakes; (*c*) develop possible methods to establish assay standards for platinum.

Mr. J. F. Gidley (Imperial Metal Industries (Kynoch) Ltd., Birmingham) showed how copper alloy production could be controlled at the casting stage by rapid X-ray fluorescence analysis for copper, the alloying constituents and impurities. After suitable preparation the cast sample was examined for fluorescent radiation characteristic of copper, chromium, maganese, iron, nickel, zinc, arsenic and tin. The interpretation of the alloying constituents was straightforward, but content determinations of copper required compensation for the interference effects. Comparison of chemical and routine X-ray results showed an accuracy of 0·20 per cent copper and the accuracy of the constituents was sufficient for control purposes. Results and recommendations for additions to the melt could be reported back in about 5 min. Further developments were required before miscellaneous samples can be adequately analysed. Mr. K. M. Bills (International Nickel Company (Mond), Ltd., Birmingham) described how this analysis technique could be applied to the work of an analytical section dealing with alloy development work involving various sample forms. Both in the talks and discussion, such advantages as the non-destructive nature of the test and the disadvantage of inter-element effect were stressed. The relative merits of X-ray and optical emission methods formed one of the more interesting parts of the symposium.

The extended use of direct-reading spectroscopic instruments in a steel works was described by Mr. L. Gwilliam (Steel Co. of Wales). Samples from the furnace, after some preliminary treatment, were spectroscopically examined for carbon, sulphur, manganese, nickel, copper, tin and phosphorus, and results could be reported by in between 3 and 8 min with some fifty–seventy samples being handled in an 8-h shift. Development work was being carried out to extend these automatic methods to the analysis of iron and steel-making slags. Eventually, it was hoped to replace much of the present routine chemical analysis on refractory materials and slags by spectrographic and X-ray fluorescence methods.

Mr. C. Frith (Brown Bayley Steels, Ltd.) described how control analysis of silicon, manganese, chromium, nickel, molybdenum, copper, niobium, titanium and vanadium for stainless steel production was carried out using direct reading spectroscopic instruments. Attention to calibration procedures and suitable means of applying corrections enabled satisfactory results to be consistently obtained. Out of four thousand analyses of various types of stainless steels made in the past three years only three involved errors which arose from the direct reading method.

Similar applications were found in other metallurgical industries. Some of these were noted by Mr. D. Jukes (Henry Wiggin and Co., Ltd., Hereford) in his description of "Spectrographic Control of Nickel Alloy Production". He emphasized that success in application of direct reading methods depended on meticulous attention to standards and calibration procedure. X-ray fluorescence methods for the determination of major alloying constituents such as chromium, cobalt and molybdenum were adopted and up to 400 determinations of a single element could be made by this method in an 8-h period. Although production analysis will in future place less emphasis on conventional chemical methods, the analytical chemist will be increasingly concerned with the provision of reliable standards.

Both in these papers and the discussion which arose, sample preparation and uniformity of procedure was shown to be extremely important for consistent results. One aspect of this was described in the paper by J. R. Brown and D. Wilkinson (Guest, Keen and Nettlefolds Group Research Laboratory, Wolverhampton) on "The Analysis of High Silicon Steels with a Vacuum Spectrometer". The presence of polythene tubing in the argon circuit (used to flush the spark chamber of a direct reading instrument) increased the impurity-levels of oxygen and water vapour in the gas and this contamination could cause erroneous results in the analysis of steels containing more than 1 per cent of silicon. Replacement by copper tubing and purification of the argon over heated magnesium reduced impurity-levels to 7 and 1 parts per million of water vapour and oxygen respectively, and these levels allowed satisfactory analyses for carbon, silicon, phosphorus, manganese and tin in the steels.

The chairmen during the symposium—E. H. S. van Someren (British Welding Research Association), F. R. Bareham (Aluminium Laboratories, Ltd.), H. T. Shirley (Brown-Firth Research Laboratory) and W. Ramsden (Applied Research Laboratories)—successfully supervised the discussion which arose after the papers. Both the comments made then and at more informal sessions showed the values of such a gathering as a means of exchanging information on common problems, but some disappointment was expressed that very few of the more junior analysts were present to profit from hearing the various points of view. It is expected that the papers and comments will be published by Hilger and Watts later this year.

L. BOVEY

THE ONTARIO RESEARCH FOUNDATION

THE annual report for 1961 of the Ontario Research Foundation[*] includes the reports of the director, Dr. A. D. Misener, and the director of research, Dr. A. E. R. Westman, together with the accounts, lists of scientific and technical papers published in 1961 and of professional and technical staff. Dr. Misener claims that in all categories the growth of research and development in Canada is substantial and compares favourably with the rate of growth in the United States and the United Kingdom. During 1956–58, that financed by industry itself in Canada increased by about 20 per cent per annum; in the same period the Foundation's contract research for industry increased by 22 per cent per annum, but research expenditure by both Government and industry is low by a factor of 3–4 when expressed as a percentage of the gross national product, and in research performed by industry but financed by Government is low by a factor of 11 compared with the United Kingdom and 19 compared with the United States.

Dr. Westman notes an overall expansion in sponsored research in 1961, particularly in chemistry, a reduction in science activities, particularly in engineering and metallurgy, and difficulties in recruiting junior staff. The Department of Biochemistry concluded a study of the effect on the serum cholesterol-level in rats of a diet containing hydrogenated sunflower oil, but a new investigation was commenced on the effect of agitation on the isomeric fatty acids produced in hydrogenation. Basic research was continued in the Department of Chemistry on the study of stereo-specific catalysts for polymerization and the mechanism of the polymerization of butene by the heterogeneous catalyst aluminium triisobutyl-titanium trichloride, as well as work on phosphate glasses, while in physical metallurgy, unsponsored research included studies in metal physics, the magnetic properties of iron ore and copper alloy surfaces. The Department of Parasitology completed a study of the throat bots of deer and established the mode of transmission of 'brain worms' of deer with certain land snails as intermediate hosts. A survey of fish parasites in lakes was extended, and a new study on a warble fly, the larvæ of which live under the skin of certain wild animals, showed that the activity of the animal is reduced if it harbours many of these parasites, which may cause death directly or make the animal unable to escape predators. The Department of Physiography made further examination of the Algonquin Lake plain, and in the Department of Textiles unsponsored research was concerned with the chemical modification of cotton cellulose, wool and hair to give improved fabrics.

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[*] Ontario Research Foundation. Annual Report, 1961. Pp. 64. (Toronto: Ontario Research Foundation, 1962.)

ENGINEERING DEVELOPMENTS IN THE BRITISH BROADCASTING CORPORATION

THE excellent series of monographs describing the work of the Engineering Division of the British Broadcasting Corporation (*Nature*, 191, 1356; 1961), has been supplemented by five further issues[*] since September 1961.

As their titles indicate, these monographs deal with a range of subjects from basic research in the techniques necessary for a high-quality broadcasting service, to the actual design and development of the equipment installed at the steadily increasing number of transmitting stations in Britain.

In Monograph No. 39, the director of engineering, B.B.C., Sir Harold Bishop, presents a comprehensive review of the development of the television service in Britain, starting with a reminder of the early history of the subject, thus: "Although all the fundamentals of an electronic transmitting and receiving system were put forward by A. A. Campbell-Swinton in his famous letter to *Nature* as far back as June 1908 [Vol. 78, 151], no practical realization of his proposals was attempted until the early 'thirties".

The earliest B.B.C. television transmissions started in 1926 when pictures from John Logie Baird's laboratory were radiated experimentally; but for nearly ten years thereafter there was much divergence of opinion among engineers and scientists as to the most effective means of scanning a picture or scene, and as to the band-width required in the radio-spectrum for its transmission. However, what has since been generally conceded to be the best public television service in the world was formally opened by H.M. Postmaster-General on November 2, 1936. Sir Harold Bishop's monograph describes in a most interesting manner the fundamental techniques of the British television system, the growth and progress of the public service over the past quarter of a century, together with a brief review of current and future development. In the latter connexion, reference is made to research in colour television, and the problems which will be encountered in the extension of television broadcasting into the ultra-high-frequency bands, and the use of a 625-line scanning standard. This monograph is illustrated with 14 plates, four of which are in colour.

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[*] B.B.C Engineering Monograph No. 38: "Operational Research on Microphone and Studio Techniques in Stereophony", D. E. L. Shorter. Pp. 23. No. 39: "Twenty-five Years of B.B.C. Television", Sir Harold Bishop. Pp. 41. No. 40: "The Broadcasting of Music in Television". Part I, Introduction. R. F. A. Pottinger. Part II, Sound and Vision, L. Salter. Part III, Operational Technique, E. G. M. Alkin. Part IV, Acoustic Treatment of Television Studios, C. L. S. Gilford, pp. 23. No. 41: "The Design of a Group of Plug-in Television Studio Amplifiers", K. J. Austin. Pp. 19. No. 42: "Apparatus for Television and Sound Relay Stations", F. A. Peachey, R. Toombs and D. L. Smart. Pp. 22. (London: British Broadcasting Corporation, 1961 and 1962. 5s. each.)

With the extension of the sound and television broadcasting ervice towards the objective of full national coverage, limitations of staff have made it necessary for the B.B.C. to develop remote and automatic control facilities for unattended relay stations. Monograph No. 42 describes the development of such stations, which vary in radiated power from a few watts to several kilowatts, and derive their programme modulation 'feed' either by direct line or radio link, or by normal broadcast reception from the appropriate regional transmitter. Some eleven such stations are in service, and another fourteen are under construction; many of these are in remote areas where difficulties have been experienced by the listening and viewing public in obtaining satisfactory reception. With regard to maintenance, the B.B.C.'s plan is for a regular call to be made by an engineer at each of the new unattended stations. While attempts are made to anticipate a breakdown, such as the replacement of a valve seen to be approaching the end of its useful life, no steps are taken towards major repairs in the field. When necessary, the faulty unit is removed and replaced by a spare; a practice which causes the minimum disruption to the service.

The work described in these and other monographs indicates the realization by the B.B.C. of its responsibility to provide the best and most complete national broadcasting service from a technical point of view; and to take all practicable steps to keep this service up to date. With the encouragement given in the Government's recent White Paper (H.M.S.O., Cmnd. 1770, July 1962), it can be confidently expected that the service will continue to develop in the future on the existing high standard. R. L. SMITH-ROSE

THE JOHN INNES INSTITUTE

THE satisfactory progress made in genetical studies of various economic plants is recorded in the annual report of the John Innes Institute*, and work on cellular mechanisms is aided by the Department of Cell Biology—now a functional unit.

The long-term project of improving varieties of apples has continued, and there is hope eventually of selecting seedlings with resistance to both scab and mildew. The work on selection of cherry varieties has been helped by the discovery that inoculation of the bacterial canker organism into 2-year-old wood in December give results closely resembling those found in the field, and progress has been made in development of reliable methods of testing the resistance of first-year seedlings. Some selections of strawberries show resistance to mildew and give heavy yields of large fruit. It is hoped to combine these attributes with good quality and resistance to other diseases. The possibility of breeding tomatoes resistant to tobacco mosaic virus is being explored; but the occurrence of symptomless carriers is a drawback because a commercial variety with complete tolerance of the virus is undesirable since it is a reservoir of infection.

In the Department of Genetics investigations of genetic control of the enzyme triphosphopyridine nucleotide-linked glutamic dehydrogenase of *Neurospora crassa* has been continued. and methods developed for obtaining pure preparations. Work has continued on the mechanisms of recombination and mutation in *Ustilago maydis* and mutability in *Antirrhinum majus*. From a stock of tomato seed stored at 27° C for 14 years it has been established that there is a much higher incidence of rogues from plants grown from old seed than among those raised from one-year-old seed. Prolonged storage of seed also affected earliness but not total yield of fruit.

In the Department of Potato Genetics advances have been made in classification of cultivated varieties and an extensive range of behaviour has been revealed in the interspecific relationship of Central American wild species. Assessment of scab resistance has been advanced by perfection of a pot test which gives results comparable with field trials. Some scab-resistant clones have been revealed in Andigena stocks and it is hoped to transfer this resistance to a *Tuberosum* background.

The main theme of research in the Department of Cell Biology has been the examination of the structure and function of the ribonucleic acid of the nucleus. There is evidence that nuclear RNA is not necessarily a precursor of the cytoplasmic RNA but is mainly broken down within the cell to low-molecular weight compounds.

The growth rooms and cabinets developed in the Department of Physiology and Plant Culture are now in routine use for various investigations. One of these is the effect of temperature and light-intensity on the development of the shoot apex of the tomato. Considerable enlargement of the shoot apex always takes place during the vegetative phase before the first inflorescence is initiated. The processes of formation of leaf primordia and apical enlargement appear to be independent. More leaves are produced at 25° C before flowering than at 15° C, and the number of extra leaves produced is greater the lower the light-intensity. Experiments have been initiated to define more precisely the nature of the rogue characters in tomato and to express it in quantitative terms.

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* John Innes Institute. Fifty-second Annual Report, 1961. Pp. 42. (Bayfordbury, Hertford: John Innes Institute, 1962.) 3*s*.

INTERNATIONAL WEIGHTS AND MEASURES

THE Proceedings of the forty-ninth session of the International Committee of Weights and Measures held during October 4–20, 1960, have now been published*. It contains the reports of the secretary of the Committee covering the period September 1, 1958–August 31, 1960; the director, Mr. Volet, of the International Bureau of Weights and Measures, on the activities of the Bureau during the same period; the commission for the revision of the treaty of the metre; the consultative committee for ionizing radiation standards; the commissions on system of units; and the commissions on the work and administration of the Bureau.

The International Committee recommended, and this was later adopted by the eleventh General Conference on Weights and Measures, that the metre be the length equal to 1,650,763·73 wave-lengths *in vacuo* of the radiation corresponding to the transition between the levels p_{10} and $5d_5$ of krypton-86; the definition of the metre in vogue since 1889, based on the international prototype of platinum iridium, be abrogated; and the international prototype sanctioned by the First Conference of Weights and Measures in 1889 be preserved at the International Bureau of Weights and Measures under the same conditions as those fixed in 1889. The Conference also confirmed the recommendation that the International Bureau undertake the work of international unification of the standards of measurement of ionizing radiation, and the extension of the laboratories of the Bureau and the increase of its equipment for this purpose. In this connexion, the Bureau would take charge of the international standard of radium No. 5430 which the Radium Institute of the University of Paris was willing to have transferred. The Committee was asked to undertake without delay the necessary investigations to fix precisely the units and ideas used in regard to ionizing radiation and to submit its findings to the twelfth General Conference to be held in 1966.

It was agreed that the cubic decimetre and the litre were not equal, but differed by about 28 millionths, and that a report on this matter should be presented to the twelfth General Conference.

The full texts of the sixteen resolutions adopted by the eleventh General Conference are printed in the *Comptes Rendus des Séances de la Onzième Conférence Générale des Poids et Mesures*†. Appendix 5 to this volume is the official French version of the International Practical Scale of Temperature of 1948 (amended edition of 1960), and Appendix 6 gives a detailed description of the progress of the International System of Units based on the metre, kilogramme, second, ampere, degree K and candela.

S. WEINTROUB

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* Bureau International des Poids et Mesures, Sèvres. Comptes Rendus des Séances de la Onzième Conférence Générale des Poids et Mesures, Paris, 11–20 Octobre 1960. Pp. 144. (Paris: Gauthier-Villars et Cie., 1961.)

† Comité International des Poids et Mesures. Procès-Verbaux des Séances, 2ᵉ Série, Tome 28 (49ᵉ Session, 1960). Pp. 161. (Paris: Gauthier-Villars et Cie., 1962.)

A RADIOISOTOPE TECHNIQUE FOR TRACING AIR MOVEMENTS IN CLEAR AIR AND IN CLOUDS

By R. A. FOOKES and J. S. WATT

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AND

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A NEW technique using radioisotopes for tracing air movements in clear air and in clouds is being developed principally for use in experiments related to cloud seeding. The tracer is released from an aircraft and, during a series of passes, the γ-rays emitted by the material about the aircraft are detected by a large crystal scintillometer. From the measurements of γ-ray intensity, the movement of the tracer may be followed and information obtained on its spatial distribution.

The method has been tested in ten separate flight experiments. In the first two xenon-133 was used; but this was abandoned in favour of the more readily available copper-64 (half-life 12·8 h) in the form of finely powdered copper oxide. Up to 2-curie quantities of ^{64}CuO have been released and traced with an omnidirectional scintillation detector for periods up to 50 min. A typical flight record of one pass through a cloud is given in Fig. 1. It shows the variation of detected radiation intensity as the aircraft passed the radioisotope about 1·6 min after its release. The

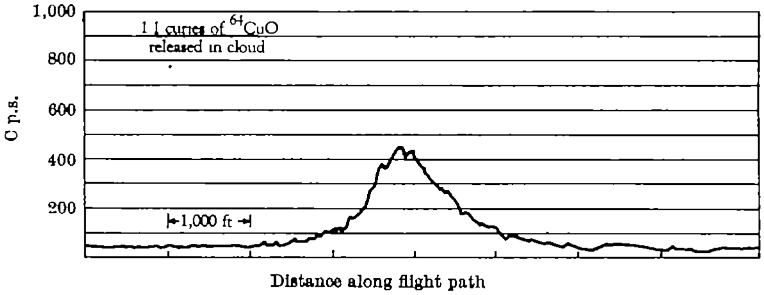

Fig. 1. Record of variation of γ-ray intensity along flight path

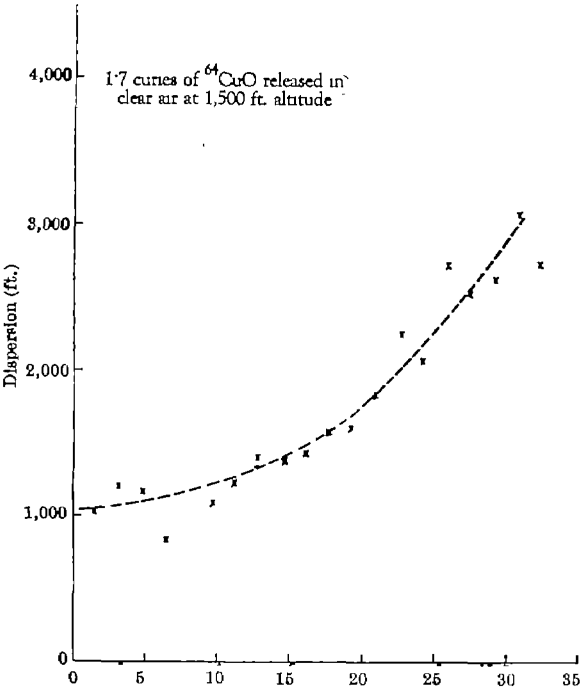

Fig. 2. Dispersion as a function of time after release

background count-rate measured by the scintillometer (the crystal is a cylinder of sodium iodide of 4-in. diameter and 3-in. height) was about 40 counts per second. For altitudes between 4,000 and 12,000 ft., it is usually between 40 and 100 counts per second. Fig. 2 shows dispersion as a function of time after release of the ^{64}CuO for a flight on May 16, 1961. Dispersion is defined as the length of flight path corresponding to the distance between the points on the flight record where the count-rate, after background has been subtracted, has fallen to 10 per cent of that at the peak intensity of the pass.

From Fig. 2 it can be seen that about 30 min after release of the ^{64}CuO its γ-rays were detected throughout 3,000 ft. of flight path. However, soon after release, when the volume containing the radioisotope should be small, the dispersion measured was about 1,000 ft., varying little in the first few passes. These large apparent values of dispersion, indicating poor resolving power of the technique along the line of flight, are due to lack of directivity of the detection device and the large range of the γ-rays in air. To improve the directivity of the system, a collimated detector has been made. It consists of the scintillation crystal sandwiched between two circular lead plates which are mounted in planes perpendicular to the flight axis. If the collimated detector is designed to 'see' only those γ-rays emitted within an angle of ± 10° to the plane perpendicular to the direction of flight, then the resolving power along the line of flight should be improved by about an order of magnitude.

As the radioisotope disperses in the atmosphere, air currents sweep it away from the point of release and so it becomes increasingly difficult with time to return to the region of greatest activity. In clouds, the tracer may be lost completely within a short period. To help locate the activity and hence guide the aircraft back to it, a device can be used which indicates whether the bulk of the radioisotope is above or below to the left or to the right of the aircraft. A prototype unit, giving only left–right indication, has been successfully tested in flight experiments. A more advanced unit has since been constructed, comprising four scintillation detectors, each with a large plastic phosphor. Lead plates, in the form of a cross (×) and mounted with their line of junction in the direction of flight, separate the detectors so that each one effectively views one quadrant of a sphere. Each detector is shielded by a thin lead sheet, which absorbs few of the unscattered copper-64 γ-rays, but most of those scattered by the air. This reduces the number of γ-rays which, although emitted in one quadrant, are scattered into the detector of another quadrant.

There is an upper limit to the volume throughout which quantitative measurements of γ-ray intensity may be made with the present technique using the omnidirectional detector. Consider the case of the radioisotope uniformly dispersed in this volume. The upper limit will depend on four factors; the sensitivity of the scintillometer, the absorption of γ-rays in air as measured by it, the background detected by the scintillometer, and the maximum amount of activity that may be released. The first two factors have been determined by measuring the count-rate of the scintillation detector as a function of distance between it and a calibrated source of copper-64. To reduce interference by the ground, both source and detector were suspended more than 100 ft. above ground-level. From the measurements made, it has been calculated that the scintillometer will record a count-rate of about 70 counts per second above background when in air containing a uniform concentration of 30 μμc. of copper-64 per cubic foot. This count-rate is sufficiently greater than background for quantitative measurements to be made. It is possible to handle and release at least 20 curies of ^{64}CuO. Hence the upper limit of volume throughout which measurements of radioisotope concentration can be made is at least four cubic miles.

MECHANISMS OF THE WEAR OF GRAPHITE SLIDING ON STEEL—WITH AND WITHOUT AN ELECTRIC CURRENT PASSING ACROSS THE INTERFACE

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THE reasons for the low coefficient of friction of graphite under normal atmospheric conditions, and the nature of the mechanism of the wear of graphite, have been considered and discussed by various authors. Savage[1] suggested that various vapours, for example, water vapour, form a thin surface layer on the graphite, which provides boundary lubrication. Deacon and Goodman[2] have discussed experimental results with various vapours and the effects of temperature, in terms of weak bonding between the planar faces of graphite platelets and strong bonding at their edges.

Recently, it has been shown by Bollman and Spreadborough[3] that groups of graphite planes can roll up to form small roller bearings. They consider that such rollers are responsible for the low frictional properties of graphite. Further work by Spreadborough[4] with a small copper stylus sliding over graphite has suggested that lubrication by this method involves the surface orientation of crystallites, and the subsequent formation of these rollers. The mechanism of wear is, presumably, the formation of these rollers by a 'ploughing' action.

A detailed investigation has been carried out by us[5] into the factors influencing the rate of wear of one grade of graphite brush sliding on steel slip-rings. The aim of the present article is to report the results of further studies on one aspect of this work—the mechanism of wear. By using various electron-microscope replica techniques, it has proved possible to confirm that the wear mechanism changes with the sliding conditions, and to identify the mechanisms of wear by means of subsidiary experiments. Examination of wear debris collected from the apparatus, the surface of the brush, and the surface of the slip-ring failed to show the presence of graphite rollers. However, abundant evidence of such rollers (though usually of smaller dimensions) was found on examination of thin sections of new brushes produced with an ultra-microtome, suggesting that the mechanism of wear involving roller production is probably confined to low speeds of sliding, and involves ploughing or cutting actions.

Rate of Wear

The apparatus was designed to measure accurately the rate of wear of graphite brushes sliding on a steel slip-ring of 6-in. diameter. These brushes ran on two separate tracks and the rate of brush wear could be obtained from two sensitive dial gauges, the plungers of which operated on the rear surface of the brushes (Fig. 1). Brush loading was applied by means of a system of pulleys and weights, so that the load remained constant as the brush wore away. Electric current could be passed across the interfaces.

The results of particular interest here involve the dependence of the rate of wear on load, brush area, and current. With no current, equilibrium wear conditions are established fairly rapidly, and the rate of wear is then proportional to load and independent of the apparent area of contact. This means that the surface conditions remain the same as the load is changed, and no additional variables are introduced[6]. An electric current passing across the interface removes the proportionality with load, and its effect is, therefore, to change the nature of the interface. The current has a greater effect on the positive interface than on the negative interface. Earlier results were confirmed by using brushes of the same grade, but from different batches. The magnitude of the results differed by about 20 per cent, but the same shape and character of the curves remained.

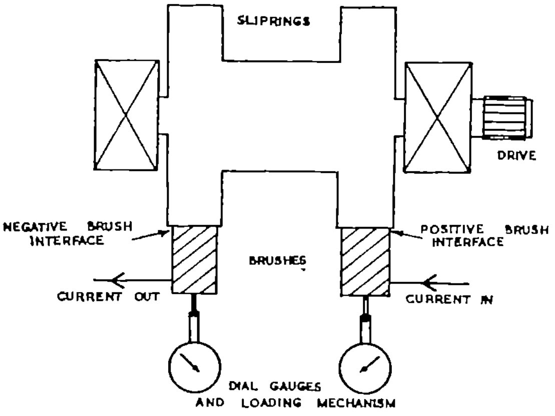

Fig. 1. Diagram of the wear apparatus showing arrangement of the brushes

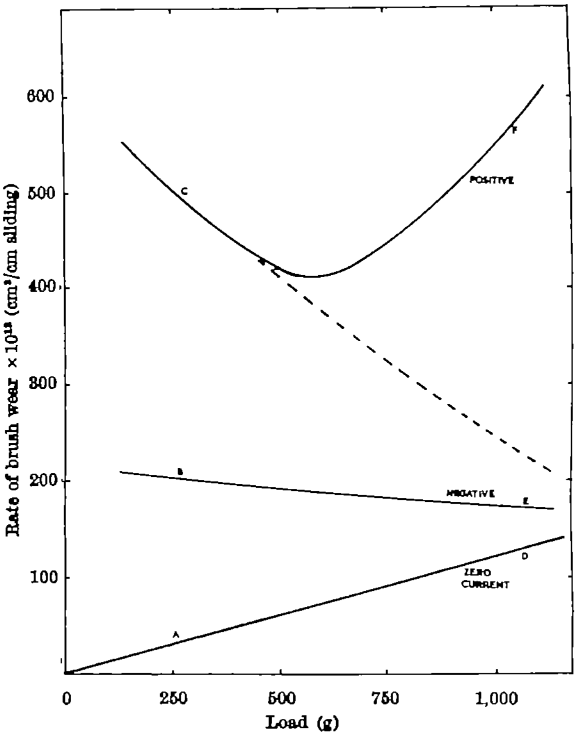

Fig. 2. Rate of brush wear versus load (*HM* 6 brush; 4,000 cm/sec; 60 amp and 0 amp)

The results for the (total) rate of wear of Fig. 2, with current passing through the brushes, can be considered as being composed of two parts : a contribution from a mechanical process of wear, and a contribution due to the passage of current (an electrical process of wear), though this may be merely a modification of the mechanical process. At low loads, as the load is increased the mechanical contribution increases, the electrical contribution decreases, so that the total rate of wear for both negative and positive brushes decreases. This is true for the negative brush, even at higher loads, though for the positive brush the rate of wear suddenly increases rapidly. This suggests that a change in the mechanism of wear takes place at higher loads under the positive brush. To examine this point, brushes were run under various conditions (points *A*–*F* in Fig. 2,) and the brush faces were examined by means of replica techniques and the electron microscope.

Electron Microscope Investigation

Replicas of the brushes after running under various conditions were made in the following manner.

The brushes were masked off with 'Lacomit' stopping-off medium, leaving only the rubbed faces exposed. Primary copper replicas were taken from each brush by electro-deposition of copper from a solution containing 250 g/l. copper sulphate, 75 g/l. sulphuric acid, at a current density of 150 m. amp/in^2. to produce a deposit approximately 0·002 in. thick. The copper replicas were carefully stripped from the brush surfaces, together with a large quantity of adhering graphite. A considerable amount of this graphite was removed by pressing adhesive tape on to the copper surface, then stripping off the tape, together with some of the graphite particles. Repeated application of the adhesive tape was carried out until no further graphite could be removed. Any residual adhesive from the tape was removed from the copper by a thorough washing in warm petroleum ether. Secondary carbon platinum replicas, self-shadowed at 3 : 1 parallel to the rubbing direction, were then taken from the primary copper replicas. The negative replicas thus produced were examined in an Elmiskop I microscope.

Fig. 3 shows a typical photomicrograph from points *A* or *D* on Fig. 2. The brush surface is fairly smooth, apart from tongues of graphite protruding from the surface, as might be expected from an aligned graphite layer[7]. Fig. 4 shows a typical photomicrograph from points *B*, *C*, *E*. Here the general features are of a smooth surface covered with numerous smooth-sided protuberances of variable size and frequently almost hemispherical in character. These protuberances could be formed by some erosion process, for example, by small hard particles from the metal ring (perhaps oxide). In Fig. 5 a typical photomicrograph from point *F* is shown. Many deep, sharply angled and step-sided hollows are seen, which are probably formed when particles of graphite are torn from the brush face in a brittle manner.

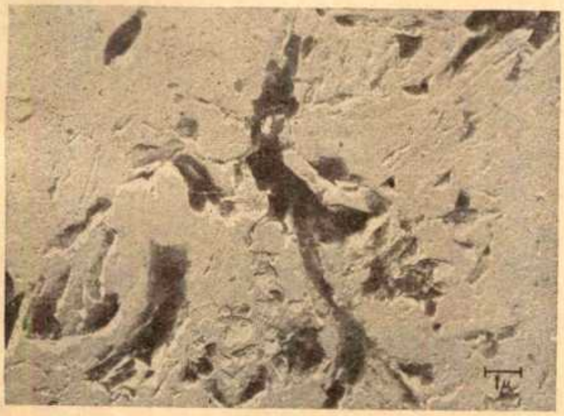
Fig. 3. Electron micrograph typical of points *A* and *D*, showing tongues of graphite present in the brush surface

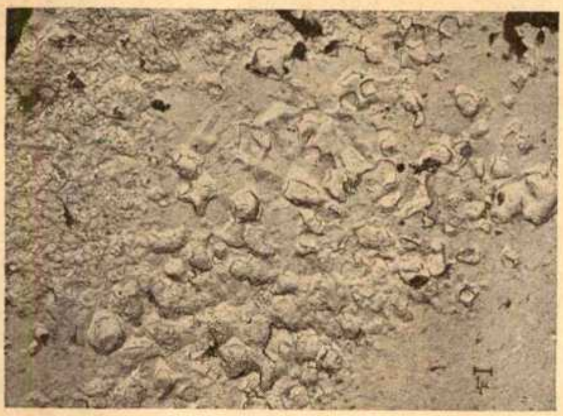
Fig. 4. Electron micrograph typical of points *B*, *C*, and *E*, showing many smooth-sided protuberances

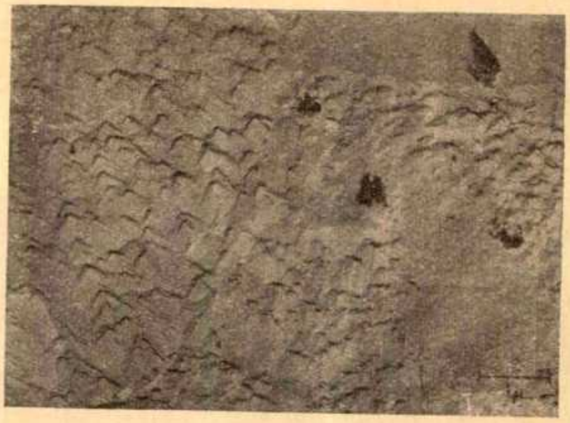
Fig. 5. Electron micrograph typical of point *F*, showing step-like fractures

To examine these interpretations placed on the electron micrographs, new brushes were rubbed on (*a*) the smooth surface of a paper card ; (*b*) a piece of glass on which a copious supply of loose 'Carborundum' powder (600 grade) had been placed ; (*c*) a piece of new 'Carborundum' paper (600 grade). The resulting photomicrographs corresponded : (*a*) above was similar to Fig. 3, (*b*) to Fig. 4, and (*c*) to Fig. 5.

Conclusions

Previous work by us[5] has shown that asymmetric wear at the positive and negative brushes was most probably due to the preferential removal of graphite wear fragments comprising graphite lamellæ held between asperities on the positive metal wear track, so that an increased filing action by the metal asperities on the positive brush arose.

The present work supports this view. Furthermore, at high loads and high currents, the wear process at the positive brush changes, so that wear particles are torn from the brush in a brittle manner.

No evidence of graphite rollers was found in wear particles collected from the wear debris, the face of the brush, and the ring surfaces, though they could be easily recognized in thin sections of brushes made with an ultra-microtome. It would appear that a mechanism producing graphite rollers, and an explanation of a low coefficient of friction arising from such rollers, are not universally applicable ; they are, perhaps, limited to low velocities of sliding.

We thank Mr. J. K. Brown, director of research, for permission to publish this article, and G. P. Buchi for development of the replica technique.

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[2] Deacon, R. F., and Goodman, J. F., *Proc. Roy. Soc.*, A, **243**, 464 (1958).

[3] Bollman, W., and Spreadborough, J., *Nature*, **186**, 29 (1960).

[4] Spreadborough, J., *Wear*, **5** 18 (1962).

[5] Thompson, J. E., and Turner, M. J. B., *Proc. Inst. Elec. Eng.*, Pt. A, **109**, 235 (1962).

[6] Archard, J. F., and Hirst, W., *Proc. Roy. Soc.*, A, **236**, 397 (1956).

[7] Midgely, J. W., and Teer, D. G., *Nature*, **189**, 735 (1961).

MODE SEQUENCES IN RUBY LASER EMISSION

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TIME-resolved observations of the intensity distribution and wave-length of the output light from a ruby laser, operated well above threshold pumping power, have already been reported[1]. This article describes a more detailed investigation, by similar methods, of the near-field patterns and wave-lengths of the first few spikes emitted by a laser operated very close to threshold conditions. Transverse mode patterns are observed, similar to those already reported for single spikes from ruby lasers[2,3] and in the output from gas discharge lasers[4,5]. The experiments recorded here show each spike to be associated with a single mode of oscillation, in agreement with the results of Hanes and Stoicheff[6]. In addition, however, they show that in the early spikes one or more sequences of modes occur, overlapping in time, each sequence being associated with a certain region of the crystal face. The steady progression towards longer wave-lengths noted previously[1] is observed during a sequence: the longitudinal mode number decreases regularly by one, or sometimes two, from one member of the sequence to the next. The transverse mode patterns do not change systematically with time, but a general trend towards more complex and extensive patterns is apparent.

A cylindrical ruby crystal 5 cm long and 0·6 cm in diameter, with the crystal axis perpendicular to the cylinder axis, was used. The polished ends were coated with dielectric multilayers of high reflectivity. The crystal was mounted inside an A.E.I. type *FA*26 helical xenon flash tube, the assembly being cooled by a flow of air at room temperature.

Figs. 1*a* and 1*b* are rotating-mirror camera photographs of the front face of the crystal, for the initial spikes of two successive pulses close to the threshold pumping energy. The modes occurring in the first few spikes appear to be fairly simple. The first four come from the same region of the crystal face. About the time of the fourth spike a new region, at the same level in the photograph, begins to emit in different modes. Soon afterwards a third region, below the first two, begins another sequence, while the earlier sequences continue. The different regions of the crystal face emit independently of each other, as was noted by Sorokin and Stevenson[7] for calcium fluoride doped with trivalent uranium and by Statz *et al.*[8] for ruby.

It should be noted that inhomogeneities and strain cause significant variations in the optical length of the crystal, amounting to several wave-lengths over the end faces of a typical crystal[9]. Thus the optical path between the two surfaces is constant only over small areas, which are the regions where the more easily excited modes may be expected to appear.

On the photographic plate, the separation of the individual mode patterns is sometimes smaller than the diameter of the image of the crystal face. Thus, the patterns from different regions of the crystal face sometimes appear on the plate in an order different from that in which they were emitted. Most of the spikes are of short duration (< 0·25 μsec), but a few persist for several μsec, as has been observed previously[1,5]. Occasionally two spikes are apparently linked in the same mode, as may be seen in Fig. 1*b*. The sequence of transverse mode patterns does not

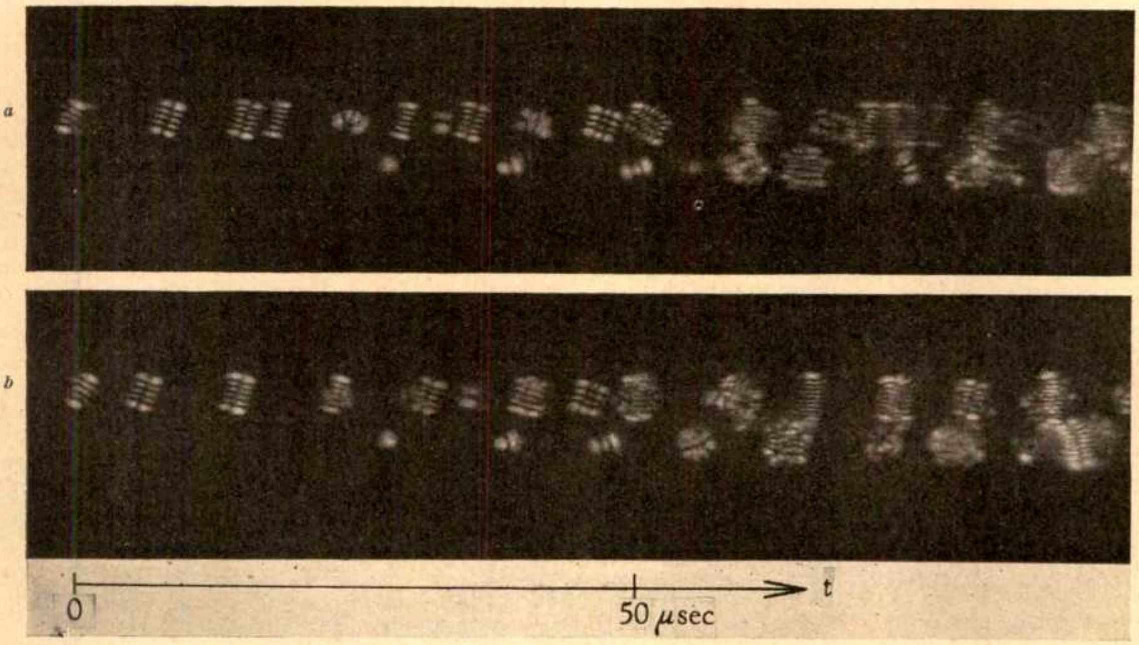

Fig. 1. Transverse mode sequences in two successive pulses

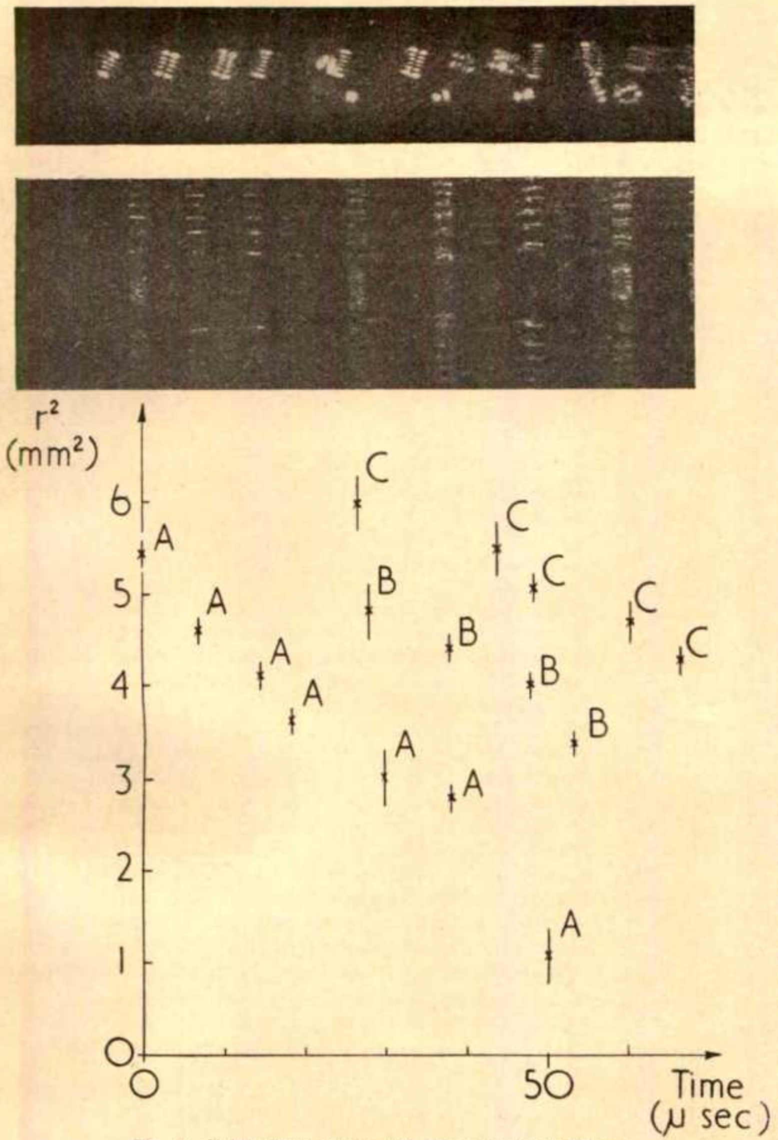

Fig. 2. Fabry–Perot fringes and transverse mode patterns

change much in a series of pulses at short intervals, though the wave-length of the first spike is known to change appreciably from pulse to pulse as a result of heating[1].

The laser was also operated with the crystal rotated about the cylinder axis into four different positions 90° apart. The mount and the flash tube were not rotated. The order in which the various regions of the crystal face began to emit depended on the position of the crystal, an effect probably due to asymmetry in the pumping light distribution, as suggested by Evtuhov and Neeland[2]. However, each region always exhibited its own characteristic mode sequence.

By splitting the output beam and passing the two parts through different optical systems it was possible simultaneously to record near-field patterns and Fabry–Perot interferograms of the spikes with the rotating-mirror camera. Fig. 2 shows part of a plate taken in this way, with a plot of the squares of the fringe radii added below. Some of the fringes were too faint to be measured. As in Fig. 1, the order of the transverse mode patterns is not always the temporal order of emission. However, the corresponding fringe patterns were obtained with a diffuser and a narrow slit, and are thus in correct time order. The Fabry–Perot etalon had an 8·29 mm spacer, for which the inter-order fringe separation corresponds to 0·29 Å at 6943 Å. The fringe-forming optical system was such that the difference in wave-length between successive longitudinal modes in the ruby crystal (0·025 Å) corresponded to a difference in the squares of the fringe radii of 0·50 mm².

As may be seen in Fig. 2, the wave-lengths of spikes emitted from a particular region of the crystal face become progressively longer. Within the experimental errors indicated the wave-length steps correspond to integer decrements in the longitudinal mode number. The effect is too rapid to be explained by thermal changes. When a new region begins to emit, an independent new sequence is started. No simple relationship has been found between the wave-lengths at which the various sequences begin. Three regions may be distinguished, giving rise to the sequences *A*, *B* and *C* in Fig. 2. The situation becomes too confused for analysis after the first twenty spikes or so, because each mode then extends over a large fraction of the area of the crystal face. The beat frequencies observed by McMurtry and Siegman[10] are likely to be due to coincidences in time between members of two sequences from different regions.

A second ruby crystal of similar dimensions and the same orientation was also studied. Its end faces were aluminized, the output surface having 2 per cent transmission. As the intensity of its light emission in each spike was only one-hundredth of that of the first crystal, the transverse mode patterns and fringes had to be examined separately. A single ill-defined transverse mode was found during the initial spike sequence. A different pattern was observed on rotating the crystal. Marked linking occurred between spikes. The wave-length variation in the early spikes followed similar trends to those observed with the first crystal.

It is suggested that a crystal will resonate in different modes in two successive spikes, because in the vicinity of antinodes of a standing wave the density of excited ions will be depleted by emission. Also, as suggested previously[1], the longitudinal mode corresponding to the eigen wave-length nearest to the R_1 line centre is most likely to reach threshold first, followed by modes corresponding to wave-lengths successively more distant from the centre. However, this still does not explain why the wave-length of the emission from a given region always increases during the first few spikes.

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ELECTRON SPIN RESONANCE IN WOOD-GRINDING AND WOOD-PULFING

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Formation of Free Radicals in Wood Grinding and Pulp Beating

It is known that the mechanical disintegration of high-molecular-weight substances results in degradation. For example, Hess and Steurer[1] have shown that the molecular vibrational energy available in ball-milling of cellulose is sufficient to rupture covalent bonds. This was substantiated by Lindberg *et al.*[2], who found that carbon–carbon as well as carbon–oxygen linkages are ruptured, and that this degradation is entirely mechanical. The majority of organic substances have been found to undergo thermal decomposition by mechanisms[3] in which free radicals are formed as intermediates. Furthermore, a recent study of the cryolysis[4] of high-molecular-weight compounds demonstrated that bond rupture and radical formation are due to the pressures deriving from phase transitions.

In view of these findings we have attempted to determine whether mechanical wood-grinding and pulp-beating, as practised commercially in the pulp and paper industry, involves the formation of macroradicals in lignin and carbohydrates respectively. This was investigated by electron spin resonance measurements in wood with increasing grinding time, and in paper pulps with increasing beating time.

Air-dried wood from black spruce (*Picea mariana*), and strong kraft pulps produced from the same wood were used in these experiments. Weighed samples of these materials in open 'Pyrex' glass capillaries were placed in the cavity of an electron spin resonance spectrometer (Varian Associates, model V-4500) at room temperature, and the signals were recorded. In one series of experiments, the wood was studied first in the initial (mechanically undamaged) state, then after it had been ground through mesh 40 of a laboratory Wiley mill, and finally after a further ball milling. The electron spin resonance signal areas were measured planimetrically. In all samples studied, free radicals were found to be present, their concentration increasing as the wood particle-size became smaller, as illustrated in Fig. 1. Care was taken to keep the spectrometer sensitivity constant throughout the experiment.

For comparison, the smaller signal (*d*) from a bleached, high-purity pulp, ball-milled to about the same fine grain as the ball-milled wood (*c*), is also shown. In addition, it was observed that the macroradicals in ground wood are relatively unstable, and that their total amount decreases rapidly when the temperature is raised.

The reason that the wood signal is so much greater than the pulp signal may be that the lignin present in wood, because of its three-dimensional, rather rigid structure, is less resistant to grinding than the cellulose. Thus, more bonds are ruptured and free radicals formed in the lignin portions of the wood than in its cellulose portions. Alternatively, more free radicals may have been trapped in the ground lignin than in the ground cellulose.

In another series of experiments the electron spin resonance of two unbleached kraft pulps was studied

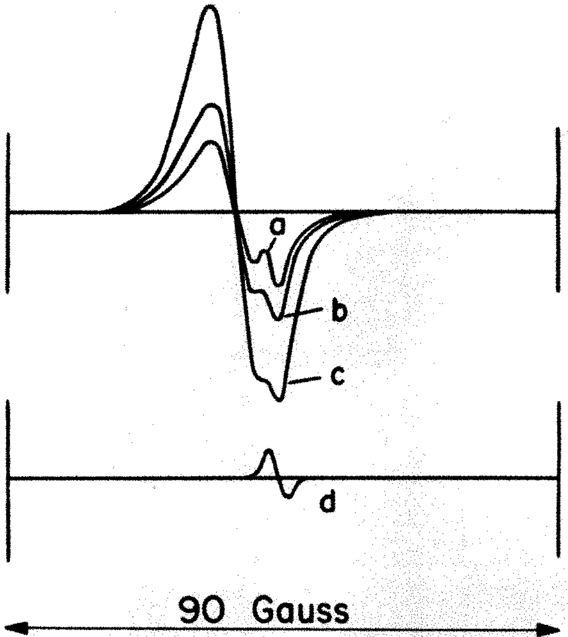

Fig. 1. Electron spin resonance signals of wood (*Picea mariana*) in progressive stages of sub-division. *a*, Initial undamaged wood; *b*, wood ground through mesh 40 of a Wiley mill; *c*, wood ground according to (*b*) and afterwards ball-milled; *d*, high-purity bleached pulp, ball-milled like (*c*).

at room temperature with increasing time (0–125 min) of beating in a Valley beater. Specimens of equal weight (0·0134 g) were cut from 1-g standard sheets of the various pulp samples, and in rolled form they were inserted into the glass capillaries. The electron spin resonance signals were recorded and evaluated. No significant difference in the size of the signals was found irrespective of the duration of beating.

Tentatively, the following explanation is given. By the mechanical damaging of the fibres in pulp beating, covalent bonds are broken; thus, macroradicals are formed, and the specific surface is increased. Concurrently, water association and fibre swelling take place, influencing the wet-web behaviour[5]. When water is removed from the wet sheet during drying the macroradicals would contribute to interfibre bonding. This theory would also explain why sheets formed from pulps which were never dried exhibit superior strength properties compared with those from pulps which had been dried. The rather uniform free radical content found in pulps beaten to decreasing Canadian Standard Freeness (CSF) is probably related to the macroradicals stabilized and trapped in the residual lignin. This is supported by the finding that the initial wood and the unbleached kraft pulps exhibit electron spin resonance signals which are similar to each other in shape, but different from the signals from isolated lignins.

Electron Spin Resonance in Alkaline Wood Pulping

We have recently carried out alkaline pulping of wood in the cavity of an electron spin resonance spectrometer (Varian Associates, model *V*-4500) and have found that strong signals were recorded at the pulping temperature, thus indicating the formation of free radical centres. Results from a kinetic investigation[6] of the alkaline delignification of wood have also suggested the participation of free radicals. Lignin preparations isolated in various ways (milled wood lignin, ethanol lignin, dioxane lignin, periodate lignin, ligninsulphonic acid, alkali lignin) were also found to contain free radical centres even after long periods of storage.

In our pulping experiments, air-dried wood from black spruce (*Picea mariana*) was ground through mesh 40 of a Wiley mill. Part of this material was soaked for 10 min at room temperature in a 4 per cent sodium hydroxide solution (ratio 1 : 20). Afterwards, the excess lye was pressed off, and the impregnated material was freeze-dried, to avoid the effect of moisture on the cavity *Q*. As found earlier[7], alkaline lignin solubilization at elevated temperature occurs whether large amounts of moisture are present or not. The spectrum of both the air-dried initial wood powder and the freeze-dried, alkali-impregnated wood material was measured in 'Pyrex' glass capillaries at room temperature and also at the pulping temperature (180–190° C). Rapid heating to this temperature was obtained by means of a stream of hot nitrogen. A single line near the free-spin *g*-value was observed in all experiments, the maximum slope line-width being approximately 10 gauss. The areas of the signals obtained in the various experiments were estimated planimetrically, and the results were calculated on a uniform weight (0·015 g).

Two series of experiments were carried out, namely, in open capillaries, and in evacuated capillaries. Alkali-impregnated wood was also examined in the presence of 10 per cent elemental sulphur and of 10 per cent 2,6-di-*tert*. butyl-*p*-cresol, the latter being known as an efficient scavenger for free radicals. As a control, a high-purity, bleached cellulose pulp was investigated at room and at pulping temperature with and without preceding alkali impregnation. As in the wood experiments, a 4 per cent sodium hydroxide solution was used in a ratio of 1 : 20, and after pressing off the excess of lye, the impregnated cellulose was freeze-dried.

In a preliminary series of experiments, it was found that both wood and pure cellulose exhibited greater electron spin resonance signals (preceding section) after grinding than did the initial materials. Interestingly, the signal of the ground wood was about ten times larger than that of the ground cellulose. However, when heating ground wood and ground cellulose respectively to pulping temperature, the electron spin resonance signals dropped in both cases to low values. On the other hand, when ground wood or ground cellulose was alkalized and freeze-dried, the signals obtained at room temperature increased in both cases only slightly. However, when the alkalized samples (wood or cellulose) were heated to pulping temperature, very strong electron spin resonance signals were found, exhibiting a maximum value when reaching maximum temperature, and afterwards decreasing with increasing time while keeping the sample at this temperature. This decay of the free radicals formed was found to follow an exponential function, and finally to level off.

Comparative experiments in open (air-filled) and evacuated capillaries showed that oxygen reduced the maximum signal intensity. It may be that the oxygen acted as scavenger for free radicals. A similar influence was found to be exerted by elemental sulphur, when the alkalized samples mixed with small amounts of sulphur were heated to pulping temperature in evacuated capillaries. This seems to indicate that the role of the sulphur in kraft pulping is partly that of a free-radical scavenger. This view appears to be supported by the fact that when adding known scavengers for free radicals to alkalized, freeze-dried wood, like 2,6-di-*tert*. butyl-*p*-cresol, and heating the mixed samples to pulping temperature, the electron spin resonance signals dropped to relatively low values, compared with those previously obtained under the same conditions.

The investigations show that in alkaline pulping of wood, lignin and carbohydrates both are being degraded with the formation of free radical centres. The fact that a relatively narrow single line was observed indicates that no free radicals of distinct structure, such as quinone methides, are formed during pulping. The macro-radicals, because of their high reactivity, undergo various secondary reactions. For example, it can be visualized that there is not only lignin condensation but also some combining of free radicals from lignin with those from carbohydrates. In other words, the possibility exists that part of the residual lignin in pulps from alkaline pulping is grafted on the fibrous pulp portions by secondary reactions. An important role of the chemicals present in alkaline pulping is probably that of scavenging the free radicals and thus avoiding these reactions. These conclusions are supported by the work of Rex[8], who has found that tannins and humic acids such as are present in soils and coals contain free radicals, and that the latter are stable over long periods of time. In air-dried samples of fresh wood, the same author found very low concentrations of free radicals, and he postulated that free radicals do not occur in protolignin. However, lignin portions solubilized from wood with acidified dioxane, and also portions of the wood extracted by water in presence of the organic acids formed from the wood itself, exhibited strong electron spin resonance. Rex suggested that semi-quinone free radicals are related to this phenomenon. Furthermore, Freudenberg[9], Adler[10] and other authors studying biogenesis of lignin, postulated that free radicals of the quinone methide type are involved in the lignification of plant tissues.

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ELECTROPHORETIC AND IMMUNOLOGICAL CHARACTERISTICS OF NATIVE AND PURIFIED HUMAN GROWTH HORMONE

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THE immunochemical characteristics of the human growth hormone antigen–antibody system have been described previously and a high degree of antigenic homogeneity demonstrated (reviewed in ref. 1). On electrophoresis in agar gel (*p*H 8·2, barbital buffer) the Raben preparation of human growth hormone (HGH) migrated as a single broad zone located between the α-$_2$ and β-1 globulin regions. Immunoelectrophoresis in agar gel of Raben HGH and rabbit antiserum to HGH gave a single, elongated ellipsoidal arc of precipitation which was considerably lengthened by increasing the voltage gradient across the gel. These observations suggested the presence in preparations of HGH of multiple components with distinctive mobilities which had similar antigenic determinants. Barrett, Friesen, and Astwood[2] and Ferguson and Wallace[3] clearly demonstrated by means of zone electrophoresis in starch-gel electrophoretic heterogeneity in purified HGH prepared by three different methods. The components assayed by Ferguson and Wallace exhibited both growth hormone and prolactin activity. In the work reported here, evidence has been found which suggests that at least some of the electrophoretic heterogeneity is attributable to artefact arising from denaturation of the hormone during fractionation or storage.

Vertical starch-gel electrophoresis[4] of Raben preparations of HGH were carried out using the discontinuous buffer system of Poulik as modified by Ferguson and Wallace[3]. HGH prepared by Raben[5], dissolved in a small amount of 0·1 N sodium hydroxide and buffer, was subjected to electrophoresis for 17 h with a voltage gradient of 5 V/cm gel at 4° C. After completion of electrophoresis the gel was scored along its longitudinal axis at intervals of 1 cm from the origin and immediately removed from the tray. A layer of the gel was sliced off the top, stained for 45 sec with a saturated solution of amido black 10*B* in methanol/acetic acid/water (45:10:45) and washed with solvent alone. Sections corresponding to the stained components were cut out of the unstained half of the gel. Eluates from the sectioned gel were examined by immunoelectrophoresis[6] and by a micro-modification of the double diffusion gel plate technique of Ouchterlony[7] using rabbit antiserum to HGH as the developing serum. The reactions of the gel eluates with antiserum were compared with the results obtained with saline homogenates of fresh human pituitary glands and with a solution of purified HGH (Raben).

Three major bands and several minor bands were detected in the stained portion of the starch gel (Fig. 1) comparable with the pattern described by Ferguson and Wallace[3]. Following immunoelectrophoresis of the eluates and development with anti-HGH serum single precipitin arcs of different mobilities were formed corresponding to each of the three major bands in the stained gel. The most cathodal of the major components (No. 1) formed a precipitin arc situated at the cathodal end of the precipitin arc to HGH, whereas the crests of the arcs to components No. 2 and No. 3 were located respectively at the mid-portion and at the anodal end of the precipitin arc to Raben preparations of HGH (Fig. 1). A composite of the three types of precipitin arcs formed to the components separated by electrophoresis in starch gel was identical to the single arc of precipitation which develops when purified HGH[1,8] is reacted with its antiserum. None of the eluates reacted with undiluted or diluted horse hyperimmune anti-human serum. Complete fusion of single bands was demonstrated by the double diffusion gel plate technique of Ouchterlony when rabbit antiserum to HGH was placed in the central well and tested against the eluates, HGH, and a saline homogenate of fresh human pituitary gland placed in circumferential wells (Fig. 2).

It is noteworthy that the immunoelectrophoretic pattern of saline homogenates of fresh human pituitary glands revealed by rabbit anti-HGH serum lacked the distal portion of the anodal segment of the precipitin arc to purified HGH (Fig. 3). The location and shape of the single arc of the pituitary homogenates was comparable with the arc formed to

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* Postdoctoral Fellow, National Institutes of Health.

† Recipient of an Investigatorship of the Health Research Council of the City of New York under contract No. *I*-180.

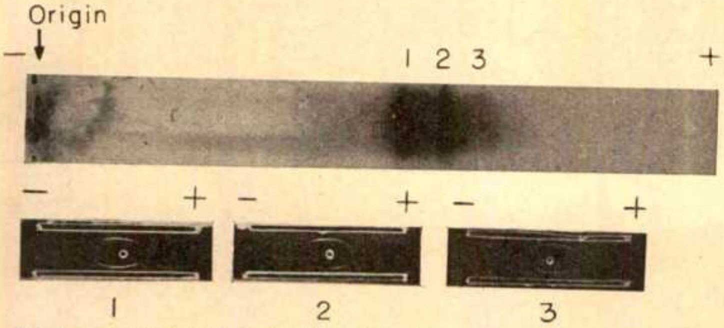

Fig. 1. *a*, Starch-gel electrophoretic analysis of human growth hormone (Raben) in discontinuous buffer system. The numbers designate the three darkest staining components. *b*, Immunoelectrophoretic analysis of eluates of the starch gel corresponding to each of the three major components of human growth hormone (Raben) placed in the central wells. The precipitin arcs were developed with rabbit anti-human growth hormone serum (in upper and lower troughs). A composite of the three precipitin arcs of the eluates corresponded to the single, elongated, ellipsoidal arc of precipitation to purified human growth hormone

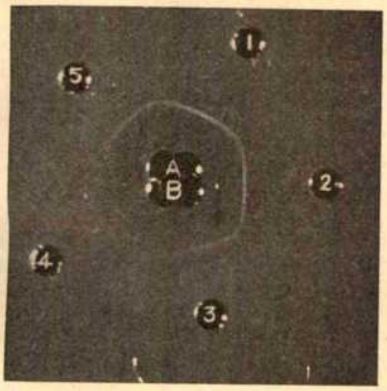

Fig. 2. A representative micro-Ouchterlony gel plate illustrating the fused single bands without spur formation obtained when: (1) component 1, (2) component 2, (3) component 3 of the starch gel eluates, (4) HGH 50 μg/ml., and (5) HGH 200 μg/ml. in peripheral wells were reacted with rabbit anti-HGH serum (*AB*) placed in the central well

component 1, the most cathodal of the major bands separated by electrophoresis in starch gel. Further evidence that the single precipitin arc which developed when pituitary homogenates were reacted with antiserum to Raben's HGH is HGH was obtained by using a modification of the technique of immunoelectrophoresis described by Osserman[9]. The line of precipitation of the reaction of HGH and antiserum to HGH fused with the arc formed to the pituitary homogenate (Fig. 4).

The results indicate that the major components of HGH prepared by Raben have similar and possibly identical antigenic determinants. Ferguson and Wallace[3] have demonstrated by bioassay both growth-promoting and prolactin activity in the three components of purified HGH and suggested that HGH has intrinsic prolactin activity, a possibility supported by the findings of Chadwick, Folley and Gemzell, Lyons, Li and Johnson, and of Wilhelmi[10]. The differences in the immunoelectrophoretic pattern of growth hormone in saline homogenates of human pituitary gland and of Li and Raben preparations of HGH suggests that the multiple components of purified HGH separated by means of electrophoresis in starch gel are mainly attributable to artefacts arising from alterations in the growth hormone molecule prior to or during isolation. The major component, located closest to the cathode, stained more darkly than the other components, and in its immunoelectrophoretic pattern was comparable with that of growth hormone in saline homogenates of fresh human pituitary glands. Additional support for the contention that the electrophoretic heterogeneity of HGH is related to physical or chemical changes[11,12] in the growth hormone molecule during purification may be adduced from the results of immunoelectrophoretic analysis carried out in our laboratory of HGH prepared by either Elrick, Li, Raben, or Wilhelmi. Differences were observed in the mobility and length of the precipitin arc(s) of these various preparations when developed with antiserum to Raben's HGH. Polymorphism might also account for the multiple protein components in purified preparations of HGH since these are obtained from large numbers of pituitary glands, from a heterogeneous population. Immunoelectrophoretic analysis of individual pituitary glands, however, does not support this idea, and the possibility that different molecules of native growth hormone may exist does not seem to account for the heterogeneity of purified HGH found by starch-gel electrophoresis.

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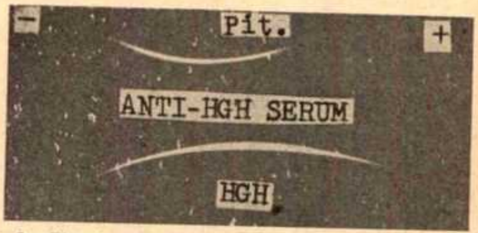

Fig. 3. The immunoelectrophoretic pattern obtained with a fresh saline homogenate of a human pituitary gland (upper arc, *Pit.*) and of 2 mg/ml. of purified human growth hormone (lower arc, HGH) revealed by rabbit anti-human growth hormone serum added to the central trough

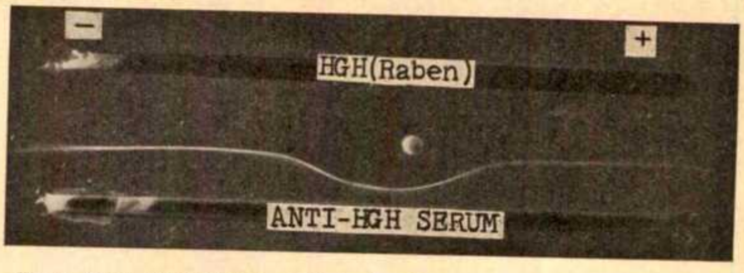

Fig. 4. Immunoelectrophoretic analysis of a saline homogenate of human pituitary gland by the Osserman technique illustrating the interreaction of purified human growth hormone, 100 μg/ml. (in upper trough), with saline homogenate of human pituitary gland (in central well) and rabbit anti-human growth hormone serum (in lower trough). The straight line of precipitation which develops from reaction of purified HGH and rabbit anti-HGH fused without spur formation with the cathodal and anodal ends of the precipitin arc of the human pituitary extract

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CYTOCHEMICAL OBSERVATIONS ON THE EFFECT OF PHYTOHÆMAGGLUTININ IN SHORT-TERM TISSUE CULTURES

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THE addition of phytohæmagglutinin (PHA) to human leucocytes cultured *in vitro* causes the appearance within a few hours of large basophilic primitive 'blast' cells, capable of synthesizing DNA[1] and of undergoing mitoses[2]. The emergence of large numbers of dividing cells, under the influence of PHA, has been widely utilized for the investigation of chromosome morphology in the peripheral blood of normal individuals.

How and from where the blast cells stem has been the object of considerable speculation. *In vitro* experiments by Nowell[3] appeared to indicate that the dividing cells in normal peripheral blood were derived either from monocytes or from some immature members of the lymphoid series. Mature lymphocytes and mature polymorphs were considered incapable of synthesizing DNA and undergoing mitoses.

Autoradiographic studies, using tritiated thymidine[4], have shown the presence in the peripheral blood of normal individuals of small numbers of large mononuclear cells which showed evidence of thymidine uptake and therefore of active DNA synthesis. These cells probably constitute a mobile pool of primitive and multipotential progenitor cells which, in special circumstances, could give rise to large numbers of dividing cells.

A recent investigation, based on morphological observations[5], suggests that the PHA-induced blast cells are derived from nucleolated intermediate lymphocytes. With the view of exploring further the nature and genesis of the blast cells present in cultures from the peripheral blood of normal individuals and with the secondary purpose of obtaining some insight into metabolic changes which occur in these cultures, we have carried out a number of cytochemical studies in cultures from 12 normal individuals. In addition to Romanowsky stains, the following cytochemical staining reactions were used: an azo-dye method for alkaline phosphatase[6], the periodic acid–Schiff reaction[7] the sudan black *B* reaction[8], a modified Graham–Knoll peroxidase technique[9], and methods for non-specific esterases[10], for phosphorylases[11] and for demonstrating succinic, DPN- and TPN-linked dehydrogenases[12].

In 8 of the 12 cases leucocytes were cultured both with and without phytohæmagglutinin. The following procedure was adopted. Twenty ml. of venous blood from a normal person were collected in a sterile Universal bottle, containing 0·1 ml. of heparin, and left to sediment in iced water for 30–60 min, depending on the rate of sedimentation. The blood was then centrifuged at 300–350 r.p.m. for 5 min and the supernatant removed. A white cell count was carried out on an aliquot of the supernatant to determine the initial cell concentration, and, following this, a calculated amount of *T.C.*199 and the subject's own serum were added to adjust the count to 1,000–2,000 cells/mm^3. The medium was then transferred to two other Universal containers, to one of which 0·4 ml. of PHA (Difco, batch No. 452131) was added.

Samples from both bottles were usually aspirated after 18–24 h, after 42–48 h, after 72 and occasionally 96 h. In one case samples were obtained initially at the second hour, and then after 4, 6, 18, 26 and 48 h. The amount aspirated was centrifuged at about 500 r.p.m. and smears made of the sediment. Smears stained by the Leishman or May–Grunwald–Giemsa methods showed, in material from cultures containing PHA, a rapid fall in the numbers of neutrophil polymorphs and degenerative changes such as conspicuous vacuolation and pyknosis in the few that survived. Monocytes, eosinophils and basophils, when present, usually persisted for the whole duration of the culture. Serial differential counts on the specimens obtained at different times showed the appearance during the first 18–24 h of varying numbers of nucleolated lymphocytes which persisted at the 48th hour but were gradually replaced and outnumbered usually by the 72nd hour by large numbers of primitive, nucleolated and basophilic mononuclear cells.

In cultures with no added PHA, neutrophil polymorphs were present in greater numbers than in specimens obtained at the same time from cultures containing PHA, and degenerative phenomena appeared much later and at a slower rate. Eosinophils, basophils and monocytes persisted in these cultures without conspicuous signs of degeneration. Lymphocytes showed no change in their morphology and only exceptionally were more primitive nucleolated lymphocytes encountered.

The alkaline phosphatase reaction, in cultures both with and without PHA, was negative in all mature polymorphs, even when an initial high alkaline phosphatase-level was observed in the peripheral blood of the same individual.

The periodic acid–Schiff reaction showed a distribution and staining pattern in the different cell types similar to that obtained from normal peripheral blood, although the reaction in mature granulocytes was coarser and more uneven. Prior treatment with salivary amylase abolished the reaction in all cell types. The reaction was studied semi-quantitatively in both lymphocytes and blast cells. In the material obtained from cultures with PHA, an increase in the amount of periodic acid–Schiff-positive material was nvariably observed in the lymphocytes. This ncrease usually reached a maximum at 18–24 h, ersisted up to 48 h, and was most conspicuous n some of the nucleolated lymphocytes, where locks of periodic acid–Schiff-positive material were resent together with coarse granulations. From he distribution of the scores, it was noted that he rise in periodic acid–Schiff positivity was due o a very rich content of glycogen in certain dividual lymphocytes rather than to a general ght increase among the lymphocytic population, although in some cases the proportion of eriodic acid–Schiff-positive lymphocytes rose coniderably. One of the most striking features observed in many specimens from PHA-containing cultures was the occurrence of clusters of lymphocytes surrounding degenerating polymorphs or clumps of platelets (Figs. 1, 2 and 3). No such appearance was seen from the cultures without PHA, where lymphocytes were more evenly distributed. Periodic acid–Schiff scores on mature lymphocytes from cultures without PHA showed a progressive decrease, more rapid in some cases than in others. Periodic acid–Schiff reactions on the blast cells were usually negative, although a diffuse tinge or a few rather fine granules were sometimes seen. Occasional strong positivity was encountered in cells which were classified as blasts because of their nuclear immaturity and cytoplasmic basophilia, but which were of smaller size than the great majority of the large periodic acid–Schiff-negative primitive cells.

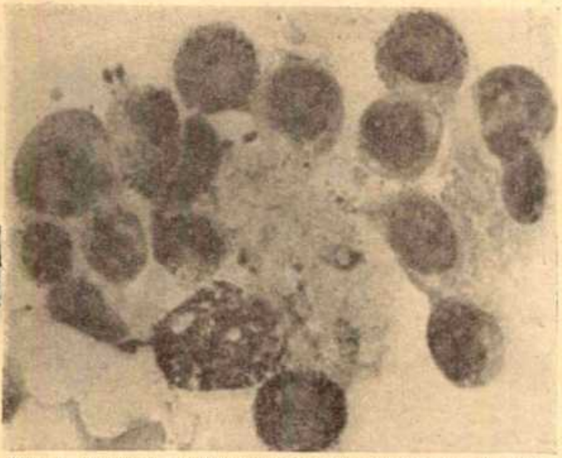

Fig. 1. Group of variably periodic acid-Schiff-positive lymphocytes, including a nucleolated cell on the left, surrounding a mass of degenerating platelets. At bottom centre is a strongly periodic acid–Schiff-positive lymphocyte next to a vacuolated polymorph

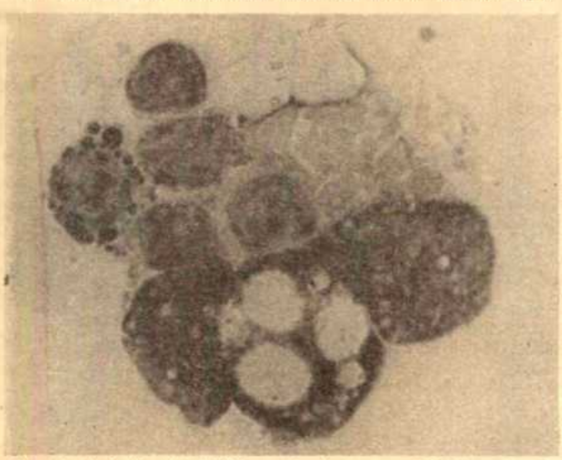

Fig. 2. Periodic acid–Schiff reaction in group of closely apposed lymphocytes and polymorphs. Of the 4 polymorphs two show gross degenerative changes, with huge vacuoles in one (lower centre) and with cytolysis and loss of granules in the other (upper right). The lymphocytes show varying degrees of periodic acid–Schiff positivity, that on the extreme left having very coarse globules or blocks

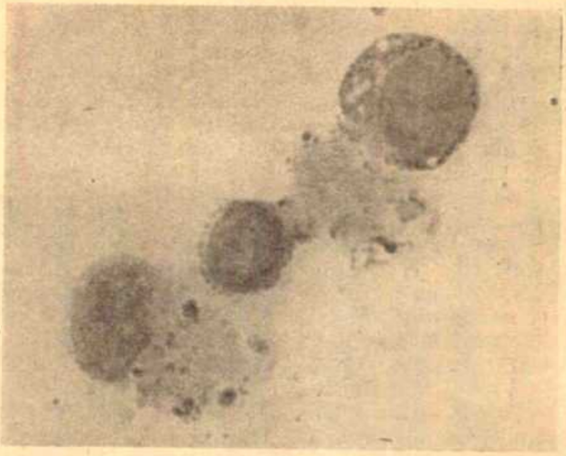

Fig. 3. A sequence of 3 lymphocytes, the upper a transitional nucleolated cell, in close relation to degenerate platelet masses, and showing variable periodic acid–Schiff positivity. The periodic acid–Schiff reaction is moderately intense in the nucleolated cell

Examination of smears stained with the sudan black *B* reaction showed the blast cells to be completely negative in all the specimens studied. Polymorphs generally showed a fairly strong reaction in the spaces between the areas of cytoplasmic degeneration and vacuolation. Some of the monocytes showed a scattering of discrete granules, comparable with that of normal monocytes, although a larger proportion, especially in smears from cultures containing PHA, showed evidence of active phagocytosis, with vacuoles, globules and sudanophilic fragments of degenerate polymorphs.

The peroxidase reaction was similarly negative in the blast cells, while monocytes showed peroxidase-positive fragments from ingested polymorphs. In contrast to the sudan black reaction, peroxidase positivity was weak in mature polymorphs, especially in those from cultures with added PHA.

Non-specific esterases were absent from many blast cells, but weakly demonstrable in others as a few fine granules of positivity. Lymphocytes sometimes showed a peripheral clump of positive material, but otherwise showed only a few minute granulations. Polymorphs reacted very weakly whereas monocytes contained strongly positive granules, similar to those seen in monocytes from normal peripheral blood, together with globules and vacuoles, which also showed a positive tinge.

The phosphorylase reaction was generally weaker than normal in most polymorphs, those obtained from cultures containing PHA being the least reactive. Platelets showed a normal, strong staining reaction, and lymphocytes showed crowns of dark blue or violet granules, similar in distribution to those seen with the periodic acid–Schiff reaction. Blast cells were substantially negative.

Dehydrogenases were studied in the cells from both types of culture, utilizing three different substrates: sodium succinate, sodium lactate and glucose-6-

phosphate. Increased dehydrogenase activity was observed in all specimens from cultures containing PHA. In these, the increase in positivity was observed in the lymphocytes in the first 18–24 h and was most intense with sodium lactate. Blast cells were invariably characterized by coarse granular positivity, most conspicuous with sodium lactate (Fig. 4), but also very intense with sodium succinate and glucose-6-phosphate. Omission of the substrate prevented the appearance of a positive reaction. No significant increase in dehydrogenase positivity was observed in the lymphocytes from cultures without PHA. The only cells showing fairly strong reactions in material from both types of culture were monocytes and eosinophils, both of which reacted most intensely when glucose-6-phosphate was used as a substrate.

All these cytochemical observations appear more in keeping with a lymphocytic than a monocytic or granulocytic origin for the blast cells. The negative peroxidase and sudan black reactions and the feeble non-specific esterase activity are of the kind found in the lymphocyte cell series, whereas if primitive cells were to be derived from monocytes or granulocytes they might be expected to show, at least during early phases of transition, some reflexion of the fairly conspicuous positivity with one or other of these reactions characteristic of those cell lines. Again, the remarkable increase in periodic acid–Schiff positivity of lymphocytes in cultures with PHA contrasts sharply with the consistent fall in positivity in similar cells in cultures without PHA, and points to the involvement of lymphocytes in the transformation and proliferation induced by PHA. It may well be that a rich supply of glycogen, either preformed or freshly synthesized, is a necessary preliminary requirement for the metamorphosis from lymphocyte to blast cell. In this connexion the observed distribution of lymphocytes in PHA-cultures around degenerating polymorphs and clusters of platelets raises the possibility that a transfer of glycogen from these formed elements to the lymphocytes may be occurring by some process of pinocytosis or phagocytosis. The cytological appearances are sometimes highly suggestive of such a process (Figs. 5 and 6). The more rapid rate of degeneration of polymorphs in cultures with PHA as compared with that in cultures without PHA, observed in this work and also by Elves and Wilkinson[5], may perhaps be related to the capture of glycogen from these cells by lymphocytes. Experiments to determine more precisely the role of polymorphs and platelets in peripheral blood cultures with PHA are now in progress.

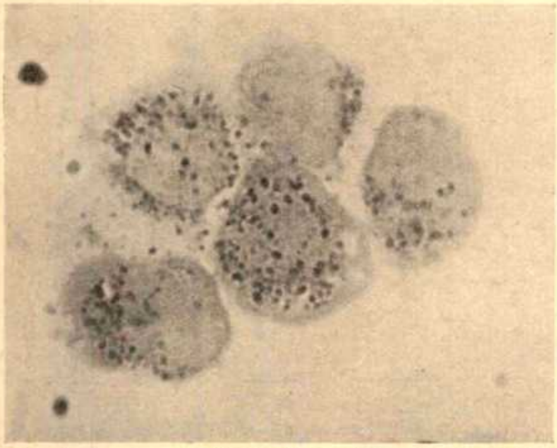

Fig. 4. A group of 5 blast cells showing a strongly positive reaction for lactic dehydrogenase

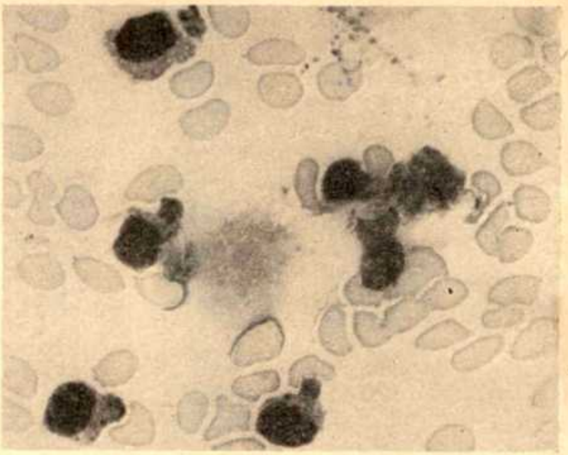

Fig. 5. Cytological relationship of lymphocytes and platelets. There is an appearance from above downwards of successive stages in incorporation of platelet material into the lymphocyte cytoplasm. Leishman's stain

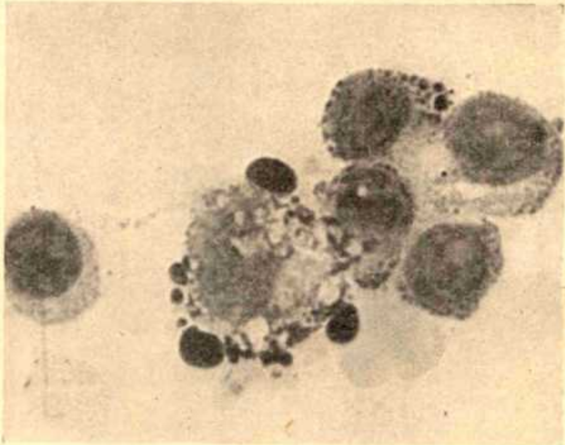

Fig. 6. In the centre is a degenerating polymorph with very coarse periodic acid–Schiff positivity at the periphery. To the right and in close contact with this cell is a clump of 4 lymphocytes, all having periodic acid–Schiff positive granules; the lymphocytes nearest to the polymorph are very coarsely positive. A separated lymphocyte to the left of the field remains periodic acid–Schiff negative

The emergence of large proliferating blast cells must call for considerable synthesizing activity with a substantial energy requirement, and the absence of glycogen from most of the developed blast cells would be expected if glycolysis provided a main energy source. Suggestive evidence that this may indeed be so is provided by the high activity of the dehydrogenase systems involved in glycolysis which is demonstrable cytochemically in both the blast cells and the lymphocytes.

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CHEMICAL STIMULATION OF THE PERIPHERAL TRIGEMINAL NERVE

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IT is generally accepted that the trigeminal nerve endings in the nasal mucosa play some part in the detection of odour[1-5]. In 1937 Allen[6] reported that dogs with bilaterally ablated olfactory bulbs would still detect the vapour of certain acids while other animals, the trigeminal nerves of which had been transected, could not do so. Katz and Talbert[5], Parker and Stabler[7], and Schiff[8] have also reported that information on the presence of certain chemicals may be transmitted by the trigeminal nerves. These investigators agree that stimulation of trigeminal nerve endings is likely to occur only with highly concentrated irritants such as volatile acids and pungent organic compounds.

Recent investigations, utilizing the methods of electrophysiology, indicate that the role of the trigeminal nerve in odour reception may be greater than previously suspected[9,10]. The sensory nerve supply of the cornea is trigeminal in origin and is historically a highly sensitive network[11]. Those who have investigated corneal sensitivity have confined their stimuli to contact and temperature change[11,12], while those who have studied the endings of the nasal mucosa have examined only the chemical stimuli[9,10]. The expanded odour responsiveness of the nasal trigeminal nerves described by Beidler[13] generates the question of a sensitivity comparison between this and other areas served by trigeminal afferents. A favourable site for examination of this question is the cornea, where many of the endings of the trigeminal nerves are known to rest between the epithelial cells[14].

Recent examinations of primary nerve data from the corneal afferents by Lele[12] and Dawson[11] have indicated that it is possible to secure the long ciliary nerve as it travels toward the rear of the orbit with the optic nerve. In the present investigation the long ciliary nerves of the frog (*Rana catesbiana*) were treated as previously described by Dawson[11].

Dissection of an animal previously anæsthetized with ethyl carbamate (3 g/kg) provided access to the long ciliary nerve in surgically produced depression at the rear of the orbit. The proximal end of the nerve was severed and dissected twigs were placed on 100μ stainless steel recording electrodes. These led, in a differential mode system, to a Grass pre-amplifier, oscilloscope arrangement through which spike potentials could be photographed or recorded by the electronic summation technique of Bernhard and Granit[15] and Beidler[16]. A second recording method utilized the spike impulses from the oscilloscope to drive a modified Schmitt trigger circuit and in turn provided uniform pulses to a Tracer-Lab electronic counter[17]. By this means, data about events per second could be obtained. The latter method is considered much more sensitive to small changes in neural activity-level than the summation technique which assumes all activity to be of the same amplitude.

Stimulation of the corneal nerves was achieved by liquid application of three chemicals. These were ammonium hydroxide, amyl acetate (1-pentanol acetate), and β-phenyl-ethyl alcohol (benzyl carbinol). The inorganic stimulus was applied in concentrations of 0·03–1·0 molar in distilled water. Saturated samples of the organic stimuli were made by equilibrating each chemical with distilled water. Solubility published for amyl acetate is 0·18 g/100 g water[18] and for β-phenyl-ethyl alcohol is 1·6 g/100 g water[19]. Fractionation of these saturated solutions provided dilute stimuli in the range of $1\cdot125–131\cdot0 \times 10^{-3}$ molar for phenyl-ethyl alcohol. The concentrated amyl acetate solution at 14×10^{-3} molar was diluted in steps to $0\cdot437 \times 10^{-3}$ molar. Animal and stimulus temperature varied between 20° and 23° Celcius. Stimulus solution *p*H values were 6·80 ± 0·25.

The stimulation procedure began by placing one drop of distilled water on the cornea from which a basal activity-level was derived by either the counting or the summating technique. Then one drop of the stimulus solution was placed on the cornea. The number of impulses produced during the next ten seconds was recorded on the electronic counter and an overall curve of activity was derived from the recording device. The activity count for this period read from the electronic counter was recorded as impulses per second. The counter used in this experiment was a hybrid type which displayed two digits electronically and three mechanically. As a result of the mechanical speed limitation, its use was confined to fibre bundles which contained only a few active fibres. Where larger bundles were examined, the data was recorded by the electronic summator. Previous reports using the summator have displayed only raw data[10,11].

Reports of maximum amplitude of the summated response are valid for only one preparation and one fibre bundle. A comparison of more than one of these implies that the nerves in each record are similarly distributed for spike amplitude and receptor sensitivity. The summated data presented in this paper were taken from many nerve bundles. However, each summated datum point is a ratio between amplitude (mm) × time (sec) for the experimental stimulus to a similar value which was produced by a control stimulus. Since the control stimuli were constant from preparation to preparation the final ratios are corrected for differences in between preparation basal sensitivity and differences in the numbers of active fibres. The time value in each case was the number of seconds from the initial increase in summated response to the point at which the response amplitude had fallen to 50 per cent of the maximum. Similar ratios were calculated

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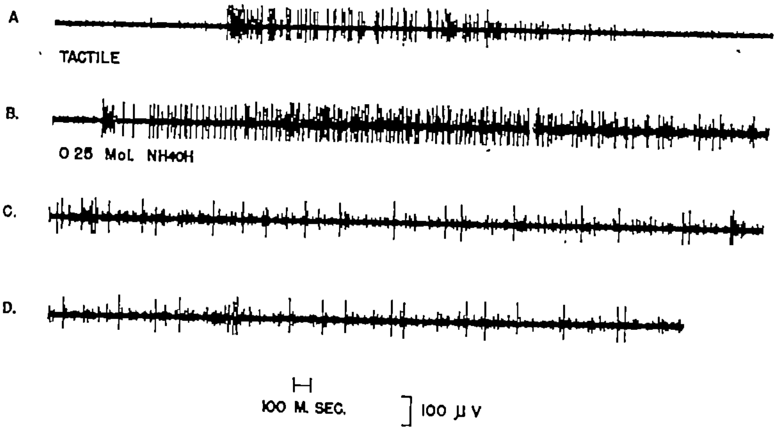

Fig. 1. Oscillographic record of discharges produced in the long ciliary nerve during stimulation of the frog cornea. Record *A* is the result of corneal contact by a polished glass rod. Records *B–D* are the result of corneal application one drop 0·25 *M* ammonium hydroxide. The artefact over the letter *B* indicates application of the chemical

for the counted impulse data where the preparation allowed this technique. The mean activity ratios which appear later in this article contain both the counted and summated ratios but only one type for a particular preparation.

In most cases the afferent activity elicited by the stimulus fell to the baseline after three minutes. In such instances the cornea was rinsed with a few ml. of distilled water and stimulated again after five minutes. At high stimulus concentrations of the inorganic solution, response-levels were frequently maintained past the three-minute duration. In these cases the cornea was rinsed and sufficient time was allowed for return of activity to the basal level.

The photographically recorded results of light tactile stimulation of the corneal surface as seen in Fig. 1 are comparable with those previously demonstrated by me for the mammalian cornea[11].

The tactile response demonstrates a different temporal character from that produced by the inorganic hydroxide. The hydroxide response begins with a relatively slow rate of spike potential output which reaches an extended peak and then gradually decreases with several large fibres remaining active for an extended period of time. The characteristic tactile response, however, shows an initial burst of activity followed by a sharp decrease which terminates as the polished glass stimulating rod is removed from the corneal surface.

A further change in character appears in Fig. 2 following stimulation on the cornea by one drop of 37 millimolar (mM) phenyl-ethyl alcohol. This

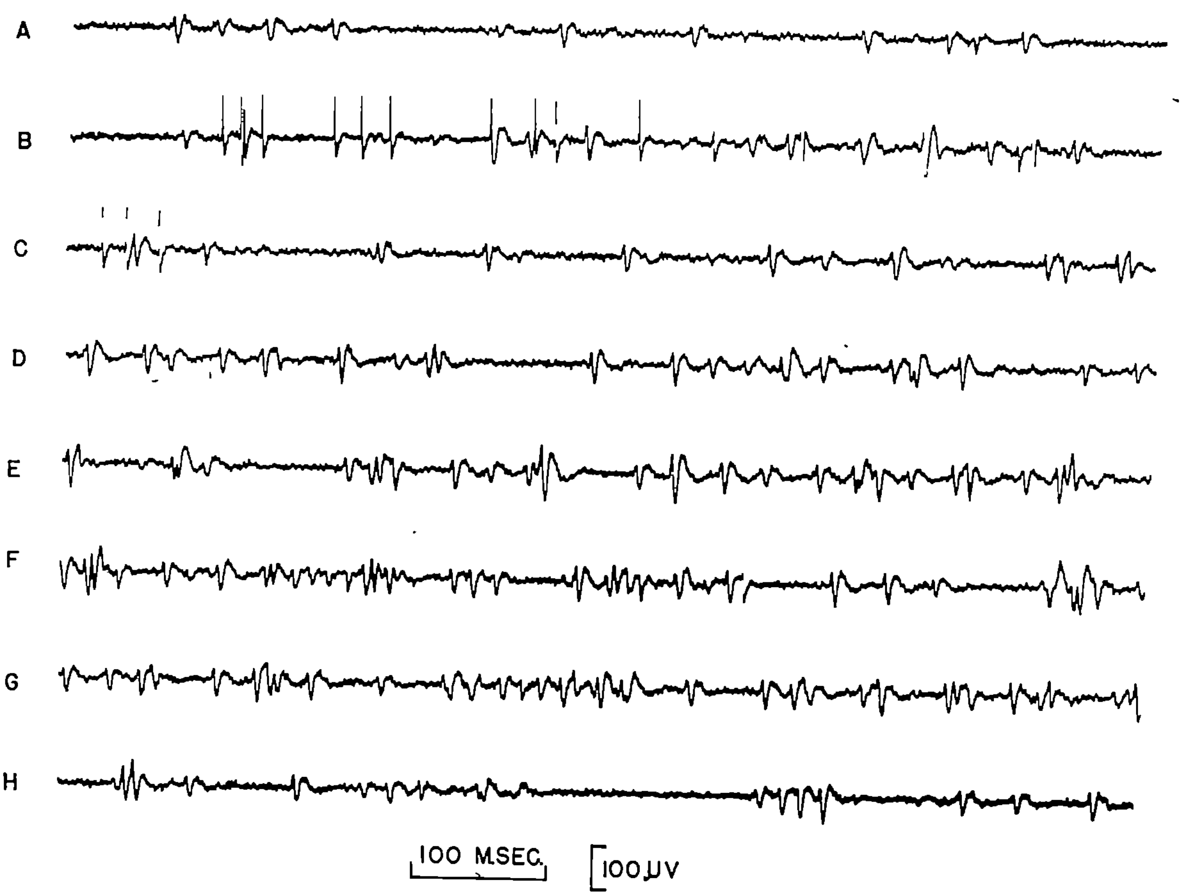

Fig. 2. A few fibre discharge records from the long ciliary nerve. Record *A* indicates resting activity-level; *B* the result of contact stimulation. The spikes at the beginning of *C* are the chemical stimulus artefact. The remainder, *C–G* are produced by 37 mM phenyl-ethyl alcohol. Record *H* is a continuation of *G* after a 30-sec lapse

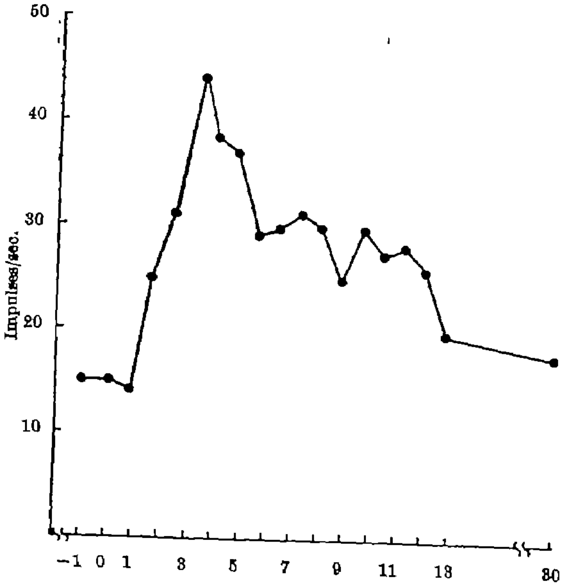

Fig. 3. The temporal course of 'slow fibre' activity produced by 37 mM phenyl-ethyl alcohol stimulation of corneal surface. Contact with the chemical occurred at time 0

few fibre record exhibits a much lower noise-level than that of Fig. 1 and also was recorded at a more rapid rate. Here 'slow fibres' are seen responding in record *A* at a basal level where no stimulation has been applied. On tactile stimulation the large, very fast spikes previously seen in Fig. 1 appear. However, these fibres do not discharge except as a tactile artefact produced by initial contact of the stimulating liquid as seen in line *C*. The burst of 'fast' fibre activity at the beginning of line *C* signals the application. Following this artefact, only small fibre activity appears which gradually increases to a maximum in records *E* and *F* and then diminishes so that after 30 sec, in line *H*, the basal level has almost returned. Examination of the spike dimensions for these two discharge types disclosed a duration of 200 μsec for the 'fast' fibres and a range of from 5–10 msec for the 'slow' fibres. These fall within the limits described for '*A*' and '*C*' fibres by Gasser and Grundfest[21].

The temporal course of the small fibre activity in Fig. 2 is illustrated in Fig. 3. As in Fig. 1 the nature of the discharges departs from those produced by contact stimulation. No high-voltage, short-duration spikes, which are indicative of large diameter axons, are seen during chemical stimulation. Earlier records of chemically excited nasal trigeminal activity indicate the presence of 'fast' fibre activity in nerve bundles[10].

An interesting initial depression of slow fibre response occurs following the stimulus artefact. Although total response time appeared related to the organic stimulus concentration most activity was maintained for at least twenty seconds, while for high concentrations excitations persisting over a minute were not uncommon.

The mean activity ratios produced by both the organic and inorganic liquid stimulants are described in Fig. 4. Each point includes activity data from five to seven preparations utilizing both the electronic summation and counting techniques. Variability in the number of active neural elements seen by the recording electrodes between preparations necessitated the development of a uniform index of response. This was accomplished by the use of the ratio of experimental response to control response. The ordinate values are then either: (1) the ratio of impulses per second produced by the (experimental) stimulus to the impulses per second produced by (control) distilled water application or, (2) a similar ratio between summated activity (Fig. 5) in mm multiplied by the duration of 50 per cent amplitude (experimental) to a similar measure for the (control) distilled water application. These datum points then reflect the means of many ratio values for both summated and/or numbers of spike potentials per unit time. During the course of experimentation the hypothesis was tested and verified (for equivalent stimulation):

$$\frac{N\text{exp./sec}}{N\text{cont./sec}} \approx \frac{R\text{exp.}}{R\text{cont.}} \approx \frac{Y\text{max.}_{\text{exp.}}\ X\ ty_{50}}{Y\text{max.}_{\text{cont.}}\ X\ ty_{50}}$$

where Ymax. = maximum amplitude of the summated potentials; ty_{50} = time required for y to reach Ymax. and fall to 50 per cent of Ymax.; and N = number of recorded spike potentials.

It is of particular interest that the curves for the organic stimulus begin and terminate prior to onset of threshold for the inorganic stimulus. Also, the resulting peak activities are lower. It appears that an optimal stimulation range is effective for the organic stimuli. Each produced an activity peak at about 1·5 log molar concentration units above the threshold. Increased stimulus concentration served to reduce rather than enhance the level of excitation. Similar losses in excitability during high concentration stimulation have been reported by Mozell, who recorded olfactory bulb potentials produced by amyl acetate and heptane[22]. It is difficult to explain this decrement on the basis of adaptation since there is no evidence of excitability loss during ammonium hydroxide stimulation. Those activities produced by ammonium hydroxide approach a power function at

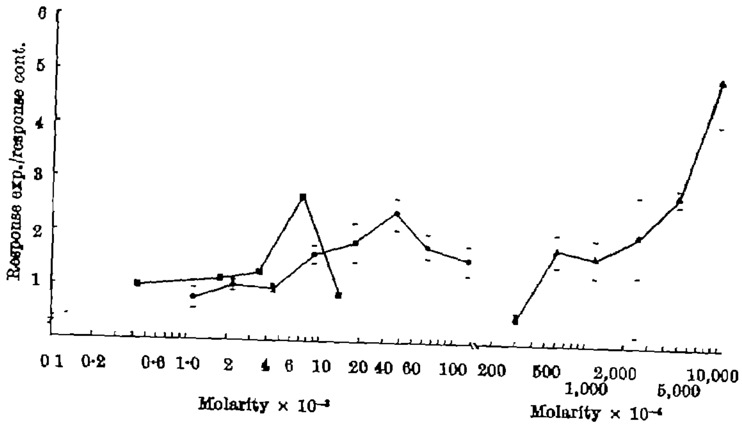

Fig. 4. Response of the corneal afferent nerves to concentrations of organic and inorganic stimuli. The ordinate ratio is discussed in the text. Values for phenyl-ethyl alcohol and ammonium hydroxide are the means of 5–9 measures. Those of amyl acetate are for two measures each. Limit marks indicate the standard errors of the mean. ●, phenyl-ethyl alochol; ■, amyl acetate; ▲, ammonium hydroxide, Ξ, σ_m

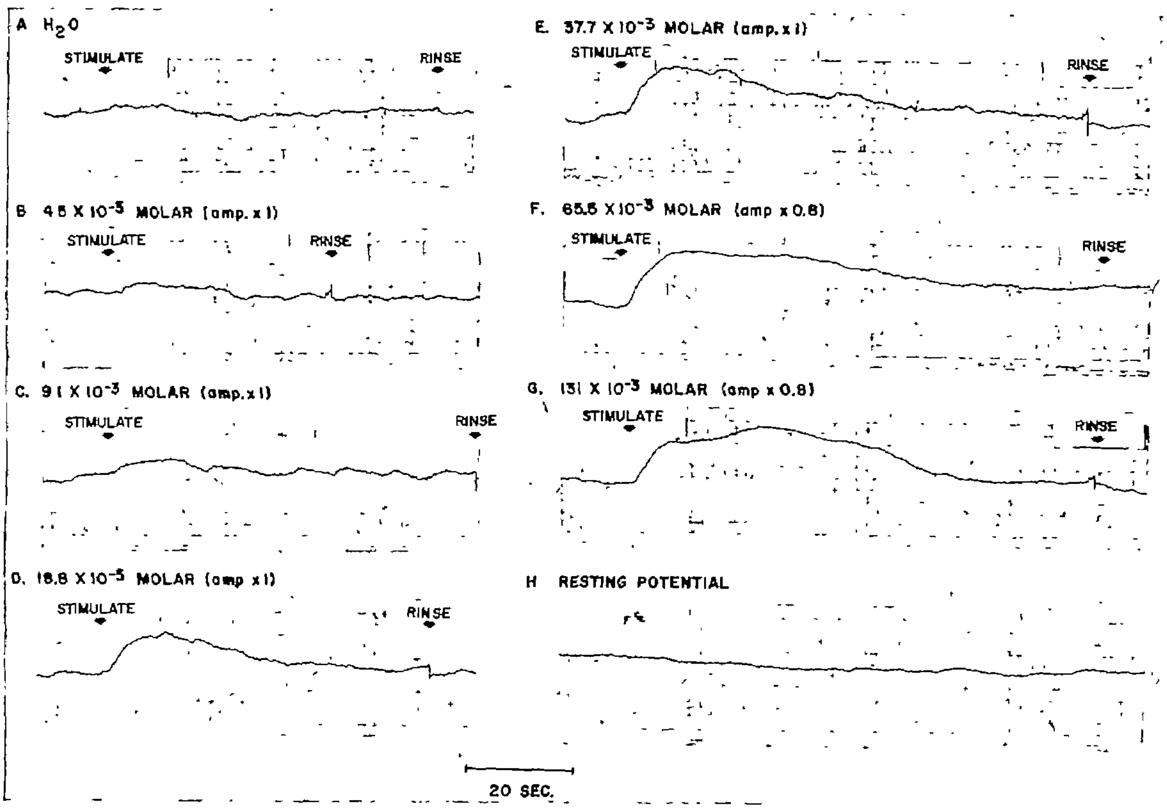

Fig. 5. Electronically summated responses of the corneal afferent nerves to liquid phase stimulation by 87 millimolar phenyl-ethyl alcohol. Record *A* is the standard response to distilled water. *B–G* are responses to increases in stimulus concentration, and *H*, variation in resting response. Amplification was reduced in *F* and *G* to compensate for increased excitation

medium and high concentrations. Activities at the one molar level suggested that, for some preparations, all fibres present were firing at very high rates. Irreversible damage to the nerve terminals may be indicated by continued rapid discharge long after the stimulus had been washed away.

Responses to the inorganic stimulus are not necessarily confined to large-diameter fibres. The records of Fig. 1 are misleading in this respect. There is a strong tendency for the high amplitude signals and the considerable noise level of these data to obscure any slow activity which might be present. However, at no time were the high-frequency, short-duration spikes seen during stimulations with the organic stimuli. If the receptor action of the organic solutions is confined only to small fibres and is reversible, it is quite likely that the irreversible action of the higher levels of inorganic stimuli is effective for fibres of both small and large diameters.

If factors influencing chemical access to the corneal nerve endings are considered with those controlling access to the endings in the nasal mucosa, the data presented can support the hypothesis that the sensory capacities of the two systems are similar. The threshold for amyl acetate from Fig. 4 is about 10 times the 40 μM psychophysical corneal threshold calculated from the earlier work of Katz and Talbert[5]. The 40 μM threshold of these authors is in turn larger by a factor of 10 than the nasal trigeminal threshold of rabbit reported by Beidler and Tucker[10].

A characteristic of the data which have been presented remains unexplained. There are peaks in response followed by a decrement in activity at higher stimulus-levels which in some cases produced excitation-levels below that of the controls. At present there is no satisfactory explanation as to what the role of this particular characteristic could be in human detection of organic vapours.

Other recent electrophysiological evidence also indicates that all stimulation of these nerves need not be from 'obnoxious stimuli' although it is recognized that the role of the trigeminal nerve in the production of odour sensations is unknown[13]. It has been demonstrated that non-noxious chemicals can produce trigeminal discharges at levels comparable and, in some cases, lower than the thresholds for olfactory stimulation. Trigeminal nerve inhibition of certain reflexes has been studied to determine if disruption might occur during odour reception[9]. Two effective stimuli were coffee and asafœtida, which have been previously considered pure olfactory stimulants[6,14].

Tucker has reported that in animal studies it is often difficult to predict whether threshold value for a specific stimulus will be lower for the olfactory or trigeminal innervation of the nose[11]. A subjective criterion based on irritability is no longer adequate for the selection of olfactory or trigeminal stimuli in chemical sense studies.

Primary afferent nerve recordings were made from the sensory trigeminal nerves which innervate the cornea of frog (*Rana catesbiana*). Temporal patterns and mean activity ratios were derived from the afferent discharges elicited by an inorganic irritant, an organic stimulus previously used as a stimulus for trigeminal fibres in the nose, and an organic solution classically considered "pure olfactory" stimulants. Thresholds were discussed and response magnitude-concentration curves presented.

The following conclusions are drawn: (1) neural elements of the corneal afferent supply which respond to surface contact are not the same elements which respond to liquid phase applications of dilute amyl acetate or phenyl-ethyl alcohol; (2) the temporal patterns of spike potentials differ for contact and NH_4OH excitation; (3) the organic stimuli used produce a decrement in afferent output at the higher concentrations; (4) threshold for amyl acetate is higher for the corneal afferents of frog than previously reported for nasal trigeminal units of rabbit.

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PROBLEMS OF ETHYLENE METABOLISM

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THE theory that ethylene plays a key part in fruit ripening as a natural hormone has received fresh support from recent investigations of ethylene production in preclimacteric fruits[1,2]. But though there is now an adequate analytical tool for measuring the small amounts of ethylene produced in plant tissues, namely, the gas chromatograph with flame ionization detector[3], no appreciable progress has yet been made towards an understanding of the mechanism by which ethylene is produced or its site of production in the cell[4].

The theory that this site lies in a sub-cellular particle received indirect support from an examination of apple tissue, where the tonicity of the enveloping solution was found to have a marked effect on ethylene production[5].

Reports that mitochondria produce ethylene have come from two groups of workers, led by Spencer and by Lieberman. Spencer[6] reported that a mitochondrial fraction from tomato fruit evolved ethylene in the presence of malic acid and co-factors, but this procedure remained unverified[7]. In a later paper Chandra and Spencer[8] showed that high rates of ethylene production could be obtained by fragmenting the particles with ultrasound.

Lieberman and Craft[9] first reported that sub-cellular particles of various types evolve ethylene under acidic conditions in the presence of thiomalic or thioglycollic acids. Burg and Burg[10] re-investigated this reaction with tomato fruit and found that the evolved hydrocarbon was not ethylene but a closely related compound. In our work with apples we reached a similar conclusion and identified the gas as ethane (unpublished). Recently, Lieberman and Mapson[11] have extended the work on the thiomalic acid reaction by finding that linolenic acid forms a component of the system. They consider that the system producing ethane bears a close relationship to the one producing ethylene in Nature.

We have now re-examined the effects on particulate suspensions of sonication and the addition of thio-acids. Our conclusions do not confirm those outlined here, because: (*a*) although preparations treated with ultrasound do evolve ethylene, along with other hydrocarbons, the reaction does not require the presence of active particles; (*b*) the production of ethane in the presence of thio-acids can be simulated without subcellular particles.

We used a detecting system and separating column which were similar in general to those used previously[7], but the amplifier was a Vibron electrometer type 33*C*/*B*33*C* and the stationary phase was squalane, which provides a better separation of the relevant compounds than silicone oil. The sensitivity of the apparatus was such that 0·004 μl. of ethylene per litre of air was detectable in a 1-ml. gas sample.

(1) *Sonication*. The method of isolating subcellular particles from tomato fruit and disintegrating them with ultrasound followed as closely as possible the procedure of Chandra and Spencer[8]. It differed at two points. (*a*) The particles were subjected to a total of 4 min in a 60 W 20 kc/s M.S.E. ultrasonic disintegrator fitted with a 19-mm diam. titanium probe and adjusted to maximum output ($\sim 1 \cdot 3$ amp). The tube containing the preparation was immersed in a freezing mixture at $\sim -20°$ and sonicated for eight 30-sec periods interspersed with periods of rapid stirring to reduce the temperature to 0°. In this way the overall temperature of the solution was kept below 7°. (*b*) The particles were incubated in 12-ml. flasks, each containing 3 ml. liquid and fitted with a short length of rubber tubing closed with a glass

bead. Samples of air were removed by connecting an evacuated 30-ml. gas sampling tube to each flask. They were analysed directly without the use of mercury perchlorate.

Initial experiments showed that not only ethylene but also acetylene and ethane were produced in appreciable quantities. (The latter two would not be detected where the mercury perchlorate method was used.) Then a sonicated preparation was fractionated by centrifugation at 105,000*g* for 30 min and it was found that the supernatant retained most of the activity of the original preparation. The effects of boiling or evacuating the mitochondrial fraction immediately before or after sonication were compared. It was found that whereas treatment before sonication had little effect, treatment afterwards resulted in a marked decrease in hydrocarbon production. Furthermore, the sonication of a solution of sucrose and adenosine triphosphate produced measurable quantities of the same hydrocarbons. The results of these experiments are given in Table 1.

Table 1. EVOLUTION OF HYDROCARBONS BY SONICATED SUB-CELLULAR PARTICLES FROM TOMATO FRUIT

Flask contents	mμl./h/ml. particulate suspension at 25° Acetylene	Ethylene	Ethane
Particulate suspension, sonicated	32·3	10·1	1·4
Particulate suspension, boiled before sonication	41·4	12·2	1·4
Particulate suspension, boiled after sonication	9·2	0·9	0·3
Particulate suspension, evacuated before sonication	25·3	12·4	1·4
Particulate suspension, evacuated after sonication	< 0·001	0·2	< 0·002
Sonicated particulate suspension, supernatant from 105,000*g* centrifugation	15·2	5·7	0·6
Sonicated particulate suspension, particles from 105,000*g* centrifugation	0·4	0·4	0·2
Solution of sucrose (0·5 *M*) and adenosine triphosphate (1·98 × 10^{-3} *M*)	7·1	1·7	0·04

Reaction conditions: each flask contained 1 ml. particulate suspension (derived from 62 g fruit) in a total volume of 3 ml with the following—0·5 *M* sucrose, 0·125 *M* potassium dihydrogen phosphate, 10^{-3} M magnesium sulphate, 10^{-3} M manganese sulphate, 0·05 M L-malic acid, adjusted to *p*H 7·0 with sodium hydroxide, 1·98 × 10^{-3} M adenosine triphosphate and 3·3 × 10^{-4} M diphosphopyridine nucleotide. Incubation period 2 h. The homogenate of the fruit was centrifuged at 3,000*g* and the resulting supernatant recentrifuged at 85,000*g* to obtain the particulate suspension.

There is at present no established method of comparing the behaviour of sonic disintegrators of different design; but our results are consistent with the known features of the disintegrating process. Thus the cavitation which occurs in the liquid is followed by the generation of extremely high local pressures and temperatures as the cavities collapse[11]. Free radical formation is usually associated with this phenomenon, and the production of small quantities of hydrocarbons can be visualized as a result. The possibility that fragmented mitochondria might produce ethylene, under conditions which we were unable to achieve, is not ruled out, but the physiological reaction should be distinguished carefully from the extraneous effects of sonication.

(2) *Treatment with thio-acids.* The ethane-producing reaction requires the presence of thiomalic or thioglycollic acids, or (as we have found) thiolactic acid. These compounds have in common the structure HS.CH*R*.COOH. An investigation by Maier and Tappel[13] into the catalysis of fatty acid oxidation by hæmatin compounds suggested to us that the ethane reaction might be analogous. When linolenic acid was incubated in a reaction mixture containing cytochrome *c* in place of subcellular particles, ethane was produced. Later it was found that adenosine triphosphate or a ferric or ferrous salt were more effective catalysts of the reaction. Typical results are shown in Table 2.

Table 2. EVOLUTION OF ETHANE BY A MIXTURE OF LINOLENIC ACID AND THIOGLYCOLLIC ACID IN THE PRESENCE OF CATALYSTS

Flask contents*	mμl. ethane/h at 30°
Thioglycollic acid + linolenic acid	4·5
Thioglycollic acid + linolenic acid + ATP + cytochrome *c*	168
Thioglycollic acid + linolenic acid + cytochrome *c*	21·4
Thioglycollic acid + linolenic acid + ATP	295
Thioglycollic acid + linolenic acid + 0·0001 M $FeCl_3$	315
Thioglycollic acid + linolenic acid + 0·00001 M $FeCl_3$	183

* Each flask, in a total volume of 3 ml., contained 1 mg linolenic acid (as the potassium salt), 0·02 M thioglycollic acid, 0·125 M *tris*-citrate buffer *p*H 5·0, 0·5 M sucrose, and where indicated, 0·002 M adenosine triphosphate (ATP) and 0·1 mg cytochrome *c*. Incubation period 2 h.

There are several respects in which this reaction resembles the particulate one.

(1) The reaction with linolenic acid is more than 90 per cent inhibited by 0·001 M ethylenediaminetetraacetic acid or 0·001 M diethyldithiocarbamate under the experimental conditions shown in Table 2.

(2) A number of other thio compounds are ineffective by comparison with the type HS.CH*R*.COOH. Thus in the presence of cysteine, 2-mercaptopropionic acid, thiodiglycollic acid and methionine the rate of production of ethane is 11, 9, 1 and 1 per cent respectively of the rate in presence of thioglycollic acid (conditions as in Table 2).

(3) The rate of production of ethane in the presence of C_{18} acids lies in the order linolenic $\gg$ linoleic > oleic and stearic. A comparison between the particulate and non-particulate reactions is shown in Table 3.

(4) Several other volatile compounds are produced by the reaction and can be separated by gas chromatography. Of these the more prominent components

Table 3. COMPARISON OF ETHANE PRODUCTION IN THE PRESENCE OF C_{18} ACIDS IN A REACTION MIXTURE WITH OR WITHOUT A PARTICULATE SUSPENSION FROM TOMATO FRUIT

Added C_{18} acid	mμl. ethane/h at 30° Without particles*	With 1 ml.† particulate suspension
Stearic acid	0·2	10·1
Oleic acid	0·2	11·7
Linoleic acid	4·5	92·3
Linolenic acid	101	437

* Reactants, in a total volume of 3 ml.—1 mg C_{18} acid (as the potassium salt), 0·02 M thioglycollic acid, 0·002 M adenosine triphosphate, 0·1 mg cytochrome *c*, 0·125 M *tris*-citrate buffer *p*H 5·0, 0·5 M sucrose.

† Each flask contained, in a total volume of 3 ml., 1 mg C_{18} acid (as the potassium salt), 0·02 M thioglycollic acid, 0·002 M adenosine triphosphate, 0·2 M *tris*-citrate buffer *p*H 5·0, 0·5 M sucrose and 1 ml. particulate suspension (derived from 25 g fruit).

Tomato fruit without seeds and surrounding pulp (250 g) were homogenized at 1° in 0·2 M *tris*-citrate buffer and 0·5 M sucrose (400 ml.), using a blender fitted with polytetrafluorethylene-covered spindle and nylon impeller blades. The homogenate (*p*H ~ 7) was filtered through four thicknesses of muslin and centrifuged at 1,000*g* to remove debris. The particles were isolated at 10,000*g*, washed once in 0·5 M sucrose and recentrifuged at 10,000*g*.

Table 4. PRODUCTION OF HYDROCARBONS BY PARTICULATE SUSPENSIONS AND BY A MIXTURE OF LINOLENIC AND THIOGLYCOLLIC ACIDS

Hydrocarbon	mμl./h at 30° Linolenic and* thioglycollic acids	Particles with† thioglycollic acid	Particles without† thioglycollic acid
Ethylene	0·5	0·1	0·4
Ethane	295	56	4·2
n-Propane	1·3	1·4	< 0·03
n-Butane	6·1	5·9	0·3
n-Pentane	56	107	10·0

* Reactants, in a total volume of 3 ml.—1 mg linolenic acid (as the potassium salt) 0·02 M thioglycollic acid, 0·002 M adenosine triphosphate, 0·125 M *tris*-citrate buffer *p*H 5·0, 0·5 M sucrose.

† Each flask contained, in a total volume of 3 ml., 0·02 M thioglycollic acid (where indicated), 0·002 M adenosine triphosphate, 0·2 M *tris*-citrate buffer *p*H 5·0, 0·5 M sucrose and 1 ml. particulate suspension (derived from 125 g fruit). The particles were prepared from tomato fruit by the method given in Table 3.

were each located on three columns of varied polarity (squalane, tritolyl phosphate, polyethylene glycol 400) and identified on this basis as *n*-propane, *n*-butane and *n*-pentane. A comparison between the particulate and non-particulate reactions is shown in Table 4.

It cannot be concluded from these results that the ethane-producing reaction bears a close relationship to the natural reaction which produces ethylene. The strongest evidence in favour of the conclusion of Lieberman and Mapson is that the particulate preparations evolve small amounts of several of the hydrocarbons which are produced in large amounts in the presence of thioglycollic acid (Table 4). Since, however, a reaction of this type can be sustained without the assistance of enzymes it is possible to visualize it proceeding in the particulate preparation in a manner irrelevant to its physiological reactions.

The production of hydrocarbons in the absence of particles might be explained as a result of peroxidation of the fatty acid, which would be followed by the formation of free radicals through homolytic fission of the peroxide. It is possible that an analogous reaction, but with different starting products, might yield the preponderance of ethylene that is characteristic of the natural process in the intact fruit.

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SYNTHESIS OF HÆMOGLOBIN IN RELATION TO THE MATURATION OF ERYTHROID CELLS

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IT is generally believed[1] that it is in the nucleated erythroid cells in the marrow that nearly all synthesis of hæmoglobin occurs, that the rise and decline of hæmoglobin synthesis follow a course parallel to the maturation of the erythroid cell, and that the non-nucleated immature red cells, whether in the marrow or blood, carry out only a small, residual hæmoglobin synthesis. Fichsel[2] and Weicker *et al.*[3] dissent from this view ; they assign 2/3–4/5 of hæmoglobin synthesis (in rabbits) to the reticulocyte state. Lajtha and Oliver have cited the strong evidence against this opinion[4].

We have been led to a hypothesis (in animals with non-nucleated mature erythrocytes) which does not make hæmoglobin synthesis depend on the ontogeny of the erythroid cell. It is as follows. The hæmoglobin-synthesizing apparatus is nearly fully generated by the end of the basophilic erythroblast stage ; and there is little, if any, increase after this stage. Synthesis persists in some cells through all maturation stages ; and also stops in some cells in all stages ; when it stops it is not resumed, neither in the same cell nor in its progeny. The rate of synthesis, its persistence or cessation, are not governed by the amount of hæmoglobin in the cell. The longer synthesis persists through maturation the more hæmoglobin does the eventual erythrocyte have. Mitosis may occur both in cells in which hæmoglobin synthesis is in process and in those in which it has stopped.

The daughter cells after mitosis do not enlarge ; with the subdivision of mass there is a corresponding subdivision of the existing synthesizing activity or inactivity. Accordingly the activity per cell is halved. The basophilic erythroblast has the maximum rate of synthesis. In each successive intermitotic phase the rate per unit mass falls off slowly and the number of cells in which synthesis has stopped increases. Thus, on the average, the active orthochromatic erythroblasts are the slowest per unit mass, as well as per cell, among the active nucleated cells, and they have the largest fraction in which there is no synthesis. These features are exaggerated by the orthochromatic phase being of the longest duration. When the stimulus to the bone marrow is greater than normal many reticulocytes arise directly from the polychromatic cells, skipping the subdivision of mitosis. The size of such a reticulocyte is that of its parent minus the volume of the nucleus ; they do not constrict ; they give rise to erythrocytes of about the same size. Accordingly they are larger than orthochromatic erythroblasts and the erythrocytes to which the latter give rise. Some orthochromatic erythroblasts become small reticulocytes and some, as Lajtha and Oliver suggest, become erythrocytes directly ; by either route their eventual size is the same.

The data for the above hypothesis have come from experiments on protein incorporation with amino-acids labelled with tritium or carbon-14 by marrow cells and reticulocytes, fractionation of reticulocytes according to their synthesizing activity, cytological observations on and measurements of the cells in anæmic and normal marrow, and measurement of the hæmoglobin content and life-span of circulating erythrocytes after recovery from severe anæmia. (Publication of the details of these experiments is in preparation.) The observations which are correlated by this hypothesis are the findings in the foregoing experiments—some of these are inconsistent with present pictures of erythropoiesis, the variations observed in the size, hæmoglobin content and life-span of erythrocytes in the normal animal, in experimental and clinical hæmolytic anæmias, and after stimulation of the red cell marrow by erythropoietin.

Tables 1 and 2 are summaries of autoradiographic data from marrow cells of a severely anæmic and of a

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Table 1. INCORPORATION OF TRITIATED LEUCINE INTO HÆMOGLOBIN OF SEVERELY ANÆMIC RABBIT MARROW ERYTHROID CELLS *in vitro*

(1) Type of cell	(2) Per cent of total erythroid cells	(3) 110–70	(4) 69–40	(5) 39–20	(6) 19–5	(7) 0	(8) Per cent of total synthesizing activity	(9) Synthesizing activity per cell
		No. of grains per cell						
		Per cent of cells in each class						
Basophilic erythroblast	8·7	45	28	11	4	12	23	100
Polychromatic erythroblast	23·3	6	22	28	6	38	38	44
Orthochromatic erythroblast	28·7	0	6	16	5	73	11	14
Non-nucleated cells (reticulocytes)	30·1	5	18	21	9	47	28	36

Table 2. INCORPORATION OF TRITIATED LEUCINE INTO HÆMOGLOBIN OF NORMAL RABBIT MARROW ERYTHROID CELLS *in vitro*

(1) Type of cell	(2) Per cent of total erythroid cells	(3) 110–70	(4) 69–40	(5) 39–20	(6) 19–5	(7) 0	(8) Per cent of total synthesizing activity	(9) Synthesizing activity per cell
		No. of grains per cell						
		Per cent of cells in each class						
Basophilic erythroblast	6·7	46	36	6	11	1	38	100
Polychromatic erythroblast	34·4	3	12	18	45	21	44	23
Orthochromatic erythroblast	41·6	0	0	2	31	67	12	5
Non-nucleated cells (reticulocytes)	17·1	0	2	8	20	70	6	6

normal rabbit. In the cells of the anæmic animal, Table 1 shows that the most active group was the basophilic erythroblast; the polychromatic were a little less than half, and the orthochromatic cells somewhat less than a quarter as active. The reticulocytes as a class were more active than the orthochromatic erythroblasts. Synthesis of hæmoglobin had stopped in cells at all stages; the fraction of cells showing no grains was larger in each successive maturation phase.

In the anæmic animal marrow the polychromatic, orthochromatic and reticulocytes were in about equal proportions; in the normal animal the orthochromatic was the largest fraction, and its increase was entirely at the expense of the reticulocytes. In the anæmic animal the synthesizing activity of the reticulocytes was more than twice that of the orthochromatic cells; in the normal the activity of the two groups was nearly the same. In the anæmic animal the reticulocytes contributed 25 per cent of the total activity, in the normal animal 6 per cent. The latter, but not the former, finding would have been expected from the view generally held.

The above hypothesis was tested by predictions regarding difference in sizes of marrow orthochromatic cells and reticulocytes in the normal on one hand and in the anæmic animal on the other. A summary of such measurements is given in Table 3. It is seen that the orthochromatic cells were the same size in the normal and anæmic marrows, as expected. Also as expected the reticulocytes were significantly larger in the anæmic marrow; $P < 0{\cdot}01$. In the normal marrow the reticulocytes were smaller than the orthochromatic cells; this difference we ascribe to the reduction in volume following the loss of the nucleus. The normal marrow reticulocytes were the same size as the circulating erythrocytes, which is evidence that reticulocytes do not constrict when they mature to erythrocytes. Brecher and Stohlman[5] had come to the same conclusion from other evidence. In the anæmic marrow the reticulocytes were significantly larger than the orthochromatic cells; $P < 0{\cdot}01$. When to this difference is added the shrinkage as a result of loss of the nucleus it is obvious that most of the reticulocytes in the anæmic marrow must have come from cells larger than the orthochromatic erythrocytes. Lajtha and Suit[6] noted that the more active reticulocytes in human marrow were larger than the nucleated orthochromatic cells. When there is a great reticulocytosis, as in our severely anæmic rabbits, Brecher and Stohlman found that the peak volume distribution of reticulocytes in the rat with an anæmia caused by phenylhydrazine was twice that of normal red cells. Double the volume corresponds to a diameter of about 4/3 normal, which, in turn, corresponds to the ratio of the diameters of the polychromatic and orthochromatic erythroblasts respectively[7]. Such abnormal cells could not have come directly from cells larger than the polychromatic erythroblasts. If they had come by skipped mitoses directly from stem cells or pronormoblasts, as Stohlman[8] proposed, their volume would have been about eight times normal, and if from the basophilic normoblasts, five times normal.

Table 3. DIAMETERS (ARBITRARY SCALE) OF MARROW ORTHOCHROMATIC CELLS AND RETICULOCYTES AND CIRCULATING ERYTHROCYTES IN ANÆMIC AND NORMAL RABBITS

	Orthochromatic cells	Reticulocytes in marrow	Circulating erythrocytes
Anæmic	16·0 ± 1·66	16·7 ± 0·98	—
Normal	16·0 ± 1·45	14·3 ± 1·41	14·3 ± 0 27

The following four tests of the above hypothesis were provided by fractionation of reticulocytes in the blood of a severely anæmic rabbit after they had been incubated with leucine labelled with carbon-14: (1) active (in hæmoglobin synthesis) and inactive reticulocytes should be found; (2) the active cells should contain more hæmoglobin because synthesis had persisted in them for a longer time; (3) the active should be larger than the inactive reticulocytes; (4) the hæmoglobin content of the most active reticulocytes should be greater than in normal erythrocytes even though they had not yet completed their synthesis of hæmoglobin. Predictions (2) and (4) are the reverse of the expected from the view that synthesis proceeds toward a final maximum hæmoglobin content per cell which is that found in the normal erythrocyte.

Some results are given in Table 4. The red cells (85 per cent reticulocytes) in the blood of severely anæmic rabbits were fractionated in a bovine serum albumin gradient[9] by a modification of the method of Vallee *et al.*[10]. The most active cells remained at the top; the cells in the pellet had almost no activity; there was a gradient of activity in the intermediate layers from the top down. The gradient of hæmoglobin content per cell was in the same direction: most at the top, least at the bottom. In a comparable experiment the cells were incubated with

Table 4. FRACTIONATION OF CIRCULATING RED CELLS OF A SEVERELY ANÆMIC RABBIT IN A BOVINE SERUM ALBUMIN GRADIENT

Position in centrifuge tube: layer number	9 (top)	5–8	3	Pellet
Per cent of total cells	1·7	45·7	19 2	33·2
Reticulocytes. per cent total red cells in the layer	100	100	100	13
Hæmoglobin per cell: (mg $\times 10^{9}$)	34·6	26·0	22·6	16·0
Hæmoglobin counts per cell: (c.p.m. $\times 10^{6}$)	35·4	12·3	4·3	0 06
Hæmoglobin (c.p.m. per mg)	1,020	470	190	3

tritium- instead of labelled leucine, similarly fractionated and then autoradiographed. Fifty per cent of the cells in top (most active) layer had no grains, which is practically the same fraction of inactive cells as was found in the autoradiography of non-nucleated erythroid cells in the marrow (Table 1); and as in the carbon-14 labelling, the number of grains per cell diminished sharply from the top down. Leif and Millette[11] had observed on fractionating rabbit reticulocytes which had been incubated with leucine labelled with carbon-14 that the specific activity of the hæmoglobin was highest in the cells of the top layer and lowest in the bottom; Miller[12] had observed by autoradiography that only 30–35 per cent of the cells had grains although 85 per cent of the cells stained like reticulocytes.) Hæmoglobin synthesis had stopped in the cells of all densities. Brecher and Stohlman showed that the hæmoglobin concentration determines the densities of these cells. As the cells at the top were the lightest and yet had the most hæmoglobin per cell they must have been the largest; conversely for the cells in the pellet; and correspondingly intermediate values for the cells between top and bottom.

Similar evidence was obtained by labelling the red cells in the blood of a rabbit recovering from severe phenylhydrazine anæmia. On the morning of the last phenylhydrazine injection a dose of valine labelled with carbon-14 was injected followed 4 h later by ten times as much unlabelled valine. On the following days the rabbit's blood was sampled and determinations made of the specific activity of the hæmoglobin (c.p.m./mg hæmoglobin) and the hæmoglobin per cell (mg hæmoglobin × 10^8/No. of cells). The findings are plotted in Fig. 1.

On day 4 all the marrow cells, which had been exposed to labelled valine for a short time, were in the blood. Declining specific activity in Fig. 1 denotes dilution of labelled hæmoglobin made in cells on day 0 by those made later. The correlation is clear between hæmoglobin content per cell and specific activity of the hæmoglobin. There was the same correlation on days 0–4 but these are omitted from Fig. 1 because the blood picture was complicated during this period by destruction of red cells with Heinz bodies caused by phenylhydrazine and by abnormally heavy labelling of circulating reticulocytes on day 0 exposed to the very high specific activity of the injected labelled valine.

In man, as in rabbits and rats, there is an increase in the average size and hæmoglobin content of erythrocytes in the peripheral blood in the response of increased erythropoiesis to hæmorrhage or hæmolysis[13].

Fig. 1 shows that on the 10th day of recovery the average hæmoglobin per cell was in the normal range, which testifies to the short life-span of the cells made during anæmia; the life-span of normal rabbit erythrocytes is 65–70 days[14]. We had made a similar observation in experiments in which polycythæmia was produced in normal adult rats by erythropoietin: the blood picture returned to normal in about three weeks after the erythropoietin administration had stopped[15]. This might have been explained on the basis of a normal life-span of rat erythrocytes of 100 days[16] and complete arrest of erythropoiesis in the marrow until the polycythæmia had subsided. But a shortened life-span of red cells produced in response to acute loss of blood had been observed by Berlin and Lotz[17] and after direct labelling of the cells by Stohlman[18]; in the latter's experiments the cells were the same which Brecher and Stohlman had shown to be larger than normal.

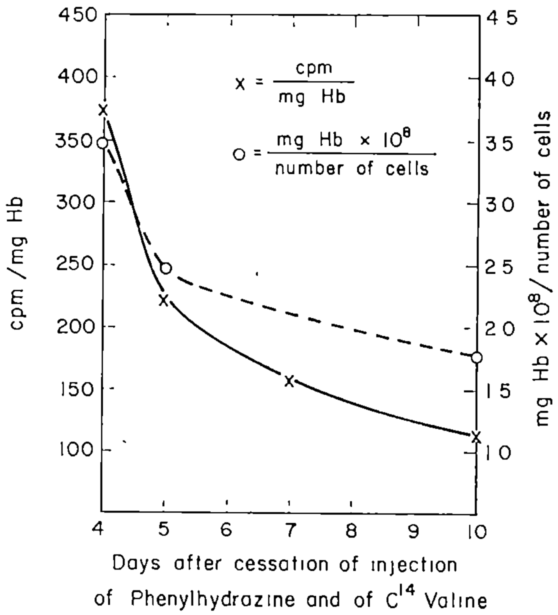

Fig. 1

It is a normal occurrence for some erythrocytes to arise directly from earlier erythroblasts than the orthochromatic; these are larger, have more hæmoglobin per cell and have a shorter life-span than normal erythrocytes. Ambs[19] gives the spread of diameters of normal human erythrocytes as 6·1–8·6μ, and of their hæmoglobin content as 15–48 × 10^{-9} mg per cell. Thorell's values for the diameters of the erythroblasts are 10, 8 and 6μ respectively for the basophilic, polychromatic and orthochromatic cells[7]. The diameter of the largest cells Ambs observed, 8·6μ, is that of the basophilic erythroblast minus the shrinkage due to loss of the nucleus; the diameter of the smallest cells, 6·1μ, is a little larger than the average of the orthochromatic erythroblasts, 6·0μ. The highest hæmoglobin content per cell Ambs found, 48 × 10^{-9} mg per cell, was higher than the average of the most active group in Table 4; in half the latter active hæmoglobin synthesis was still in progress. The lowest value, 15 × 10^{-9} mg per cell, is near to that of the pellet cells of Table 4. Ambs saw a tendency for the larger cells to have more hæmoglobin, though there was no rigorous ('gesetzmässiges') correlation. This is to be expected from findings such as those in Tables 1 and 2 that synthesis of hæmoglobin stopped in some cells at all maturation stages from the basophilic onwards. In the normal, as after severe blood loss, there is a fraction of the erythrocytes with a life span so much shorter that it constitutes a different population. London cites different pieces of evidence of an "infant mortality of the very youngest cells" and that the older erythrocytes tend to be smaller.

Analysis of hæmoglobin synthesis using incorporation of leucine labelled with carbon-14 in rabbit marrow cells and reticulocytes *in vitro* was in accord with the hæmoglobin synthesis aspect of the hypothesis here.

The reticulocytes in normal blood were about one-fifth as active as those in the blood of the severely anæmic animal. The explanation is that in the normal they came via the relatively inactive orthochromatic erythroblasts, in the anæmic a large fraction came directly from the much more active polychromatic erythroblasts and they were also larger than the normal reticulocytes.

The average activity per marrow cell was 3·1–5·2 times that of the circulating reticulocytes in the same animal. If one makes the likely assumption that the most active cells in the experiment of Table 4 were representative of the reticulocytes in the marrow then from the data in Tables 1 and 4 an expected value is obtained for:

$$\frac{\text{average activity per cell of marrow cells}}{\text{average activity per cell of marrow retics}} \times \frac{\text{average activity per cell of marrow retics}}{\text{average activity per cell of circulating retics}}$$

It is 1·07 × 5·3 = 5·6. The RNA per cell in marrow cells was about six times that in reticulocytes. The results in Tables 1 and 4 indicate that in the anæmic animal about half the activity in the basophilic erythroblast is lost by the time it becomes a reticulocyte; most of this loss is represented by the fraction of cells in which synthesis has stopped. The remainder of the original synthetic activity, about half, is lost in the maturation of the circulating reticulocyte.

New, structural ribosomal protein was synthesized by marrow erythroid cells *in vitro* at a rate that corresponded to 7·5 per cent in 24 h of the initial ribosomal protein. This small amount, which could be accounted for entirely by the pronormoblasts and young basophilic erythroblasts, supports the tenet that there is no significant increase of synthetic apparatus in the marrow erythroid cells after the basophilic stage.

The evidence is direct, and all of it concordant that abnormal demands on the red cell marrow such as acute hæmorrhage, severe hæmolysis or large doses of erythropoietin evoke cells which are larger, have more hæmoglobin per cell and are shorter-lived than normal erythrocytes. In the hypothesis we propose the polychromatic erythroblast has a dual capacity; it may subdivide and become an orthochromatic erythroblast or skip the mitosis and become a reticulocyte directly. Our evidence is insufficient to show whether the basophilic compartment may have such a dual capacity. If it has, its contribution to the reticulocytes of the quick response would be small because it is a small compartment. The orthochromatic compartment appears insensitive to stress, and in any event could not produce the abnormally large cells which are characteristic of the stress response. It is therefore only, or mainly, the polychromatic erythroblast which adapts directly to stress by changing its course of development. This explains the changed character of the red cells evoked by stress and also how, after administration of erythropoietin, the increased uptake of iron-59 in circulating cells is too quick for increased production of cells all the way from stem cells[20].

The question then arises whether it is necessary to invoke another or an additional site of action of erythropoietin. It is widely held that the sole action of erythropoietin is to stimulate the stem cell to proliferate[1,18,21]. All the development of the erythroid cell thereafter is considered to be normal. If that were the case one would expect the increased cells evoked by severe hæmorrhage or hæmolysis or erythropoietin to be normal in every respect. They are abnormal, and many of them appear too quickly to have come from stem cells in the normal way.

The stimulus to stem-cell proliferation could be a secondary effect of erythropoietin. There would then need to be another factor to carry the demand for increased cell production from the polychromatic to the stem cell compartment. An alternative hypothesis is that small doses of erythropoietin stimulate only stem-cell proliferation; large doses stimulate in addition skipping of mitoses by polychromatic cells. This would be in accord with the observation of Matoth and Kaufmann that erythropoietin stimulated mitotic activity of erythroblasts *in vitro*[22]. Either way we approach Stohlman's hypothesis[8,18] that red cell production is regulated by more than one mechanism, that erythropoietin is a stimulus evoked by 'panic' which promotes skipping of mitosis[5,8,18]. A difference between our hypothesis and Stohlman's is where the skipping of mitosis occurs. To have it occur at the stem cell offers no mechanism for the abnormal size and hæmoglobin content of the cells evoked by large doses of erythropoietin, for the speed of their appearance and the greater synthesizing activity of reticulocytes in severe anæmia than those in normal blood. Most of the abnormal cells are not large enough to have come via reticulocytes directly from cells larger than the polychromatic erythroblast. Neither our hypothesis nor Stohlman's offers a mechanism for the shorter life-span of the abnormal cells.

A little more detailed picture derived from our hypothesis would be that normal red cells come by the classical route of development through basophilic, polychromatic, orthochromatic erythroblasts and reticulocytes. A crisis evokes cells quickly by a shunt around the orthochromatic erythroblast; they are larger and contain more hæmoglobin than normal red cells, and are removed from the circulation relatively quickly after the crisis has passed. The crisis also stimulates (directly or indirectly) red cell production by the normal pathway, and after the crisis has passed, if a milder stimulus to red cell production persists stem-cell proliferation would continue to be stimulated; eventually there will be a polycythæmia mainly of normal cells. Under any given set of conditions there would be a mixture of normal and abnormal red cells in the blood, in a proportion which would vary according to the demands of the condition.

This work was supported by grants from the U.S. Public Health Service (*H*-1624) and the U.S. Atomic Energy Commission.

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RESPIRATORY AND SWIMMING MOVEMENTS IN THE CEPHALOPOD *CRANCHIA SCABRA*

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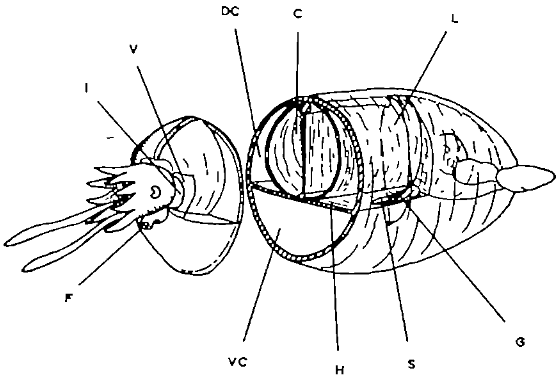
During a cruise on *Discovery II* in May–June 1962 observations were made on a living specimen of *Cranchia scabra*, a member of the œgopsid family Cranchiidae. Squids of this family differ from members of all other families in having a horizontal partition (Fig. 1, *H*) across the mantle cavity which is fused to the ventral midline of the enlarged cœlome (*C*) and to the mantle. This arrangement divides the mantle cavity into two dorsal (*DC*) and one ventral (*VC*) chamber. Each of the dorsal chambers is connected with the ventral chamber by an opening or spiracle (*S*) in the posterior end of the horizontal membrane and with the exterior by an inhalent opening at the side of the head (*I*). Each inhalent opening is limited by a thin membrane which is attached to the head along its anterior border and to the mantle wall along its dorsal and ventral borders (*V*). This is part of the nuchal collar and acts as a valve preventing water from leaving the dorsal chamber through the inhalent opening. A small funnel (*F*) allows water to pass from the ventral mantle chamber to the outside. Each gill (*G*) borders the posterior lip of a spiracle and lies on the ventral side of the horizontal membrane.

Cranchia scabra is a small, globular squid which has a very large cœlome which is divided into right and left halves anterior to the liver (*L*) by a thin membrane and has no sagittal partition behind the liver. This cavity, containing the body organs, is filled with a fluid which, if it is analogous to the fluid in the body cavities of other cranchids, makes the squid neutrally buoyant[1]. The cœlome extends the length of the mantle and is attached to it along the length of the pen and by the horizontal membranes, including their dorsally flexed, posterior ends (Fig. 1*B*, *HV*). The cœlome wall contains muscle cells and is capable of considerable contraction.

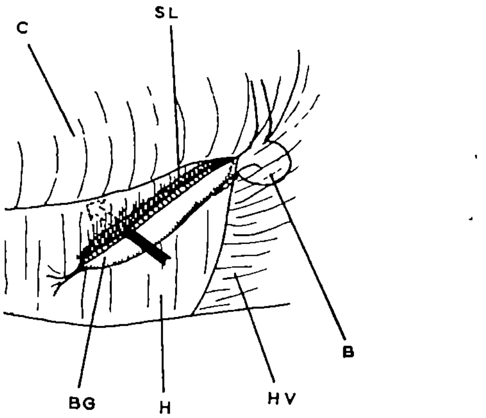
The living animal was neutrally buoyant, and changes of position and orientation (relative to light, gravity, etc.) were brought about by continual, rapid movements of the fins. Only once or twice, during its captive life of two or three hours, did the animal contract its mantle. Such contractions caused a rapid backward movement over a distance of three or four body-lengths. After the contraction the mantle remained deeply dented for several moments and only slowly regained its globular shape. This slow recovery of mantle shape and the infrequency of the movement in what appeared to be a healthy squid, suggests that such contractions are not used for continual movement but are probably only escape reactions. This conclusion is supported by observations on other small cranchids in which such movements have also proved infrequent.

Although mantle contractions are infrequent, water passes into the inhalent openings and out of the funnel continually, and it is interesting to find that the enlarged cœlome, besides imparting buoyancy, is also responsible for creating this water current. Such a current is necessary for respiration at the gills.

When the chromatophores are contracted the body is largely transparent so that the internal movements could be studied easily and the passage of fountain pen ink through the mantle cavity was observed from the side and from above with a binocular microscope. Contraction of the wall of the cœlome at its anterior end sucks in water through the inhalent openings at the same time as opening the valve (Fig. 2*A*). The contraction also stretches the horizontal membrane



A, Animal with mantle cut transversely to show, *C*, cœlome; *DC*, dorsal chamber; *F*, funnel; *G*, gill; *H*, horizontal membrane, *I*, inhalent opening; *L*, liver, *V*, valve; *VC*, ventral chamber



B, Dorsal view of the posterior end of the left side of the horizontal membrane. *B*, branchial heart; *BG*, branchial gland; *HV*, vertical part of horizontal membrane; *SL*, anterior lip of the left spiracle

Fig. 1. Diagram to show the arrangement of membranes in the mantle cavity of *Cranchia scabra*

For clarity, tubercles present on the outside of the squid have been omitted

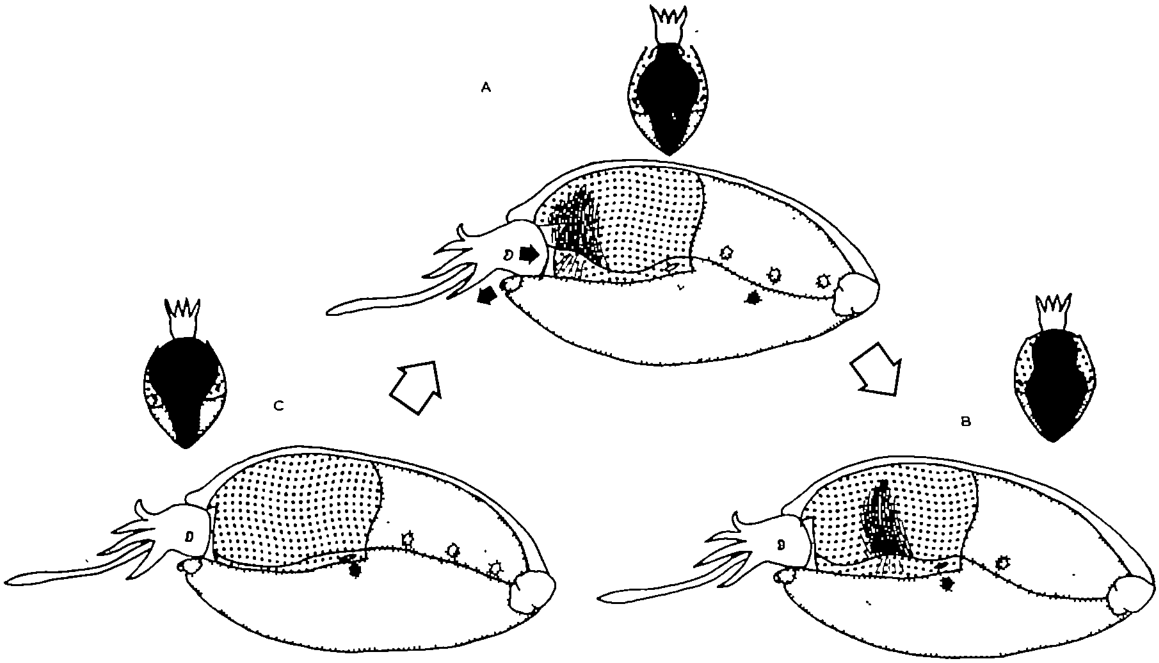


Fig. 2. Diagrams which show three stages in the respiratory movements of *Cranchia scabra*. The small diagrams show the movements from the dorsal aspect and the large ones show them from the left side. Solid arrows indicate water movements. Open arrows and shading indicate movements of the cœlome wall. Large and small dots indicate the extent of the left dorsal and ventral mantle chambers respectively

locally and so prevents it from moving upwards during the suction. Contraction at the front of the cœlome causes the posterior end to bulge and, as this lies in the ventral mantle chamber, water is forced out of the funnel. The gill, which forms a flap hanging below the spiracle (Fig. 1*B*), probably acts as a valve which prevents water from passing up through the spiracle at this stage. The area of contraction moves back along the cœlome and at the same time the region which was contracted at first, relaxes (Fig. 2*B*). The dual effect is to move water in the dorsal mantle chambers backwards and through the spiracle and also to shut the valves over the inhalent openings. The pressure necessary to close these valves may result from the expansion of the anterior end of the cœlome but, as water is being forced through the spiracle at this stage, the whole part of the cœlome adjacent to the dorsal mantle chambers must be expanding (presumably because the cœlome behind this is starting to contract) and this closes the valves by creating a positive pressure in the dorsal mantle chambers. The region of contraction increases in length when it reaches the partition at the posterior end of the dorsal mantle chambers so that the whole of the more posterior cœlome appears to contract simultaneously (Fig. 2*C*). Whether this is an active contraction or whether the cœlome wall is just changing shape by passive relaxation is difficult to decide but the wall of the posterior and anterior parts of the cœlome contain similar contractile elements. This complete movement, which is rather like a peristalsis having a large wave-length in comparison with the cœlome length, is repeated continuously.

During part of the cycle, water passes out of the funnel at the same time as water passes into the dorsal chambers and this, of course, is quite different from the timing of these currents in squids using the mantle muscle to draw water in and out.

Many cranchids, including *Cranchia scabra*, have no valve in the funnel and clearly there is no need for one with this water-circulatory system.

Similar movements of the cœlome wall have been observed in *Liocranchia* and *Megalocranchia*.

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ELIMINATIVE RESPONSE IN THE ALBINO HAMSTER

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THE eliminative response as an indicator of individual differences in temperament was first systematically studied by Hall in the rat[1] and was afterwards studied by him[2] and others[3-6] in the mouse. Hall defined the 'emotional' defæcatory reaction as defæcation in an unfamiliar environment which ceases on repeated experiences in that environment[7]. The possibility of the occurrence of this phenomenon in various species of the rodent family is of theoretical and experimental interest. A relationship between this behavioural pattern and 'anxiety' has been suggested as generally applicable to mammals[7] on the basis of the autonomic involvement in the response. Easy measurement of the response has also added to its popularity. Delineation of the characteristics of the reaction in other rodent species may lay the basis for further inter-species comparisons. Accordingly, the following investigations of the responses of the albino hamster to the standard open-field situation were carried out.

12 albino hamsters from a mixed laboratory stock were used. All the animals were individually housed in wire mesh cages with food and water *ad libitum* except as indicated. The animals were exposed to the normal day–night cycle during the months of September and October 1961, in New York City. A standard rat diet pellet with a supplement of lettuce was fed the hamsters, except for seeds offered in the open-field situation. Animals were observed between the hours of 9 and 10.30 a.m. Each animal was placed individually in the centre of a circular arena 28 in. in diameter, with a wall 24 in. high. The arena was made of sheet metal covered by pastel green flat enamel paint. An overhead fluorescent fixture with two 40-W bulbs was suspended 100 in. above the floor. During the 4-min observation, incidence of defæcation and urination; locomotion; leaving and returning to the wall of the enclosure; feeding; and grooming were recorded. This procedure was followed once each day for 11 days. On days 12 and 13 the animals were not observed. The animals were again observed in the enclosure on days 14–16.

On days 17–19, an overhead buzzer and light on the perimeter of the enclosure were presented to the animal during the 4-min observation. The buzzer was an Edwards No. 15-0 mounted on a plastic arm 34 in. above the floor of the enclosure and measured at 77–78 db. when tested with a General Radio model 1551*A* sound-level meter on the centre of the floor of the arena. The added light stimulus was provided by a 200-W bulb 44 in. above the floor of the enclosure.

The animals were not deprived of food or water in their home cages until day 19. On that day, during the observations, all food was removed from the home cages. None of the animals had food stored in the pouches during the observation on day 19. On day 20, the animals were not observed. On days 21–23, 2 min after the hamster had been placed in the open-field, a plastic tray (2½ × 1½ × ½ in.) containing 6 seeds, of different variety, was placed in the centre of the enclosure. After 2 min of further observation, the animal was removed.

During days 21–23, each animal was assigned to one of two groups on the basis of their defæcatory response during days 1–11. Although exact matching was not possible, the ranges and distributions of scores in the two groups were such that there was no statistically significant difference between the two groups in regard to frequency of defæcation or micturition during the first 11 days of observation. Group I was observed in a glass fish tank (16 × 12 × 12 in.) which they had not previously experienced, although at one time they had lived in groups in cages similar to the observation apparatus. Group II was observed in the same enclosure used prior to this period.

Animals were removed from their home-cages by the use of a round plastic container measuring 4 in. in diameter and 3¾ in. in height. Air holes were punched in the lid of the cup. The cup and enclosures were swabbed with a 70 per cent alcohol solution before each observation. During days 21–23, approximately 1 h after observation, the animals were fed *ad libitum* for 1 h.

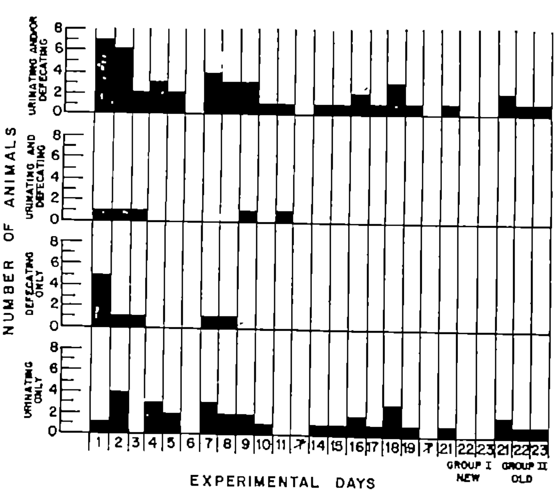

Fig. 1. During days 17–19, auditory and visual stimuli were introduced into the open-field situation. Group I animals were observed in an enclosure different from that in which they had been studied during days 1–19. Group II animals were observed in the same enclosure throughout the experiment. Crossed *T*, not tested

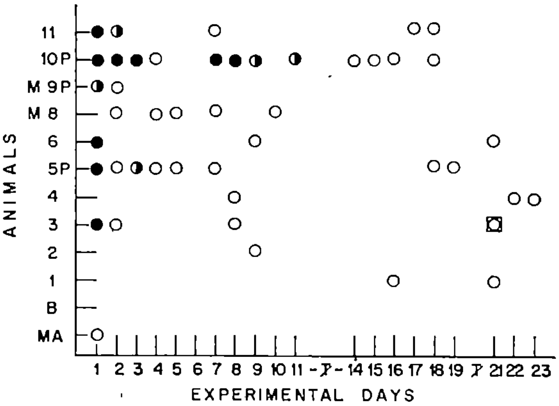

Fig. 2. Pattern of defæcation and urination of hamsters in open-field situation. See Fig. 1 for an explanation of treatment on different experimental days. ●, Defæcated; ○, urinated; ☐, newly introduced enclosure; *M*, male; *P*, pup; crossed *T*, not tested

Although the sample studied was not homogeneous as to age and sex, analyses of the dependent variables (for example, eliminative behaviour, grooming and activity parameters) showed no differences when males were compared with females, or pups were compared with adults. It should be noted, however, that some relationship between age and sex may exist in that the two female pups showed the highest frequency of eliminative behaviour. The sample was too small to warrant more precise analysis.

As can be seen in Fig. 1, the number of animals which only defæcated declined from day 1 to day 2 and except for days 7 and 8 no animal defæcated thereafter. This was not true of micturition: a low level was maintained throughout the experiment. Accordingly, the bar graph shows some decrease in number of animals defæcating or urinating, or both in the same session. There was a wide range of individual patterns of eliminative reaction as may be seen in Fig. 2. One animal stopped urinating after one day, but for the most part, individuals either persisted or showed sporadic patterns of elimination in the open-field. One animal never urinated or defæcated.

Defæcation and urination took place so infrequently before or after observation that no analysis was warranted.

Fig. 3 shows the mean, median and range of number of seconds between the animal's entry into the open-field at its centre and its arrival at the wall of the enclosure. There was a significant trend (Kendall concordance test, $P < 0\cdot05$) for this duration to decrease from trials 1–11. There was no significant difference between the group's latency on day 11 and on day 14 (Wilcoxon matched pairs test, $P > 0\cdot05$). From days 14 to 16 there was also no significant increase in latency ($P > 0\cdot05$). From days 17 to 19, the trend was significant in its tendency to decrease ($P < 0\cdot01$). The group in the newly introduced enclosure showed a downward trend on days 21–23 whereas the group in the customary enclosure did not. Both groups showed an increase in latency when days 19 to 21 were compared ($P = 0\cdot05$ for both groups). The animals in the newly introduced enclosure took about twice as long to reach the wall as did the animals in the customary enclosure ($P = 0\cdot05$), although the maximal distance in the fish tank was one-half that of the circular arena.

There was no relationship found between latency to wall and eliminative responses.

It was found that there was a significant decrease in the number of cross-overs during the first eleven days (Fig. 4) (Kendall concordance test, $P < 0\cdot001$). After two days without open-field observation there was a significant increase in the number of cross-overs when days 11 and 14 were compared ($P = 0\cdot05$). Days 15 and 16 were not different from day 11 ($P > 0\cdot05$). The trend was significantly in a downward direction when days 14, 15 and 16 were analysed ($P < 0\cdot02$). When auditory and visual stimuli were added on day 17, there was an increase in the number of cross-overs, but this was not significant ($P > 0\cdot05$). No pattern was discernible in either group on days 21–23.

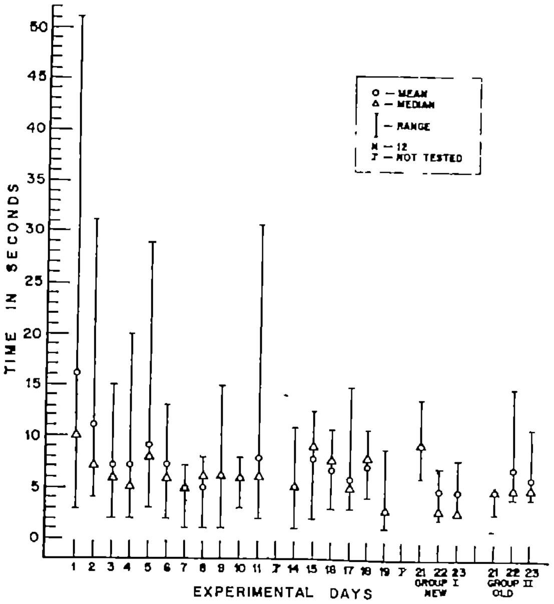

Fig. 3. Time to wall from centre of enclosure at start of observation (latency in sec)

See Fig. 1 for an explanation of treatment on different experimental days

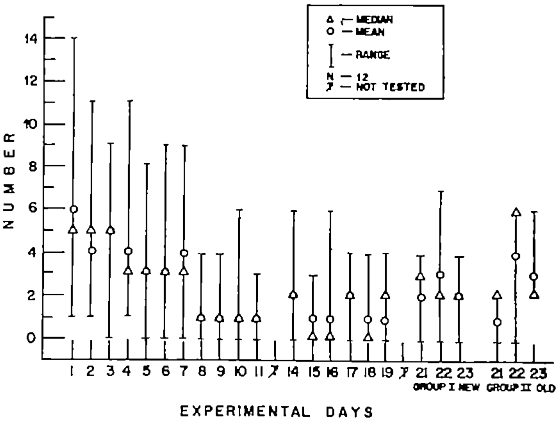

Fig. 4. No. of times animals left and returned to wall of enclosure (No. of cross-overs)

See Fig. 1. for an explanation of treatment on different experimental days

The only significant trend in grooming behaviour was evidenced in the group which was observed throughout in the same enclosure. This group showed a significant decrease in the amount of grooming during days 21–23 ($P < 0\cdot01$; Kendall concordance test).

There was no difference between the two groups in their latency measure on days 21, 22 or 23 (Mann-Whitney U test, $P > 0\cdot40$). There was no significant trend towards a decrease in the latency of this response in either group (Kendall concordance test, $P > 0\cdot05$). No behavioural measure correlated with the latencies of the combined groups.

When the animals were ranked as to eliminative responsiveness during days 1–11, it was found that the latencies of the animals in the newly introduced arena on day 21 were not related to their eliminative reaction ($RHO = 0\cdot086$; $P > 0\cdot05$). When the same analysis was made for the animals which were observed in the same arena throughout the study, it was found that there was a positive and significant correlation between latency to approach the food dish on day 21 and the eliminative reaction on days 1–11 ($RHO = 0\cdot94$; $P < 0\cdot02$).

None of the analyses of quantitative aspects of feeding activity proved to be statistically significant.

Certain aspects of the open-field behaviour of the albino hamster are noteworthy. In regard to eliminative reactions there was an unexpected persistence in micturition, accompanied by the expected decrease in the number of animals defæcating. This finding may be compared with the persistence of defæcation in the mouse[6], and the reported decrease in the rat[1].

The introduction of additional auditory and visual stimuli did not result in an increase in eliminative responsiveness, as might have been expected from the literature[5]. This may have been a function of the intensity of the stimuli used, although the buzzer increased the ambient noise-level from 66 db. to 77–78 db. It may also have been a reflexion of the level of adaptation of these animals to such stimuli because of previous experience. To our knowledge, the animals had not been presented with stimuli of this nature before. Further investigation of this variable is indicated.

In the early work of Hall[1], the relationship between the eliminative response and the feeding response was considered very important. For this reason, the observations on days 21–23 were made. On the basis of past work, it was anticipated that the animals in the new enclosure would not feed as readily as those in the customary arena. This was not found; all animals fed in both arenas. There was also no difference between the two groups with regard to the latency in responsiveness to the seed tray. It was found, however, that for the animals in the new enclosure, there was no relationship between the eliminative reaction during the first eleven days of observation and latency of response to the seed tray, while in the group in the familiar arena there was such a relationship. In other words, in the 'old' enclosure the animals which were previously high in eliminative reaction took the longest to approach the seed tray when it was presented to them under conditions of food deprivation and this did not maintain in the group in the new arena.

It was also expected from past studies that when the group was placed in the new environment, the number of animals defæcating and urinating would increase. This did not occur, possibly because they had lived in rectangular tanks of the type used in the experiment for approximately three months prior to the work recorded here. The change in enclosures was therefore possibly less significant than anticipated. It would still appear, however, that the eliminative response is particular to specific situations, in view of the fact that there was a relationship between elimination and latency of response to the seed tray in the customary observation arena, but not in the newly introduced observation arena. This finding is similar to that found in the study of open-field behaviour of the mouse[6].

We thank Amy Ziegler for help in the observation of the hamsters.

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IMMUNE RESPONSE IN THE CALIFORNIA HAGFISH

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THE hagfish, an agnathan cyclostome, is the most primitive of the true vertebrates. Since it is the first species in the phylogenetic scale with formed, circulating red blood cells and hæmocytopoietic tissue in the submucosa of the gut tract[1], definition of its immune response seemed particularly relevant to our investigation of the phylogeny of immune responsiveness. Our interest in these animals was stimulated by Jordan and Speidel's description[1] of blood cells of the Atlantic hagfish, *Myxine glutinosa*. Specimens of *Eptatretus stoutii*, the California hagfish, were obtained from Dr. David Jensen of the Scripps Institution of Oceanography, University of California, La Jolla, and were shipped in cold sea-water to our laboratories. The animals were kept at 10° C in well-aerated sea-water, except where specified, without feeding, for up to five months in good condition. In all, about 120 hagfish were used in these experiments.

Immunological responsiveness in mammals may be characterized by three parameters: the formation of circulating specific antibodies (γ_2-, β_2A- and β_2M-immunoglobulins) following antigenic stimulation, development of delayed sensitivity after appropriate sensitization, exemplified by the tuberculin reaction; and rejection of grafts from donors of differing genotype. A particularly important feature of these responses is anamnesis or immunological memory, which is reflected in the rapid increase in antibody concentration during the secondary response and by the second-set rejection of grafts.

Formation of Circulating Antibodies. Attempts to induce formation of circulating antibodies with diverse antigens were uniformly unsuccessful in the hagfish. Antigens used included killed *Brucella abortus*, typhoid–paratyphoid *A* and *B* vaccine, bovine serum albumin, hæmocyanin, and the bacterial viruses T_2 (*E. coli* phage) and *MSP*-8 (*Streptomyces griseus* actinophage). Although one, two, and, in some animals, several injections of antigens were given over periods of up to two months, and although a number of the antigens were incorporated in Freund's adjuvant at the time of injection, no agglutinating, complement-fixing, neutralizing antibodies, or antigen-combining globulin[2] were found. Up to 10 injections of T_2 phage by the intramuscular and intracœlomic routes, and under the skin into the subcutaneous lateral sinuses characteristic of the intermediate open-type circulation in this species, were given over a three-month period, without evidence of circulating neutralizing antibody at any time. Efforts to demonstrate such antibody included heat-inactivation of serum of injected hagfish for 30 min at 56° C and incubation with T_2 phage for as long as 72 h at 37° C and 0° C.

The persistence of antigen in the circulation of the hagfish was demonstrated with two of the antigens. Live infectious particles, in a concentration of 4,000 particles/ml., were found in the circulation 14 days after intramuscular injection of 10^{10} phage. Similarly, circulating hæmocyanin was detected in the serum

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14 days after injection by capillary precipitin tests with rabbit antihæmocyanin antibody.

As a control on conditions of temperature and starvation, a fresh-water teleost fish, *Ameiurus melas*, the bullhead of the mid-western United States, was immunized with T_2 phage at 10° C. Phage were cleared from the circulation within four days, and circulating antibody was detected in a 14-day heat-inactivated sample (Fig. 1). Heterologous phage (PLT_{22}–*S. typhimurium*) were included in reaction tubes as a control on the specificity of the inactivation.

The thermal death point of the hagfish, which normally inhabits the deep waters at 4° C, is approximately 28° C. To evaluate the effect of temperature on ability to form circulating antibody, hagfish and bullheads were kept at 20° C for seven days. Stimulation with 10^{10} T_2 phage failed to elicit antibody production in the hagfish, and large numbers of circulating phage were present in serum samples taken both 4 and 7 days after injection. In the bullhead, on the contrary, a fourth-day serum sample showed no particles, and heat-inactivated serum showed neutralizing antibody (Fig. 1). Heterologous phage were again included in the reaction tubes as a control for specificity of inactivation.

Delayed Sensitivity. Attempts to elicit delayed sensitivity in hagfish were also unsuccessful. Complete Freund's adjuvant containing *BCG* was injected, in 0·5-ml. amounts, by the intramuscular or the intraperitoneal route. The fish were challenged at 14 and 30 days with *BCG* and old tuberculin. Regardless of the site of injection of the challenging dose, no evidence of either a local or systemic reaction was detected.

Homograft Immunity. Grafts of skin 1 cm² from the caudal regions were exchanged between pairs of hagfish, and a number of autografts were also done. The grafts did not remain in place, with the single exception of a homograft which was in place when the animal was killed 28 days after grafting. There was no gross evidence of rejection, and microscopic study of sections disclosed neither evidence of inflammation nor indications of healing of the graft bed or the suture areas. Although the skin of the hagfish is in direct contact with the subdermal lateral sinuses, it is avascular and does not adhere to the musculature on the sides of the animals.

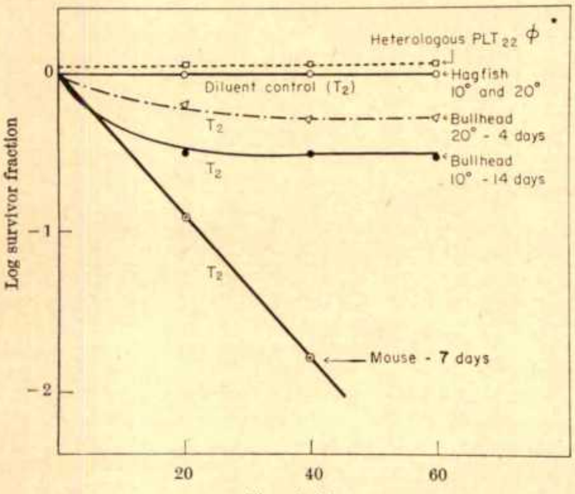

Fig. 1. Relative neutralization-rates of bacteriophage T_2 by antisera from the hagfish, bullhead and mouse. Antisera were collected after a single primary injection of 10^{10} particles on the days indicated (see text for hagfish). Dilutions of antisera were as follows : hagfish, undiluted ; bullhead, 1 : 2 ; mouse, 1 : 50

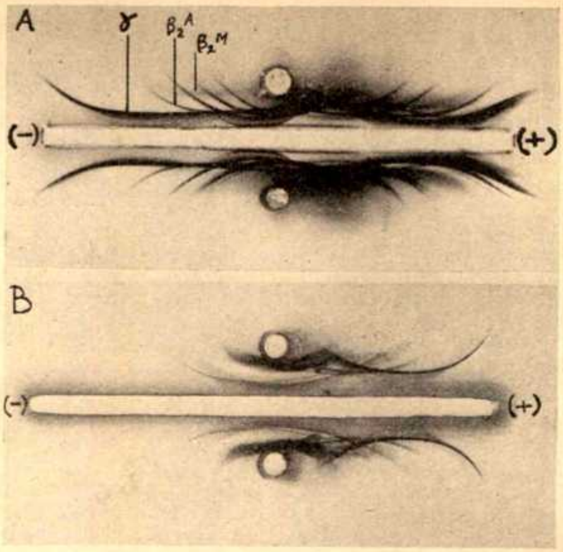

Fig. 2. Immunoelectrophoretic patterns of serum proteins from human (*A*) and hagfish (*B*). Patterns were prepared with rabbit antisera

Additional efforts to elicit homograft immunity included autografts and homografts of muscle (small sections of myotomes) and liver tissue (small implants and intrahepatic injections of liver cell suspensions). Twelve to 14 days after grafting, histiocytic infiltrations were found around all the myotome grafts, but in none of the liver implants. No morphological differences between autografts and homografts could be detected.

The poor healing in grafted animals led to death 7–14 days after the operation, and made a study of second-set reactions to grafts impossible. A similar failure to heal has been observed by Jensen[3] in the wound resulting from amputation of the tail of the hagfish.

Serum Proteins. Paper electrophoresis, starch-gel electrophoresis, and immunoelectrophoresis revealed an absence of proteins with the mobilities of albumins and γ-globulins in the hagfish. Immunoelectrophoretic analysis showed complete absence of bands corresponding to the albumin, cæruloplasmin, β_2A-, β_2M- and γ-globulins of higher animals (Fig. 2). The paper electrophoretic patterns were comparable with those of Rall *et al.*[4] on the lamprey. Further investigations concerning the chemistry of the serum proteins and electrophoretic analyses of concentrated serum samples are being completed.

Inflammatory Response. The response of the hagfish and the bullhead to an inflammatory stimulus was also examined, with the fish being maintained at temperatures of 10 and 20° C. Complete Freund's adjuvant, containing 30 mg/c.c. of *Mycobacterium butyricum*, and indian ink, was prepared, and 0·5 ml. amounts injected intramuscularly into hagfish and bullheads. By the fourth day, gross evidence of inflammation—swelling, erythema and induration—was noted in the bullhead at 20° C, and by the eighth day such changes were seen in animals kept at 10° C. There were no gross signs of inflammation in the hagfish after 7 days at 20° C or after 8 and 30 days at 10° C. The site of injection over the myotomes, marked with indian ink, was smooth and non-indurated. Microscopic sections of tissue from these sites showed infiltration of erythrocytic cells and of white cells best described as phagocytic histiocytes.

Hæmatology. Morphological studies of peripheral blood and imprints of liver and gut confirmed the original observations of Jordan and Speidel[1] that there are no apparent differences between peripheral circulating cells and cells of the presumed hæmocytopoietic centres. Our observations differed from theirs, however, in that we found no cells in the California hagfish which seemed identical with the lymphocytes of higher fishes, birds or mammals. Cells classifiable as lymphoblasts, medium-sized or large lymphocytes, or plasma cells were completely lacking in our material. A cell type resembling the small lymphocyte of higher animals, with a rim of dark basophilic cytoplasm, was observed and interpreted as a possible primitive precursor of the erythrocytic series. The cells we observed in the California hagfish did not differ significantly from those described by Jordan and Speidel, and we consider that our differences are largely differences in terminology regarding lymphoid cells. In contrast, well-developed, easily recognized lymphocytes of all stages of development and occasional plasma cells were found in the bullheads.

Examination of serum bactericidal substances and complement were not attempted. This form of immunity, often included in descriptions of natural resistance[5], has been found in invertebrates[6], and may account for protection from invasive parasites and organisms in the hagfish. Our investigations were concerned exclusively with adaptive immunological responses. In the hagfish, apparent inability to form circulating antibody, to develop delayed sensitivity, and to reject homografts was correlated with the lack of lymphoid cells commonly associated with the immune response. Additional findings in the hagfish and higher species, correlating the evolution of the immune response and lymphoid tissue, will be presented elsewhere.

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EFFECT OF PARASITISM ON GRAM-POSITIVE BACTERIA, WITH REFERENCE TO THE ORIGIN OF CERTAIN TYPES OF VIRUS

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IN a previous communication[1], one of us proposed the hypothesis that Gram-positive bacteria, under the influence of a parasitic mode of life, evolved in a parallel manner in different groups, with the progressive loss of such organs as spores and flagella, of Gram-positivity, catalase and other enzyme systems, with the effect that, from a morphologically complex saprophyte is thus derived an anaerobic, Gram-negative, nutritionally exacting parasite (for example, *Veillonella* or *Fusobacterium*). It is not suggested that all Gram-negative bacteria are derived from Gram-positive forebears; but it seems to be true of most oral and similar mucous-membrane parasites (cf. also ref. 2).

One group of such bacteria, parasitic on mucous membranes, not discussed previously, in which a comparable series appears to occur, is *Corynebacterium–Haemophilus*, and here the degenerative series can be observed to proceed even further, in a dramatic manner, in the production of members of the group of parasitic micro-organisms known as mycoplasma or pleuropneumonia-like organisms.

It seems certain that neither *Haemophilus* nor *Corynebacterium*, as at present defined, is a natural genus; both are assemblages of morphologically similar bacteria of diverse origin. However, there may be considered to exist a group of *Corynebacteria*, characteristically parasitic on mammalian mucous membranes, and including several well-known species, *C. diphtheriae*, *C. cervicis*, etc., and a group of *Hæmophilus*, of similar habitat, and including *H. influenzae*, as well as numerous, less-well-defined species.

Considerable evidence has recently accumulated to show that many strains of pleuropneumonia-like organisms are derived from bacteria of these two genera, to which they bear the same relationship as do *L*-forms to *Proteus* and similar Gram-negative bacteria[3–8]. The differences, usually quoted, between pleuropneumonia-like organisms and *L*-forms[9], are not so great as between *Corynebacterium* and *Proteus*, the *L*-forms of which latter provide the basis for most such comparisons (cf. refs. 3, 8). Descriptions of these and similar strains of *Haemophilus* provide reason for concluding that they are, in fact, structurally and nutritionally degenerate *Corynebacteria*[10–12], and we have ourselves observed the dissociation of *Haemophilus*-like organisms from *Corynebacteria*, and vice versa. Another superficially Gram-negative parasite of the mammalian nasopharynx that has been proved, by dissociation studies and immunological reactions, to be derived from *Corynebacterium*, is *Streptobacillus monilformis*[8]; its well-known *L*-form is antigenically related to the pleuropneumonia-like organisms described as *Mycoplasma hominis* Type 1 (ref. 8), and thus provides further conclusive evidence that pleuropneumonia-like organisms are of bacterial origin. This has previously been suggested (ref. 13 and others) but not proved.

The transformation of bacteria into pleuropneumonia-like organisms or *L*-forms occurs in a series of stages, of which the first is little more than the loss of the cell wall, leaving a naked, sometimes flagellated protoplast[14,15]. Subsequent stages may be so reduced in size and synthetic ability as to resemble viruses by ordinarily accepted definitions.

These transformations are influenced by the presence of antibiotics, notably penicillin, in artificial media or in the body fluids of the host. It is probable that the cell-wall free stage is induced, or at least selected, by the action of penicillin, which is mainly on the cell envelopes[14,16].

Apart from the pathogenic pleuropneumonia-like organisms originally described as such, notably the actual organism of bovine pleuropneumonia, a pleuropneumonia-like organism derivea from a *Corynebacterium* has been implicated in neonatal pneumonia[3] and the so-called Eaton agent, one of the causes of virus pneumonia, has been shown to be a pleuropneumonia-like organism[17]. Patients recovering from this type of infection frequently develop serum agglutinins reacting with a bacterium which has been isolated from cases of virus pneumonia at post-mortem, although it is not regarded as the primary infective agent. This micro-organism is usually described as a *Streptococcus* (*Streptococcus M.G.*), but it is not a typical member of this genus, and somewhat resembles the coccal stages observed in the transformation of other bacteria to and from pleuropneumonia-like organisms[3,4]. In this it is strangely analogous with *Streptobacillus moniliformis*, which presents the appearance of a semi-stabilized, intermediate *L*-form stage of a *Corynebacterium*[8], and which is responsible for a variety of rather obscure infections that have, at various times, been suspected of a virus origin.

Association of a virus with a bacterium in infective conditions, especially of the respiratory organs, is by no means exceptional. The classical example is a *Haemophilus* (*H. influenzae*), and the relationship between the bacillus and the virus has been the subject of considerable speculation, especially in the early days of the knowledge of the association; but comparable conditions are known to exist as swine influenza and fowl coryza, and the bacterial member of the infective complex is in every case referable to the *Corynebacterium–Haemophilus* degenerative series.

The actual nature of viruses is now reasonably clear, since the results of the research work of the past ten years, in this field and in that of nuclear biochemistry, have become understood. In its essentials, a virus is a system of nucleic acids, capable of genetic but not physiological expression, that is enabled to invade the nuclear processes of a host cell and to divert its metabolic activities to the production of the virus. Such a system of nucleic acid (with or without a variable amount of associated, non-genetically active material) may be derived from rogue genes of the host type of cell itself, or it may have an extrinsic origin. Plant viruses and bacteriophage are widely believed to be intrinsic in origin, and this may be true of some animal viruses. The suggestion we wish to make, however, is that the viruses of the type we have been discussing are derived, not from the host itself, but from a parasite already adapted to the host, in this case a bacterium, and that these viruses originate as pleuropneumonia-like organisms or *L*-forms, being the final stages in a generative process that affects bacteria of the *Corynebacterium–Haemophilus* series. This process is itself a manifestation of a comparable degenerative phenomenon that affects several other groups of parasitic, Gram-positive bacteria.

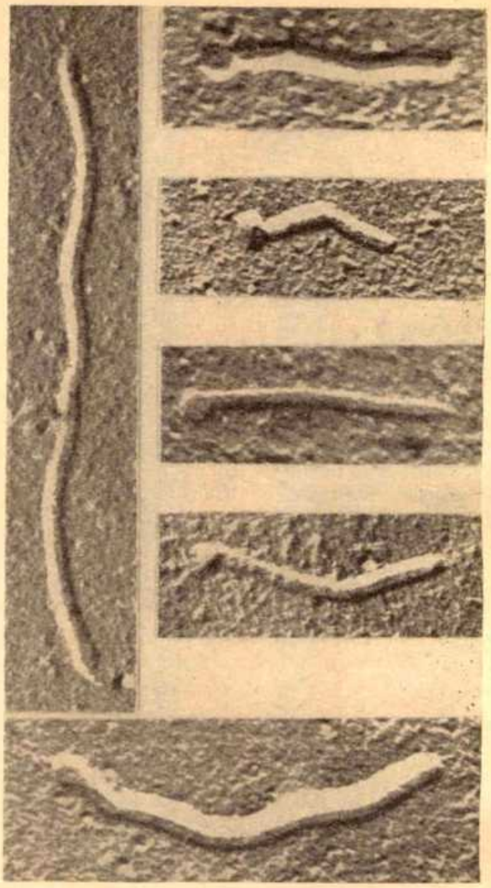

Fig. 1. Filamentous particles exactly resembling those of the influenza virus, observed in preparations of the *L*-form of *Haemophilus influenzae* induced by the action of penicillin. Note the enlarged terminal granule and obtuse-angled breaks. (Electron micrograph, gold–palladium shadowed, × 30,000)

Such a suggestion is not entirely original. Apart from the speculations, already referred to, on the nature of the association between the influenza bacillus and the influenza virus, a general theory of virus evolution from bacteria, by such a degenerative process has been suggested by Burnet[18] and others. There can also be observed the separate process of evolution of an almost virus-like condition in rickettsiæ, that appear to be, in origin, bacterial symbiotes of blood-sucking arthropods, adapted by this habitat to an existence in mammalian tissues. Such originality as we can claim is in the suggestion of a dynamic mechanism for the development of viruses and near-viruses of one type.

Experimental verification is not entirely lacking. Some strains of *Haemophilus*, and *L*-forms derived from other strains exhibit the hæmagglutination phenomenon characteristic of the influenza virus[7,19,20], and freshly isolated influenza viruses have been shown to include relatively large bodies, almost identical with the 'large bodies' of bacterial *L*-forms[21]. Lastly, one of us (P. E. P.) has succeeded in demonstrating the regular occurrence, in *L*-forms (or pleuropneumonia-like organisms) derived by exposure of *H. influenzae* to penicillin, of small filamentous particles that exactly resemble the filamentous particles of the influenza virus (Fig. 1). They are of similar dimensions, and most filaments have the regular, obtuse-angled breaks, one or more according to length, and the enlarged body at one pole (described as the 'warhead' by Burnet[18]), that characterize the authentic myxoviruses[22,23].

In the light of these observations, it is reasonable to speculate whether the greatest danger arising from indiscriminate use of antibiotics may not lie in the adaptation and selection of resistant strains of bacteria but in the artificial creation of new viruses.

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GENETIC CONTROL OF TOLERANCE TO TETRA- AND PENTA-CHLORONITROBENZENE IN *HYPOMYCES SOLANI* F. *CUCURBITAE*

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COLONIES of *Hypomyces solani* f. *cucurbitae* Snyd. and Hans. inhibited by tetrachloronitrobenzene (TCNB) and pentachloronitrobenzene (PCNB) often give rise to sectors which show relative insensitiveness to the presence of the inhibitor. (In the work reported in this article the 2,3,5,6 (also designated as 1,2,4,5) isomer of TCNB was used.) Regardless of the first appearance of the sector in the presence of the one or the other of the two fungistats, its subsequent growth shows tolerance to both, and such tolerance is not lost even after many transfers to inhibitor-free medium. TCNB is much more inhibitory to the wild-type *H. solani* f. *cucurbitae* than PCNB, while strains obtained from sectors are only slightly less tolerant to the first than to the latter compound. Therefore, scoring for tolerant versus sensitive is more clear cut when TCNB containing medium is used. Work reported previously[1] has shown that this tolerance is inherited in a Mendelian manner and that all the tolerant strains from sectors that were tested contain one gene for tolerance. This last conclusion, originally based on testing random ascospores, has since been confirmed by analysing the progeny of single asci: whenever a tolerant strain obtained from a sector was mated with a sensitive one of the opposite mating type and single asci were dissected, 4 of the ascospores of each ascus gave tolerant and 4 gave sensitive isolates.

Though considerable differences with respect to tolerance to TCNB were not observed among these tolerant strains, it was considered desirable to investigate whether or not they all contain the same gene for tolerance. In preliminary tests different tolerant strains were mated with other tolerants and random ascospores were tested. Though from many such crosses only tolerant ascospores were obtained, a considerable percentage of those obtained from some other crosses were sensitive. In the case of this latter category of crosses, sensitive ascospores were obtained from every one of a number of the resultant perithecia chosen at random. Therefore, the appearance of sensitiveness in the progeny of parents which are both tolerant could not have been due to an occasional protoperithecium or fertilizing conidium having lost its tolerant gene. It suggested that one of the parents contains a gene for tolerance which is non-allelic to the tolerant gene of the other parent.

A better understanding of this phenomenon was achieved by single ascus dissection. The spores of 45 asci taken from different perithecia which resulted when protoperithecia of the tolerant strain 12*a* were fertilized with conidia of the also tolerant strain 4*b* were analysed for tolerance to TCNB. Three kinds of asci were thus recognized (Fig. 1). In the first kind all the 8 ascospores from one ascus gave tolerant isolates. Only 6 of the 45 asci belonged to this category. Each of the 11 asci of the second kind found contained 4 tolerant and 4 sensitive ascospores. The remaining 28 asci were of the third kind, which gave 6 tolerant and 2 sensitive spores. Likewise, 4 of the 20 asci from the cross 5*a* × 4*b*, which were dissected, were of the first, 4 of the second, and 12 of the third kind. All the 3 kinds of asci can be found in the same perithecium. These results are in agreement with the hypothesis that the two parents, in each of the two crosses here, contain genes for tolerance at different loci. In such a case, the 4 tolerant ascospores of any ascus from such a cross segregating 4*T*:4*S* (as well as 2 of the 6 tolerant ones of any one segregating 6*T*:2*S*), though not more tolerant than each of the parents, should contain both genes.

To test this hypothesis further, the 4 tolerant ascospore isolates from ascus 21 of the cross 12*a* × 4*b*,

Table 1. SEGREGATION OF PROGENY FROM CROSSES BETWEEN TCNB-SENSITIVE STRAINS OF *Hypomyces solani* f. *cucurbitae* AND THE ASCOSPORE ISOLATES A_{43} AND A_{44}

Cross	Asci analysed	Non-segregating (8*T* : 0*S*)	Segregating 4*T* : 4*S*	Segregating 6*T* : 2*S*	Ratio *T*:*S*
9 × A_{43}	27	5	2	20	3·50
10 × A_{43}	8	2	1	5	3·57
9 × A_{44}	32	6	3	23	3·42
40 × A_{44}	7	2	1	4	3·66
	74	15	7	52	

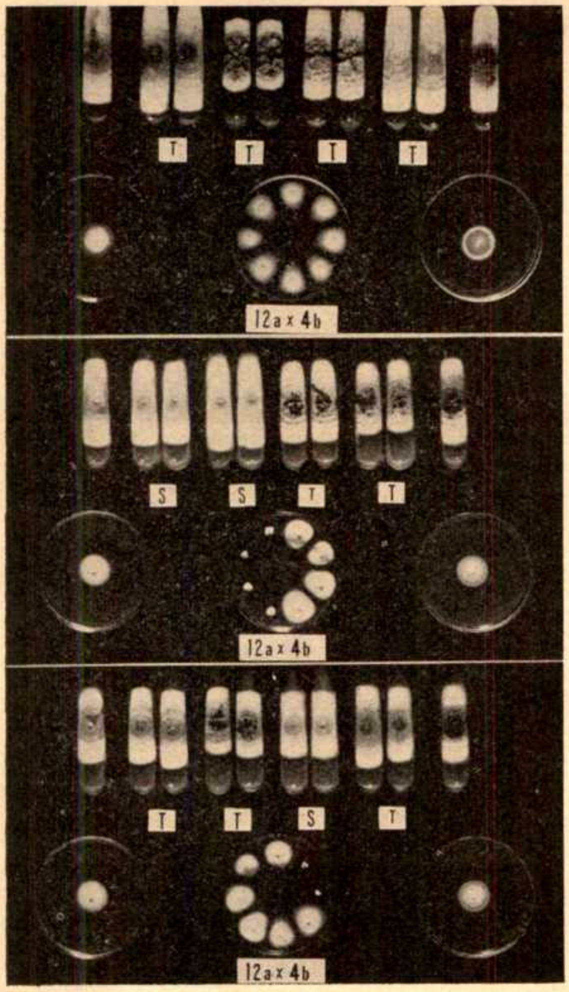

Fig. 1. The 3 kinds of asci produced from the cross $12a \times 4b$. Parents and progeny on PDA slants and TCNB agar plates. In each set of cultures on the left is strain $12a$, on the right strain $4b$, and between the two parents 4 pairs of isolates—the progeny of a single ascus. Top, $8T : 0S$; centre, $4T : 4S$; bottom, $6T : 2S$; T, TCNB-tolerant ; S, TCNB-sensitive

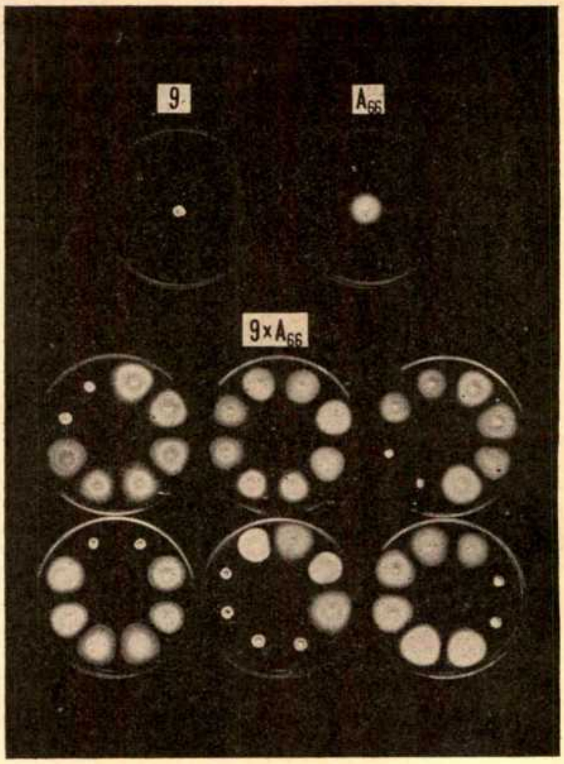

Fig. 2. Colonies on TCNB-agar of sensitive strain 9, tolerant ascospore isolate A_{66}, and the progeny of 6 asci from cross $9 \times A_{66}$. The colonies of 9 and A_{66} were started with single conidia. In each of the bottom 6 plates the 8 ascospores of one ascus were transferred

the progeny of which is shown in Fig. 1 (centre), namely A_{65}, A_{66}, A_{67}, and A_{68}, were chosen and attempts were made to mate them with different sensitive strains. Of these 4, the 2 sister ascospore isolates A_{67} and A_{68} were found to be neuters. Since strain $12a$ is a female and strain $4b$ a male, the appearance of neuters among the progeny is in agreement with previous findings[2,3]. The genetic results of crosses of isolates A_{65} and A_{66}, both of which are males and belong to compatibility group A, with sensitive strains are shown in Table 1. An illustration of the kind of segregation for tolerance to TCNB which such crosses give is given in Fig. 2. These results, together with those obtained from the cross $12a \times 4b$, show that each of the isolates A_{65} and A_{66} contain two genes for tolerance to TCNB and prove beyond any doubt that tolerance to this compound in *H. solani* f. *cucurbitae* is controlled by at least two different genes which operate independently of each other. The relative position of the two genes has not yet been determined. However, from both crosses, tolerant × tolerant and sensitive × double tolerant, the non-parental ditype ascus was produced quite frequently. This suggests that the two genes are probably on different chromosomes or different arms of the same chromosome.

I thank W. C. Snyder and P. St. Lawrence for advice.

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SUBJECTIVE VALUE OF A 'BIT' OF INFORMATION

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INFORMATION theory has had its chief impact on psychology in relation to studies of channel capacity and analysis. In this article we consider another psychological aspect of information theory, namely, the determination of the subjective value of a 'bit'.

(1) Subjects were given a page on which was printed a number of small circles. They were told the

number, and that the experimenter was thinking of a particular circle. Their task was to say how many questions they would have to ask in order to identify the circle for certain. Only questions which could be answered by 'Yes' or 'No' were allowed. Ten such pages were presented to each individual, the number of circles on any given page ranging from 2^1, by successive powers of 2, to 2^{10}. Half the subjects had pages with circles arranged regularly, and half with circles arranged irregularly, and there were four age-groups, namely, 9-year-old ($N = 79$); 12-year-old ($N = 61$); 15-year-old ($N = 93$); and 22-year-old ($N = 267$). The three younger groups were chosen from the brighter streams at school; the members of the oldest group were students at teachers' training colleges.

Theoretically, the number of questions which must be asked in order to locate any particular circle for certain is given by $\log_2 n$ (where n is the number of circles), that is, the number of 'bits' of information on the page. Actually the number of questions which our subjects believe to be necessary is in excess of this, and it increases logarithmically in all four age-groups, as the number of 'bits' increases. For example, at 8 'bits' of information (256 circles) the 9-year-old children require 70 questions; at 9 'bits' they require 89 questions; and at 10 'bits' they require no less than 450 questions. Fig. 1 shows the median number of questions plotted against the number of 'bits', for each age-group.

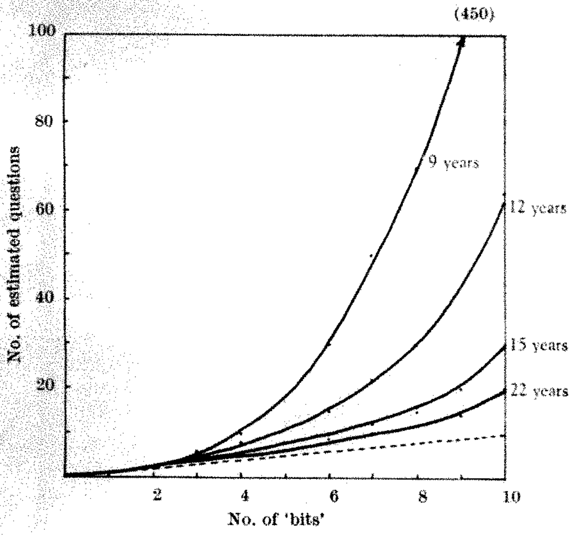

Fig. 1. Median number of estimated questions in relation to number of 'bits'

Over the entire range of 10 'bits', the mean number of questions per unit 'bit', at each age-level, indicates the extent to which the discrepancy between 'subjective' and 'objective' information steadily declines with age. This number may be regarded as a measure of subjective 'information' or, alternatively, of subjective 'uncertainty': at 9 years it is 13; at 12 years 4; at 15 years and thereafter it is approximately 2.

So far as responses to regular and irregular arrangements are concerned, the youngest group require about three times as many questions for the latter as for the former patterns. This difference is blurred in the older groups.

Every subject was also asked to say (*a*) what his first question would be, and (*b*) to mark the particular circle he thought the experimenter had in mind. In respect to (*a*) we found that 15 per cent of the 9-year-old children asked whether the given circle was located in one or other half of the page; in other words, they adopted a binary principle. The corresponding proportions in the other age-groups were 25, 65 and 54 per cent. Having regard to the fact that only one in two of the 22-year-old group realized the correct procedure, it is rather surprising that as many as one in six or seven of the 9-year-old children did so. In respect to (*b*) it might be supposed that every circle had the same 'chance' of being chosen. In fact, we find that 50 per cent of the subjects choose a circle from the 20 per cent of the circles clustered near the middle of the page.

(2) The foregoing results were obtained from group experiments. In addition, we examined 90 subjects individually on a more restricted range of 'bits', 30 aged 9, 24 aged 12, and 36 aged 15 years. Each individual was required to locate the particular circle by asking the experimenter appropriate questions. We are now in a position to make a comparison between the group and individual situations respectively, as in Table 1.

Table 1. MEDIAN NUMBER OF ESTIMATED (E) AND ACTUAL (A) QUESTIONS IN RELATION TO NUMBER OF 'BITS'

	No. of 'bits'							
	4		6		8		10	
Age	E	A	E	A	E	A	E	A
9	10	6	30	8	70	13	450	17
12	8	5	15	9	30	13	64	15
15	5	5	10	8	15	10	30	13

The main outcome is that the median number of questions actually asked is considerably less, at each age, than the corresponding number of estimated questions to be asked, obtained in the group experiments. This suggests that performance in a task such as this cannot be invariably judged from an estimate in advance.

At all three age-levels a small, but significantly larger, number of questions was required for the irregular as compared with the regular arrangements.

Over the range of 4–10 'bits', the mean number of actual questions per unit 'bit' is 1·6 at age 9; 1·5 at age 12; and 1·3 at age 15. The reciprocals of these values represent the mean proportions of a 'bit' eliminated at each question.

Inspection of the results, together with observation of the subjects at their task, shows that any given strategy which they adopt is not necessarily sustained unchanged from beginning to end. Furthermore, the strategy for a residual number of 'bits' is not necessarily equivalent to the strategy for the same number of 'bits' facing the subject *ab initio*. For example, the final 16 circles, when n initially is 1,024, are not invariably searched in the same manner as when n is initially 16. A random search may become systematic or vice versa.

These experiments raise a number of further problems for research; for example, the determination of the subjective value of a 'bit' at much larger values of n, the rate of work, the effect of presenting information in other forms and modalities, and a more detailed scrutiny of strategies at different ages in tasks of varying complexity, and at different stages of the same task.

LETTERS TO THE EDITOR

COSMOLOGY

Finite Universe and Mach's Principle

RECENTLY, Ozsváth and Schücking found an exact solution of Einstein's field equations of gravitation, which represents an instructive new model of a universe (finite rotating universe)[1]: Although in the entire cosmos matter is at rest relative to a suitably chosen co-ordinate system, nevertheless everywhere Coriolis forces do exist. This property of the Ozsváth–Schücking model is common to a solution already found by K. Gödel in 1949[2]; whereas Gödel's solution represents a universe of infinite space extension and, besides, closed time-like world lines exist, the Ozsváth–Schücking model represents a spatially finite universe in which time-like world lines cut the closed space like hypersurfaces only once. Therefore, no reasons exist for excluding this model as physically meaningless.

Ozsváth and Schücking call their world model explicitly an 'anti-Mach solution', because the inertial compass and the light compass do not coincide. They have constructed their model with the intention of disproving the 'creed', that in a finite closed universe Mach's principle is necessarily being fulfilled (*BFH* version of Mach's principle).

According to the interpretation of the O.–S. model given by us in Freiburg/Br., this solution deals with a generalization of the static Einstein cosmos, to which a circulating gravitational wave is superimposed. Since the gravitational wave is transporting energy and momentum, it gives rise to a Coriolis field (similar to the momentum density of matter), although matter is at rest everywhere.

This physical meaning follows immediately, if one writes the line element $\mathrm{d}s^2 = g_{\mu\nu}\,\mathrm{d}x^\mu\,\mathrm{d}x^\nu$ of the given solution in the following way ($x^4 = ct$):

$$g_{\mu\nu} = \left(\frac{R}{2}\right)^2 (\overset{0}{g}_{\mu\nu} + k\overset{1}{g}_{\mu\nu} + k^2\overset{2}{g}_{\mu\nu})$$

whereby

$$\overset{0}{g}_{\mu\nu} = \begin{pmatrix} -1 & 0 & 0 & 0 \\ 0 & -1 & -\cos x^1 & 0 \\ 0 & -\cos x^1 & -1 & 0 \\ 0 & 0 & 0 & \left(\frac{2}{R}\right)^2 \end{pmatrix}$$

$$\overset{1}{g}_{\mu\nu} = \begin{pmatrix} -\cos\Phi & \sin x^1 \sin\Phi & 0 & 0 \\ \sin x^1 \sin\Phi & \sin^2 x^1 \cos\Phi & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{pmatrix}$$

$$\overset{2}{g}_{\mu\nu} = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & -2\cos^2 x^1 & -2\cos x^1 & 2\alpha\cos x^1 \\ 0 & -2\cos x^1 & -2 & 2\alpha \\ 0 & 2\alpha\cos x^1 & 2\alpha & 0 \end{pmatrix}$$

The following abbreviations have been used:

$$\Phi = \alpha ct - 2x^3,\ \alpha = \frac{2}{R}\sqrt{\frac{2}{1-4k^2}}$$

(Φ phase, k means a parameter with $|k| < \frac{1}{2}$; $k = 0$ corresponds to the Einstein cosmos, R is the curvature radius of the universe). Regarding k as a small quantity ($|k| \ll 1$), the gravitational wave $k\overset{1}{g}_{\mu\nu}$ represents a first order disturbance of the Einstein cosmos, the time average of which being equal to zero. First in the second approximation according to $k^2\overset{2}{g}_{\mu\nu}$ we get a time-independent deformation of the Einstein cosmos. This interpretation is still valid if $|k|$ is no longer small against one.

As the calculation shows the momentum components of the gravitational field t^1_4 and t^2_4 are equal to zero. Since the 'basic metric' $\overset{0}{g}_{\mu\nu}$ makes no contribution to t^i_4 ($i = 1,2,3$), energy propagation of the gravitational wave is therefore determined, and that is in accordance with phase propagation:

$$\mathrm{d}x^1 = \mathrm{d}x^2 = 0,\ \mathrm{d}x^3 = \frac{\alpha c}{2}\,\mathrm{d}t$$

Thus the 4-velocity of energy-momentum propagation becomes:

$$u^\mu = \frac{\mathrm{d}x^\mu}{\mathrm{d}s} = \sqrt{\frac{2(1-4k^2)}{1-2k^2}}\left\{0, 0, \frac{\alpha}{2}, 1\right\} \quad (u_\mu u^\mu = 1)$$

The wave-length of the gravitational wave is equal to 1/4 of the circumference of the universe.

The 4-velocity of energy and phase is time-like, and therefore it is possible to introduce a co-ordinate system, in which the $g_{\mu\nu}$'s become independent of t. For that reason one may call this model 'stationary' (similar to the fact that an electromagnetic wave passing a dispersive medium with a velocity $< c$ may be transformed to a stationary condition). u^μ is a time-like and geodesic killing-vector. Corresponding to this, the $g_{\mu\nu}$ have constant values along the stream-lines of energy.

According to the field equations of gravitation, the Coriolis forces are uniquely determined by the energy-momentum components of the gravitational wave (boundary conditions do not exist). The field intensity ω of the Coriolis force is constant in the whole cosmos:

$$\frac{\mathrm{d}\vec{v}}{\mathrm{d}t} = 2\cdot\vec{v} \times \vec{\omega},\quad \omega = \alpha c\,\frac{k^2}{\sqrt{1-k^2}}$$

Surely ω is so small (in the order of the circulation frequency of the light around the universe, $R/c \simeq 10^{10}$ years) that one cannot think of an experimental proof even under extreme conditions.

We are of the opinion that Ozsváth and Schücking's interesting world model does not contradict Mach's principle, and especially not the idea that a finite and closed world model is in accordance with Mach's principle. Certainly it will not be possible to uphold the dogmatic form of Mach's principle containing the exact accordance of kinematics and dynamics since each gravitational wave (like all other disturbances of a homogeneous and isotropic metric field) shows a tendency to become self-consistent. In interpreting Mach's principle as a selectional principle for world models the accordance of kinematics and dynamics has to be postulated only on a large scale, which might be satisfied by all finite closed world models[3].

A detailed paper on this subject will appear in the *Zeitschrift für Physik*.

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[1] Ozsváth, I., and Schücking, E., *Nature*, **193**, 1168 (1962).

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IN our previous communication to *Nature* we gave an exact solution of Einstein's field equations contradicting a certain well-defined version of Mach's principle (*BFH* principle). Dehnen and Hönl now state "that a finite and closed world model is in accordance with Mach's principle (*BFH* principle)".

But our example showed that a finite and closed world model may not be in accordance with *BFH*.

Dehnen and Hönl write in their following sentence: "Certainly, it will not be possible to uphold the dogmatic form of Mach's principle". The 'dogmatic form' is the *BFH* version, which postulates "the exact accordance of kinematics and dynamics". Therefore, this would seem to contradict their first statement.

It may be that Dehnen and Hönl now put their faith in another version of Mach's principle. But so far we have not seen a precise mathematical statement of such a new form of this evasive principle. We do not agree with Dehnen and Hönl's interpretation of our solution. Their arguments depend completely on the co-ordinate system used, are not invariant and therefore inconclusive.

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GEOPHYSICS

An All-Sky Photometer

DURING the period of the International Quiet Sun Year, better opportunities may be found for the examination of airglow in the middle latitudes at a time when little or no auroral contamination is expected. With the view of measuring the intensities of the night airglow radiations emitted from the whole dome of the sky, a simple photoelectric type of photometer has been designed and built, in the Upper Atmosphere Research Section of the National Research Council, Ottawa, Canada.

The basic objective is to provide a kind of index of the airglow intensity at specific wave-lengths. The instrument will be operated on all clear moonless nights to obtain the hourly or half-hourly 'airglow index' for the station. The all-sky photometer basically differs from the conventional airglow photometer in that the former measures the intensities up to 80° zenith angle and at all azimuths (about 10° from the horizon are cut due to the tree tops on the site), while the latter usually covers a narrow angle and hence has to scan the sky to obtain complete information. It is agreed that the scanning type of photometer, subtending narrow angle, can reveal a great deal of information regarding the finer and detailed structure of the airglow. With the present apparatus, however, it is felt that a measure of the integrated intensity over the whole dome of the sky will be a useful factor in understanding some processes in the upper atmosphere.

The basic assembly of the electric clock, the convex mirror and the stand which holds the camera unit, is that of the all-sky auroral camera[1]. The camera unit is replaced by a cylindrical housing containing an end-on type (EMI 9528*B*) photomultiplier tube, a filter disk with three filters and a blank in the four quadrants of the disk, a 6 r.p.m. motor to rotate the filter disk and a cam-microswitch assembly to position the filters over the cathode of the photomultiplier.

The clock-timer mechanism is located in the base of the instrument. Every 5 min it supplies a pulse to the filter-disk motor which rotates the disk 90° in 2·5 sec. The photometer is turned on and off automatically by the clock at preset times. When the photomultiplier tube is turned off, the motor continues to operate until the blank segment is in front of the photocathode. Furthermore, a phototransistor circuit is included that can be adjusted to turn the photomultiplier off when the ambient illumination exceeds a given value.

The filters used in the present apparatus are shown in Table 1.

Table 1

Filter centred at (Å):	Half-width (approx., Å)	Peak transmission (per cent)
6300	40	73
5577	48	55
5400	46	55

The filters turn in the following sequence, 6300 Å, 5577 Å, 5400 Å and 'blank'.

The intensity of the airglow is recorded on a paper chart for 5 min in each wave-length band. The blank space in the disk permits recording of the zero intensity. A complete cycle is repeated every 20 min. In order to accommodate a wide range of intensity, say due to an aurora, the output of the photomultiplier tube is recorded as the logarithm of intensity of the incident light falling on the photocathode. At present, the full-scale recording covers a range of 10^5 in intensity, which may be reduced to 10^2 or 10^3 for convenience.

Fig. 1 gives a sample record obtained with the all-sky photometer on the night of June 27/28. Time shown on the record is E.S.T. Radiation intensity is recorded from sunset to shortly before sunrise. It

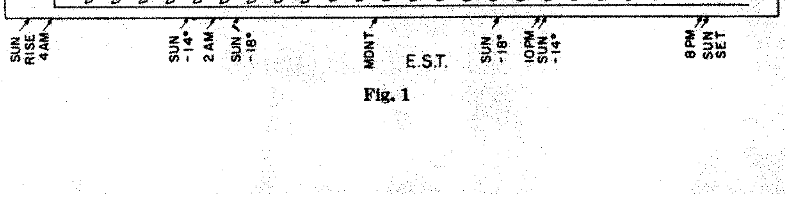

Fig. 1

may be assumed that the night conditions are almost reached at 0957 p.m. when the Sun is 14° below the horizon and the night prevails until the Sun is in the similar position on the morning side.

Although no absolute calibration is given, it is quite striking to note that the relative intensities in the three wave-lengths show a wide variation with time. Independent variation of the individual radiations can also be observed. Throughout the night 5400 Å radiation shows random and small fluctuations. 6300 Å radiation shows higher intensities at the beginning and end of the night, with a minimum around 0020 E.S.T. The 5577 Å radiation shows some decrease in intensity in the early part of the night with a distinct maximum at about 0045 E.S.T. and falling off again towards dawn. The ratio of maximum to minimum intensity of 5577 Å radiation during the night is 2·3 : 1. Photometers subtending narrow angles have also shown maxima of intensity around midnight, both at the middle latitudes[2] and in the tropics[3].

Table 2

	Beginning of night	When I_{5577} is maximum	End of night
$\frac{I_{5577}}{I_{6300}}$	4·5	8·9	4·75
$\frac{I_{5577}}{I_{5400}}$	4·3	8·4	5·3

Table 2 gives the ratios of intensities of I_{5577}/I_{6300} and I_{5577}/I_{5400} for the three epochs of the night; (1) at the beginning of the night; (2) at the time when 5577 Å radiation had maximum intensity; (3) at the end of the night.

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Fitting the Continents

THE discussion[1] by Dr. C. H. Barnett and Sir Harold Jeffreys calls for comment. First, the original suggestion that the Earth might be expanding, on the basis of experimental fit of the 200-m depth contours of the continental shelves, was made by O. C. Hilgenberg in 1933[2]. Further, Sir Harold Jeffreys's objection that there is too much implied distortion can be met. The pseudo-viscosity of the crust[3,4] and observed distortion by shear[5,6] would lead one to expect continental distortion in any event; and the outline of the continental margins may have changed in geological time, by accretion of the continents[7,8], by loss of continental crust[9], or both.

These points must be borne in mind when seeking evidence for or against Earth expansion and continental drift.

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GEOLOGY

Kenya Ignimbrites

IN a recent communication[1], Dr. G. J. H. McCall gave his views on some ignimbrite-like rocks found in the Kenya rift valley. The full details of these rocks, particularly those in the Menengai area, are awaited with great interest as they should prove to be a valuable contribution to the subject of ignimbrites and ignimbrite-like rocks. Some comments are called for in the light of an investigation[2–4] made on similar rocks in the area immediately to the east of that studied by Dr. McCall.

The hypothesis that the lenticles (or lithophysæ) are due to a drawing out by flow of formerly continuous laminæ cannot be accepted for two reasons. The first is that the lenticles would show a preferred direction of elongation when viewed in the foliation plane if they were due to a drawing out by flow; in the same way that gas vesicles drawn out by flow in a normal lava show elongation in both horizontal and vertical sections. Such an elongation has not been noticed in any of the several hundred specimens of Kenya ignimbrites examined. The second reason for rejecting Dr. McCall's explanation of the origin of the lenticles is that it fails to account for the vertical transition of the lenticles from lens-shaped vesicle-free blebs near the base of a flow unit, to equidimensional pumiceous blocks near the top; all transitions between these two extremes being present in a single unit. This transition is particularly well seen in the ignimbrite which forms the bank of the Melawa River 5 miles south-east of Ol Kalou. This ignimbrite also shows a thick development of incoherent unwelded ash as its upper member, which is indistinguishable from normal vulcanian ash. Such a deposit could not have been produced by the collapse of a lava froth. It is noteworthy that the vertical zonation of the ignimbrites in this region can be matched with those described by Marshall[5] and more recently by Martin[6] from the type locality for ignimbrites in North Island, New Zealand.

The xenoliths, which Dr. McCall attributes to the break-up of a brittle solidified lava, are common features of Kenya ignimbrites. However, in any one flow, many different kinds of lava may occur as xenoliths and they can sometimes be matched with underlying lavas. In the case of the Gorge flow of the Lower Oramutia volcanics[4] it is possible to relate the distance of the ignimbrite from its presumed source to the increase in xenolithic debris. Besides lava fragments, clods of earth and weathered lava have been found in many Kenya ignimbrites. There is obviously no reason for abandoning the orthodox view that the xenoliths are fragments of country rock torn off during the eruption or during transport above ground-level.

One of the Pleistocene 'ravine flow' occurrences mentioned by Dr. McCall is the subject of a recent paper[4]. Dr. McCall describes these as 'watery'. Does 'watery' in this context mean a high water content or an exceptionally low viscosity? It is important to realize that these 'ravine flows' are of two different kinds: ignimbrites with a texture suggestive of coalesced liquid blebs and true froth lavas. The latter are of low density and closely resemble pumice tuffs.[4]

Composite flows showing eutaxitic structure and normal lava in one unit are apparently restricted to Menengai crater. Most of the ignimbrites occur in the main body of the Kinangop tuffs. However, the phonolite and phonolitic trachyte flows in the Ol Kalou–Ol Joro Orok region sometimes show local development of ignimbrite-like structure on their upper surfaces. This would seem to be due to fluidized systems breaking out on the surface of volatile rich lavas. Some process akin to the second order *nuée ardentes* described by Cotton may be responsible[7]. Recently, Molloy and Kerr[8] described reconstituted flows of apparently normal lava derived from air-fall material. Has Dr. McCall fully considered this possibility for the Menengai occurrence ?

Dr. McCall's dissatisfaction with the term 'welding' is shared by me, although for rather different reasons. Welding implies a softening of solid bodies by heat, causing them to stick together. Many of the more massive Kenya ignimbrites show relict shard structure only in the upper and lowermost parts of the flow unit. The central part is composed of an extreme kind of eutaxitic structure (parataxitic) or by a kind of 'blobby' structure in which no definite outlines can be discerned. It is thought that the shards in these rocks were liquid at the time of deposition and that they coalesced as the gas cushion leaked away. Near-solid or plastic shards produced the zones with typical eutaxitic structure. In some cases the eutaxitic mass appears to have been invaded by the molten glass derived from the coalesced molten shards, so producing a two-phase system of shard glass in matrix glass. The operative process in these rocks is coalescence and not welding. The presence of two glasses was wrongly attributed to liquid immiscibility by Steiner[9,10].

It may be concluded from the foregoing remarks that there is no reason for giving the Kenya ignimbrites a totally different origin to the ignimbrites elsewhere. A form of Marshall's[5] original *nuée ardente* theory modified along the lines suggested by Fitch[11,12] and others is adequate to explain all the textures and structures seen in these rocks.

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Mr. C. M. Bristow's pertinent comments on my communication dealing with froth-flows in Kenya underline a fundamental difference in interpretation of these rocks between me and geologists of the Ministry of Works, Kenya. The latter have attempted to fit the evidence from this field into a framework based on the orthodox concepts of ignimbrite genesis—following Fenner[1], Marshall[2], Mansfield and Ross[3], and Gilbert[4]. I have come to the conclusion that certain features of the flows in question are incompatible with these concepts: and that some departure from the concept of ignimbrites (as clearly defined by Marshall[2]—products of deposition from immense clouds or showers of intensely heated but generally minute fragments of volcanic magma) is necessary, in this case. Grange[5], working in the same field as Marshall, remained unconvinced that the process suggested by Marshall had been responsible for the unusual rock types, and preferred to invoke an origin in effusive processes, and Steiner[6] has lately invoked such processes to explain anomalous features in certain rocks of this field. Even the foremost, active exponents of welding and compaction (lithostatic load) processes, Ross and Smith[7,8], have come a long way from Marshall's original concept. The description of an eruption of Cotopaxi quoted by them[7] as possibly a typical 'ash flow' eruption suggests effusion rather than the process envisaged by Marshall. This eruption could equally well have represented vesiculation, expansion and partial or complete rupturing in a rising magma column and effusive flow, quite independent of welding processes —a froth-flow eruption as envisaged by Kennedy[9] and Boyd[10].

I have suggested that, in the Kenya field, there is evidence that processes other than welding of sticky, hot fragments can produce a rock characterized by *fiamme*, and that it is even possible that the evidence from this field indicates a need for the complete reappraisal of the concept of welding and compaction—a concept already questioned (either directly or by implication) by Kennedy[9], Hausen[11], Steiner[6] and Boyd[10].

The idea of welding seems to have stemmed from the assumption that coherent, indurated and fragmental volcanic rocks, resembling lavas and in which glass shards were conspicuous, must of necessity have been derived from tephraitic products—fragments which had undergone air-travel in a dispersed state—by redeposition and reconstitution in a coherent mass. Nowadays, it is becoming apparent that pyroclastic material derived from magma ruptured by extreme vesiculation and autoclasis can flow, both before extrusion, and over the surface in the form of overland avalanches. Further, Steiner[6] has logically concluded that shards can stem from processes of autoclasis within partly cooled magma. The basis for considering shards and other fragments within a tabular, coherent body, resembling a lava flow, as of necessity deposited from clouds or showers may thus be questioned. The advocates of ash-flow, while retaining the idea of welding related to compaction, have moved towards the idea of overland flows. I go a stage further and question whether there is any real need to invoke welding at all ? The alternative froth-flow hypothesis allows for gradations, within the limits of single flows, from well-crystallized lava, to banded glassy lava, to mottled eutaxitic and partly fragmental lava, and even to incoherent, pulvurulent material. The welding hypothesis invokes a reverse process, fragmental material derived from rupturing processes in a gas-exsolving magma being restored to a cohesive state. There are cogent objections to this hypothesis on physical grounds, and it involves a more complex sequence of events than the froth-flow hypothesis.

Bristow accepts the fact that there are, in this field, structurally composite flows—part well-crystallized or glassy lava, part eutaxitic material indistinguishable from his 'ignimbrites'. He suggests an origin in *nuée ardente* activity. I differ in believing that these phenomena have nothing to do with *nuées ardentes*, and in believing that they provide the key to the problem of genesis of these eutaxitic rocks. Though they have been examined in especial detail in Menengai, such structurally composite flows are widespread, occurring particularly where phonolitic-trachyte rather than quartz-trachyte has been erupted—though also present to a limited extent in the latter setting. The recorded transitions from flow-banded lavas characterized by vesicular swells or lithophysæ within the laminæ to discontinuous, eutaxitic patterns in which the lithophysæ appear as shreds or *fiamme* (transitions recognized within the limits of single hand specimens) seem difficult to relate to observed *nuée ardente* eruptions, low-temperature phenomena, which have given rise to unsorted avalanche deposits never yet observed to be welded. There is strong reason to believe that the mechanism of *nuées ardentes* primarily involves entrapped air, not gas exsolution[12], and the latter process is clearly evidenced in the eutaxitic portions of the composite flows. There is no reason for believing that the material forming the fragmental, eutaxitic layers in structurally composite flows ever passed through a stage of air-travel in a dispersed state, or that it was ever completely incoherent.

The zonation seen throughout the thickness of these flows is not, in my view, as regularly progressive as in the example cited by Bristow. It is true that obsidian blebs or *fiamme*, devoid of vesiculation cavities, tend to occur at the base of flows, but I interpret this as a chilling phenomenon, the volatiles in the lava having congregated, but vesiculation prior to solidification having been negligible. Such *fiamme* also occur, however, near the upper surface of flows. Transition from coherent to incoherent material is not only seen at the tops of the flows—bands of incoherent, pulvurulent material have been observed near the base of some flows. The ash referred to by Bristow may not be a vulcanian shower deposit: it is my contention that the bulk of the so-called pumice tuffs in this field are pumice avalanche deposits, products of extreme vesiculation and rupturing—collapsed bubble-froth flows—and such material may well be found in continuity with the so-called ignimbrite within a single flow. The most convincing evidence that the flattening of the *fiamme* is not connected with compaction (lithostatic load) effects is to be found in the fact that such flattened *fiamme* are to be found at the top surface of flows, and also in very attenuated flows, in both cases where nothing has ever been superincumbent.

In connexion with these *fiamme*, Bristow's point of criticism concerning flow-lineations is interesting. It is true that no regular lineation appears on the upper surface of cubes cut from hand-specimens (with upper and basal surfaces parallel to the foliation plane). What is apparent is a swirling pattern, a distinct arrangement of sinuous linear trains of *fiamme*, other fragments, and felspar phenocrysts. This flow structure (if such it be) seems to be primarily a flow-foliation, the flow having been strongly laminar, but the fragments, *fiamme* and phenocrysts moved turbulently in the lamination planes without any strongly preferred direction. Usually side-faces of these cubes show a greater elongation of the *fiamme* on one face than on the other. It is not unknown for vesicles in lavas to reflect a similar combination of primary foliation and lineation: a specimen of nephelinite in my own possession shows strongly aligned amygdales—the structure expected by Bristow—but on the side faces of the cube it is seen that these are both elongated and flattened; that is, they are drawn out in both horizontal directions.

That some of the well-crystallized material present as angular 'xenoliths' in these flows is accidentally accrued is accepted by me. It is also agreed that all these enclaves appear as if more weathered than the host, but this does not mean, necessarily, that they were weathered before incorporation in the flow. The consistent presence of numerous, abruptly defined enclaves of crystalline material in the eutaxitic rocks would, however, seem to require an explanation involving the essential process of genesis of these rocks—particularly as these inclusions show some degree of sorting as to size, and frequently are more evident in the higher levels than the lower levels of the flow. Petrographic evidence indicates that the majority of these are nothing more than sharply defined areas of complete crystallization in the flow, either *in situ* or to some extent moved within the flow in the manner of rafts. In any event, it is agreed that these are not the fragments of the cloud or shower that is progenitor to the ignimbrite: Where then are those fragments?—if the *fiamme* are actual or embryonic vesiculation areas showing considerable uniformity with their neighbours, as I believe, and not pyroclastic fragments.

Reconstitution and processes involving 'liquid shards' have been invoked to explain relationships such as I have described. I regard these hypotheses as involving excessive 'stretching' of the original welding hypothesis to fit the evidence. Certainly, reconstitution is difficult to accept in the case of the almost holocrystalline, intersertal/intergranular textured parts of the Menengai composite flows. I consider that their similarity of texture with many nearby lavas, and the temperature required for such a crystallization, strongly support an origin in direct crystallization from a cooling silicate melt.

I have had no experience of rhyolite ignimbrite fields: while I accept the fact that there is, in these fields, a wealth of indirect evidence which has led geologists to formulate ideas of welding related to compaction, and of reconstitution, I know of no criterion which distinguishes, unequivocably, whether a particular rock is the product of partial fragmentation or of complete fragmentation followed by partial reconstitution. But while this has led me to question the concept of welding, I consider it to be a mistake, perhaps, in the present state of knowledge, to be too dogmatic, and to conclude that any worker in this or any other field is completely in the wrong. It is clear that the term ignimbrite will continue to be preferred by those who, like Bristow, adhere to welding concepts, but equally, I consider that Marshall's definition of the term makes it impossible for me to apply it to any of the rocks which I have studied in this field. The term 'ash-flow' would be acceptable allowing for the fact that in this case it would be applied to effusive rocks in which no welding is believed to have taken place, and which may never have passed through a fully incoherent stage.

The occurrence of rocks of 'ignimbrite' aspect as repeated intercalations in sequences of marine sediments is not unknown and such relationships present a problem, since it is difficult to believe the 'ignimbrite' mechanism could operate under water. Repeated emergence coinciding with volcanic outbursts or some other such unlikely process has to be invoked. The froth-flow hypothesis is one which incorporates a process equally compatible with terrestrial or submarine eruption.

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PHYSICS

Radiation Enhancement following Johnston Island Thermonuclear Explosion

We wish to report the observation, made at Hobart, Tasmania, of an enhancement in the counting-rate of a balloon-borne Geiger counter at the time of the high-altitude thermonuclear explosion above Johnston Island on July 9, 1962.

The counter, designed for operation at the temperatures encountered in night flights of balloons (down to − 60° C), was 10 cm long and 4 cm in diameter. Thicknesses of the glass wall and internal nickel cathode were 1 mm and 0·1 mm respectively. At the time of the explosion, 0900 U.T., the balloon carrying this counter (mounted horizontally) and associated circuitry had reached an atmospheric depth of 80 g cm^{-2} and was rising in altitude at the rate of 2·5 g cm^{-2} min^{-1}. The balloon burst prematurely at 0927 U.T.

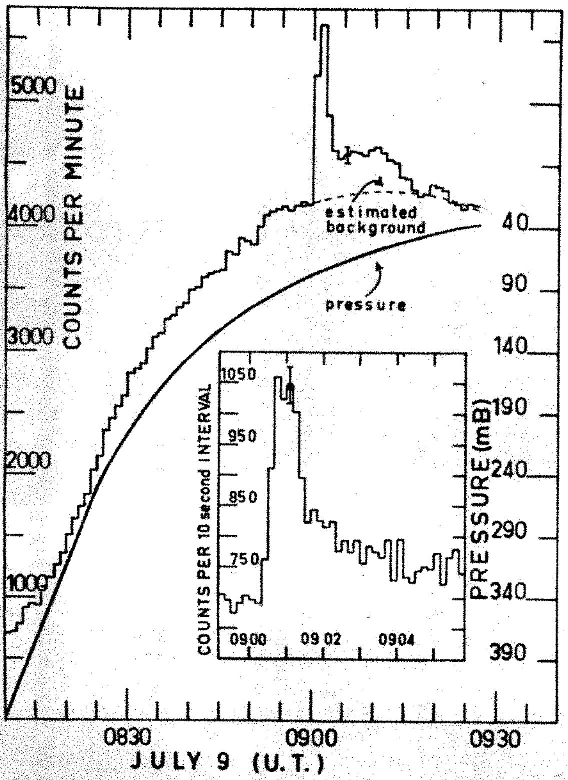

Fig. 1. Main diagram, 1 min count totals from balloon-borne Geiger counter and atmospheric pressure level versus time; inset, variation of 10-sec count totals through time of Johnston Island explosion (0900 U.T.)

Fig. 1 summarizes the observations. The maximum error in timing is estimated at ± 2 sec.

Significant features of the event are the considerable atmospheric depth at which it was observed, the delay of between 20 and 30 sec between the instant of the explosion and commencement of the enhancement, and the peak intensity (averaged over 10-sec intervals) of about 50 per cent above the normal background-level near the Pfotzer maximum of the cosmic ray transition curve. There seems little doubt that recovery to the normal background intensity was not complete until at least 0915 U.T. No effect was observed by cosmic ray neutron monitors and meson telescopes at ground-level in and near Hobart.

It is clear that if the enhancement were due to protons incident vertically at the top of the atmosphere their minimum energy was 350 MeV. However, the line of force through Hobart (geographic coordinates 43° S., 147° E., geomagnetic latitude 51° S.) crosses the equatorial plane at 2·5 Earth radii, and thus passes outside the region in which protons of energy exceeding 70 MeV were observed by *Explorer VI*[1]. The possibility that other types of radiation were responsible for the enhancement must be examined, but we consider that an attempt at this time to interpret the observations from the information available to us would be premature.

We wish to acknowledge the assistance of Mr. H. E. G. Dyer, who manned a duplicate radio telemetry station.

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Laboratory Test of the Finlay-Freundlich Red Shift Hypothesis

A new round of discussion and experiment has been set off by a recent suggestion made by A. Ward[1]. He points out that recent developments in physics make possible a laboratory test of a proposal by E. Finlay-Freundlich[2] for an alternative interpretation of the galactic red shift. The Finlay-Freundlich hypothesis was based on anomalies in various stellar red shifts and suggested that these, as well as the Hubble–Humason red shift in galactic spectra, commonly interpreted as Doppler shifts in an expanding universe, are in fact the result of loss of energy by observed photons traversing a radiation field. No generally accepted physical mechanism has been proposed for this loss. It may be noted that an as yet unknown photon–photon interaction has been suggested in which the energy lost somehow reappears as lower frequency radiation, or alternatively, it has been proposed that the energy lost reappears as neutrino pairs resulting from the exchange of a graviton between two photons[3]. The empirical relation first offered by Finlay-Freundlich may be written as:

$$\frac{\Delta\lambda}{\lambda} \cong 0{\cdot}26 \times 10^{-14} \int \rho_{\text{rad}}\, dl \qquad (1)$$

where ρ_{rad} is the energy density in ergs/c.c. of the radiation field traversed by the red-shifted photon, and l is the path length from source to observer. The Finlay-Freundlich hypothesis was attacked strongly when it was made. M. A. Melvin[4] and others have emphasized the seemingly serious astronomical counter-evidence, particularly binary star data assembled by H. L. Helfer[5]. While holding no brief for the relation, Melvin nevertheless revaluated the numerical constant in equation (1) to obtain a better representation of the various data on which Finlay-Freundlich based his relation. The improved version of equation (1) due to Melvin is smaller by 10^{-3}:

$$\frac{\Delta\lambda}{\lambda} \cong 0{\cdot}26 \times 10^{-17} \int \rho_{\text{rad}}\, dl \qquad (2)$$

The proposal made by Ward was to verify or disprove equation (2) by an experiment combining the high sensitivity for resonant absorption associated with the Mössbauer effect with the high-radiation fields in a laboratory device for thermonuclear fusion studies.

Recently two types of experiments have been reported by R. Weiss and L. Grodzins[6] in which the 14·4-keV γ-radiation from a cobalt-57 Mössbauer source was passed through a radiation field. In one the field was that of an x-band RF cavity 1 m long, with a radiation density of 7×10^{-2} ergs/c.c., while in the other γ-rays traversed a 1 m long oven containing helium at 1,300° C, with a radiation density of $2{\cdot}1 \times 10^{-2}$ erg/c.c. In the x-band experiment it was found that the wave-length shift, if any, could not be larger than $-(0{\cdot}4 \pm 5{\cdot}6) \times 10^{-16}$, while in the oven experiment the upper limit was determined to be $+(1{\cdot}5 \pm 5) \times 10^{-16}$.

Weiss and Grodzins point out that these upper limits are substantially less than the original Finlay-Freundlich hypothesis, equation (1), as indeed they are. Thus, equation (1) would predict $\Delta\lambda/\lambda \cong 1{\cdot}8 \times 10^{-14}$ and $\cong 0{\cdot}55 \times 10^{-14}$, in the two cases, and the original hypothesis is certainly disproved. However, equation 2 would give $\Delta\lambda/\lambda \cong 0{\cdot}18 \times 10^{-16}$ and $\cong 0{\cdot}055 \times 10^{-16}$, shifts the values of which lie within the experimental error of the upper limits obtained by Weiss and Grodzins. Since one might better use equation 2 to test this effect, if one is going to make a laboratory test at all, then one is led to the unfortunate conclusion that the otherwise excellent Weiss–Grodzins experiments are not definitive after all.

It is the further purpose of this communication to suggest the possibility of using an optical maser to provide the radiation field through which to pass the photons the wave-lengths of which are to be observed. Line-widths for the 14·4 keV γ-ray from iron-57 have been found experimentally to be $\sim 1{\cdot}8 \times 10^{-8}$ eV (equivalent to a Doppler shift of 0·04 cm/sec or a $\Delta\lambda/\lambda = 1{\cdot}3 \times 10^{-12}$). The radiation field which the optical maser must provide is given by

$$\rho_{\text{rad}} = \frac{1{\cdot}3 \times 10^{-12}}{0{\cdot}26 \times 10^{-17}} f = 5 \times 10^{5}\, f \text{ ergs/cm}^3 \qquad (3)$$

where f is the experimentally desired fractional shift of the γ-ray line-width.

Consider now a light pulse (assume it to emerge uniformly) from a 1/4-in. diameter ruby laser with a power-level of 10 MW, duration 10 mμsec (a commercially available arrangement). The radiant energy is then contained approximately in a cylindrical region of 0·3 cm² area, 300 cm long, with a resultant energy density of $\sim 10^4$ ergs/cm³ (equivalent to $3{\cdot}5 \times 10^4$ °K for black-body radiation). If one settles for observing a shift equal to 1 per cent of the line-width, then path-lengths for the γ-ray through the radiation field of the order of 0·5 cm suffice. If a geometry could be arranged so that the laser pulse and the γ-ray propagation directions were essentially parallel, then shifts of the order of 6 line widths should be observed. Two comments should be made at this point. First, one can envisage a lasor-iron-57 experiment in a hard vacuum, which would minimize extraneous effects of matter. Second, the brevity of the laser pulse may require a very strong γ-ray source or a large number of repetitions of the experiment if one is to achieve a statistically significant result. Thus, let R be the γ-ray counting-rate established without the laser pulses, and suppose that the wave-length shift of the γ-rays passing through the laser field is such as to reduce the counting-rate by a factor F. Suppose further that a repetition-rate of P pulses per sec, each 10^{-8} sec long, is achieved for the optical maser so that the radiation field is on for $10^{-8}\, P$ sec/sec. With the optical maser pulsing, one has after a time T a total count of:

$$(R - FPR \times 10^{-8})\, T \pm \sqrt{RT}\, \sqrt{1 - FP \times 10^{-8}}$$

To achieve a statistically significant change in the count from that obtaining when the optical maser is not used, one requires:

$$10^{-8} \times FP\, \sqrt{RT} \gg 1$$

Thus, if $R = 3{\cdot}7 \times 10^{9}$ counts per second, $F = 1/2$, $P = 10$ pulses per second, one requires:

$$T \gg 9 \times 10^{4} \text{ sec}$$

which in turn involves 9×10^5 operations of the optical maser. Could such experiments be carried out successfully, one would then have a nearly definitive proof or disproof, 'nearly' because one could regard equation 2 as implying an equilibrium radiation field, which the optical maser radiation certainly is not.

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Elastic Deformation and the Wear of Electrographite

IT has recently been shown that when electrographite slides repeatedly over a copper surface, the worn surfaces eventually become extremely smooth[1]; the mean angles of inclination of the irregularities on both surfaces are of the order of 2–5°. The maximum slope of an irregularity which can be deformed elastically into the general plane of a surface is inversely proportional to the modulus of elasticity of the material and may be calculated from criteria given by Halliday[2]. For metals the slopes required are usually less than 1°; for carbon and graphitic materials, however, the moduli of elasticity are much lower and the maximum permissible slopes range from about 7° to about 15°. Thus, with carbons, the stresses produced by deformation of the surface irregularities during sliding are likely to be well below the elastic limit, as recently pointed out by Clark, Connolly and Hirst[3]. In these circumstances, it has been suggested that wear might occur by a 'micro-fatigue' process, resulting from repeated applications of elastic stress over the localized regions of real contact[1]. This possibility is directly supported by the experiments described here.

Two polished cylinders of electrographite, 0·25 in. in diameter and 6 in. long, were loaded together at right angles to give nominal point contact at a stress below the elastic limit. The material, supplied by Morganite Carbon, Ltd., was *EG*11 for which the elastic limit in compression is about 25 kg/mm² and Young's modulus of elasticity is about 2×10^{10} dyn/cm². One of the cylinders was then reciprocated at 45° with respect to the other at a speed of 10 cm/s; the apparatus has been described in detail elsewhere[4]. During the first few traversals, the width of the track became constant and polished in appearance, and on changing the load the track width then varied approximately as the one-third power of the load. It may, therefore, be concluded that the deformation of run-in carbon cylinders is predominantly elastic.

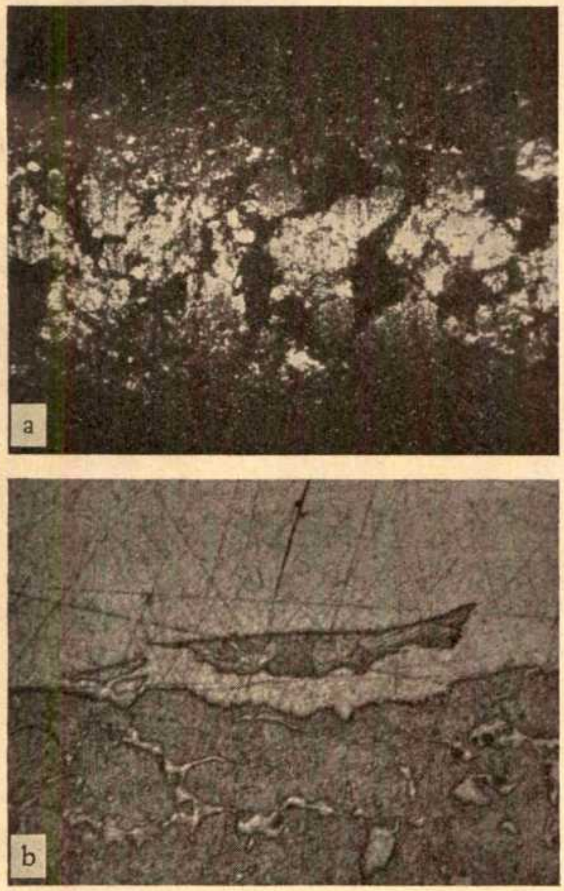

Fig. 1. Surface failure of *EG*11 cylinders after repeated elastic stresses. *a*, Pits on an *EG*11 cylinder after 6 cycles at 18 kg/mm² stress (× *c*. 22); *b*, normal section of a pit produced after 4,500 cycles at 5 kg/mm² stress (× *c*. 450)

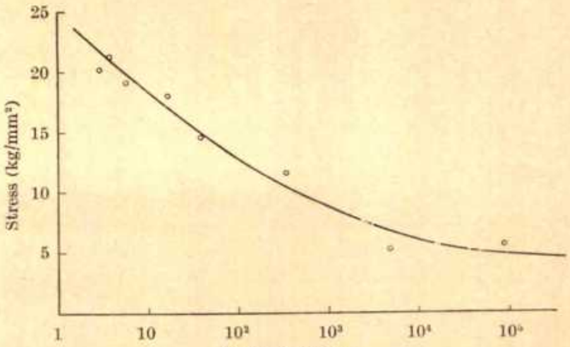

Fig. 2. Variation of the number of cycles to failure with stress for *EG*11 crossed cylinders

After a critical number of traversals of the cylinders at any one load, the surfaces within the band of contact began to break-up, leaving a number of shallow pits. An example obtained at the relatively high stress of 18 kg/mm² is shown in Fig. 1*a*, and a section through a typical pit obtained at a lower stress, 5 kg/mm², is shown in Fig. 1*b*. It may be noted that the fragment detached is in the form of a flake, and the size of the flakes decreased slightly with decreasing stress. The relationship between the mean Hertzian stress and the number of traversals required to initiate surface damage is shown in Fig. 2. The critical number of traversals increases very markedly as the stress decreases and the relationship is very similar to one characteristic of a fatigue failure.

When a carbon brush slides continuously on a metal slip-spring, the magnitude of the elastic stresses at the localized regions of real contact will depend on the number, size and shape of the irregularities supporting the load. The mean radius of curvature of the irregularities present on the surfaces of an *EG*11 brush and a copper slip-ring, after several hours of sliding at a load of 1 kg and a speed of 1,800 cm/s, has been estimated from Talysurf profiles to be of the order of 0·7 cm. The surface of the ring was largely covered by a transferred film of carbon and the wear debris was in the form of flakes, varying in size from a few hundred Angstroms upwards. If the whole of the load is assumed to be supported on a single pair of hemispherical contacts of carbon, the mean Hertzian surface stress will be about 4 kg/mm². At these low stresses, Fig. 2 shows that the critical number of contacts required to produce a wear particle will be very large, of the order of 10^6, and also depend very markedly on the magnitude of the stress. The fact that the rate of wear is proportional to the load[1] suggests that the main effect of an increase in load must therefore be to increase the number of regions of contact rather than their size. Measurements of the variation with load of the electrical constriction resistance between run-in surfaces of carbon have confirmed this conclusion.

The formation of wear particles after a critical number of repeated contacts between surface irregularities is also known to be a feature of the severe wear process of metals, for example, 60/40 brass on hardened steel[5]. The rates of severe metallic wear, however, $\simeq 10^{-7}$ cm³/cm/kg for brass on steel, are usually several orders of magnitude greater than those of carbons in the same conditions of sliding, $\simeq 10^{-10}$ cm³/cm/kg for *EG*11 on copper. The slopes of the irregularities generated on metal surfaces during severe wear are of the order of 1°, and the localized stresses must therefore be near to, or even greater than, the elastic limit. This suggests that the relatively high rates of wear of metals are a direct consequence of the greater stress on the surface irregularities. At stresses near to the elastic limit, or when the deformation is plastic, the number of cycles required to cause failure will be very much less than when the stresses are well below the elastic limit. Wear caused by micro-fatigue of the surface irregularities during repeated contacts is therefore not necessarily restricted to carbons alone; it may be equally important for metals.

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Path Integral Formulation of Relativistic Quantum Mechanics

FEYNMAN[1] has given a path integral formulation of non-relativistic quantum theory which he showed to be completely equivalent to that given by Schrödinger's equation. Although the same can be done for the existing relativistic theory[2], it cannot be unique because the latter is not consistent. The fundamental dilemma is[3]: either one adopts unitarity and all the divergences that go with it, or else admits non-Hermitian interactions and thus the non-conservation of probability.

Previously I gave a group-theoretical argument[4] in favour of the latter. Since the group in question, the exceptional Lie group F_4, is quite complicated and its infinite representations have not yet been investigated, the path integral approach is an important step toward finding not only a physical interpretation but also a representation theory of F_4.

Consider paths $z(\tau)$ in a relativistic phase space E_8 in which a geodesic corresponds to a classical relativistic oscillator. The quantum version of this problem gives, according to Huygens–Feynman principle, for the wave amplitude:

$$\psi(z/z') = \sum_{\omega} e^{i \int_{z}^{z'} z_\omega^2(\tau)\, \mathrm{d}_\tau} \qquad (1)$$

where paths ω are dense in some measure space Ω.

Such an oscillator is a model of a particle in the sense that it has a relativistic invariant which can be associated with a proper mass. But this, let us call it $k_0^2 s$, has the curious property that it depends on the space-time interval s so that if taken on the light cone it is nothing or alternatively if taken as an expectation value inside the cone it is infinite. An unambiguous result can only be obtained if one admits a metric or Lagrangian function $\mathscr{L}$, with a non-Hermitian interaction, say of the form $\mathscr{L} = (1 + i\sigma/2)z^2$, then the expectation value:

$$< k_0^2 s > = \int_0^\infty k_0^2 s e^{-\sigma k_0^2 s^2} \mathrm{d}s \Big/ \int_0^\infty e^{-\sigma k_0^2 s^2} \mathrm{d}s = k_0/(\pi\sigma)^{\frac{1}{2}} \qquad (2)$$

while the expectation value of the Laplacian of probability density is a small but non-vanishing quantity $\approx k_0^2$.

Thus σ is a very small number (the reciprocal of the cosmological constant), and to interpret it consider the following generalization of equation (1). Let $\mathscr{L} = (z_a - iz_\omega)^2$, where α is from a measure space $A \neq \Omega$. This makes it possible that the correlation $< x_a x_\omega >$ exists and equation (1) becomes the condition that it shall have the minimum value σ. If $A = \Omega$ we have essentially a phase space version of the Wiener–Siegal[5] α-representation of existing theory.

Conditions which determine A, Ω (or their generalizations) now depend on the structure of F_4. The basic fact is the principle of triality, namely F_4 acts on a vector space $M_{24} = E_8 + S_8 + S_8{}^*$ leaving invariant a trilinear form $f(z, \psi, \psi^*)$ which is also invariant under cyclic permutation P of the sub-spaces. In view of this I adopt the parametrization:

$$z_\mu = \theta_\mu^2\ (M\tau; u) \qquad (3)$$

where θ_μ runs over the 8 hyperelliptic theta functions[6] of genus three and zero characteristic. Then $u = (u_1, u_2, u_3)$ are local co-ordinates on a three-dimensional generalized Kummer surface K_3 in E_8 which, in certain neighbourhoods of u, will be equivalent to the light cone. Take $P = ST$, the operation of the modular group given by $S\tau = \tau + 1$, $T\tau = -1/\tau$.

Now suppose:

$$u = u_\gamma\ (\tau) = u_a\ (\tau) + iu_\omega(\tau) \qquad (4)$$

is a complex Wiener process in which, however, the real and imaginary parts, as above, are not independent, so that the equation for the diffusion of the probability $Pr(u/\tau)$ is not self-adjoint. If the matrix M in equation (3) is such that the θ's satisfy the same equation, then $(M; A, \Omega)$ can be considered a consistent representation in a physical sense.

To determine $(M; A, \Omega)$ uniquely requires consideration, among other things, of the cohomology structure of F_4. The essential result[7] here is that the degrees of the invariant operators (commuting with all others) are of the form $(g_i + 1)$, where g_i are the primes modulo 12. It thus appears that the determination depends on a special relation which exists between algebraic number fields, having g_i as singular divisors, and the construction of A, Ω which involves an infinite subdivision of the range of τ.

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Isotropic Exchange Integral for Cupric Acetate Monohydrate Crystals

MOOKHERJI[1] from his magnetic studies on cupric acetate monohydrate crystal predicted that there is some sort of exchange interaction between adjacent copper atoms. But in the absence of either crystallographic data or diamagnetic dilution data he could not say whether the interaction forces were of Kramers' super-exchange type acting through intermediate oxygen atoms, or whether direct spin interaction occurred. Later, from an examination of anomalous behaviour of cupric acetate monohydrate towards magnetic susceptibility and paramagnetic resonance absorption, Bleaney and Bowers[2] concluded that there is some form of strong coupling between unpaired electrons on adjacent copper atoms through exchange forces. Van Niekerk and Schoening[3] from X-ray analysis showed that cupric acetate monohydrate is a new type of complex having the formula $Cu_2(CH_3COO)_4.2H_2O$. The Cu–Cu distance is 2·64 Å, showing that there is direct interaction between the copper ions, because the distance for super exchange of Kramers type (Cu–Cu distance) should be of the order of 4 Å. Figgis and Martin[4] have shown that exchange integral $J = 1 \cdot 6\ kT_c$, where T_c is critical temperature at which $\Delta\chi/\Delta T = 0$ (χ being the gram molecular susceptibility).

We have measured the principal magnetic susceptibilities of single crystals of cupric acetate monohydrate from 300° K to 90° K. Room temperature values for the principal anisotropies were obtained by the method of Krishnan and Banerji[5] and the principal susceptibility in a convenient direction by a micro-balance as devised by Neogy and Lal[6]. Temperature variation of the susceptibilities was studied by using a cryostatic device as devised by Bose[7].

Our mean molar susceptibility values are in good agreement with those of Figgis and Martin[4], but below 200° K these values deviate considerably from those of Guha[8] and Foex *et al.*[9]. Our measurements show that the critical temperature T_c for the three principal magnetic axes of the crystal is the same and is equal to 271° K. This brings out the interesting point that the exchange integral is isotropic and equal to -299 cm^{-1}.

The value of J as given by Bleaney and Bowers (*loc. cit.*) from paramagnetic resonance data varies from -315 cm^{-1} at room temperature to -255 cm^{-1} at 90° K, while Abe and Shimada[10] suggest a constant J value. In the absence of any specific thermal expansion data or X-ray data at a low temperature it is rather difficult to say anything definite about the variation of J with temperature. Bose *et al.*[11] from their work on several cupric salts came to the conclusion that thermal variation in the crystal field makes the g values depend on temperature. We also find that the angle between the two paramagnetic units in the unit cell of the crystal changes with temperature, indicating a change of crystal field. However, theoretical susceptibility values, from the expression due to Bleaney and Bowers[2], on the assumption of a constant value of J but different values of g at different temperatures, agree well with our experimental values.

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CHEMISTRY

Vibrational Spectra and Structure of the Tricyanomethanide Ion $C(CN)_3^-$

THE tricyanomethanide ion $C(CN)_3^-$ has been known for some time[1], but its chemistry has only recently been investigated[2], and its vibrational spectra and structure have not been previously reported. We give here a summary of our recent investigation of the infra-red and Raman spectra of the potassium salt of this ion.

The infra-red spectrum was investigated both in solution and in the solid state over the range 375–2,500 cm^{-1}. For the solution spectra the following solvents were used: *cyclo*hexane (375–600 cm^{-1}), benzene and carbon disulphide (500–600 cm^{-1}), water (800–2,500 cm^{-1}), ethanol (900–2,150 cm^{-1}) and methanol (2,100–2,250 cm^{-1}). The solid-state spectra were obtained in 'Nujol' (500–2,250 cm^{-1}) and in potassium bromide disks (375–2,500 cm^{-1}). The Raman spectra were obtained from aqueous solutions. The solution spectra were very simple, but in the spectra of the solid many of the bands were split. For structural analysis the infra-red and Raman spectra based so far as possible on solution work only are given in Table 1.

Table 1

Infra-red	—	2,175 *vs sh*	1,250 *s sh*	967 *w br*	—	610*	567*	—		cm^{-1}
Raman	2,225 *vvs sh* *pol*	2.175 *vvs br* *dp*	1,242 *vvw diff* *dp*	967 *vvw* *wpol*	658 *s sh* *pol*	610 *vw* *dp*	—	478 *vvw* ?	162 *s vdiff* *dp*	cm^{-1}

* Solid state

Two structures are possible for the ion, a planar one (D_{3h}) or a pyramidal one (C_{3v}). The selection rules for the two structures are:

D_{3h} : in-plane : $2a_1'$ ($R.p$), $1a_2'$ (inact.), $4e'$ ($R.dp.$ i.r.)
out-of-plane : $1e''$ ($R.dp$), $2a_2''$ (i.r.)

C_{3v} : $4a_1$, ($R.p.$ i.r.), $1a_2$ (inact.), $5e$ ($R.dp.$ i.r.)

As D_{3h} requires seven Raman active fundamentals and six infra-red active fundamentals with only four coincidences, whereas C_{3v} requires nine infra-red and Raman active fundamentals all coincident, the vibrational spectra should enable the two possibilities to be distinguished.

The spectra quoted here support the D_{3h} configuration. Three Raman lines (2,225, 658 and 478 cm^{-1}) have no counterpart in the infra-red. As two of these are strong, sharp and polarized they are the a_1' frequencies and the third is the e'' frequency. One infra-red band has no Raman counterpart and is thus class a_2''. The other non-coincident infra-red band would be expected to lie below 375 cm^{-1}, the limit of the present investigation. There is, however, a third, weakly polarized Raman line at 967 cm^{-1} which has a coincident infra-red band. If the D_{3h} structure is accepted this cannot be a fundamental, but it can be satisfactorily explained as the first overtone of the e'' fundamental (2 × 478 = 956) which will be both infra-red and Raman active ($(e'')^2 = a_1' + e'$). This leaves three coincidences between the Raman and infra-red spectra above 375 cm^{-1}, namely 2,175, 1,250 and 610 cm^{-1}, and these are three of the e' fundamentals. The fourth e' fundamental is the Raman frequency 162 cm^{-1}, which is outside the infra-red region studied. In addition to this satisfactory correlation of the spectra it should be noted that although —C≡N vibrations are always intense the infra-red spectrum most certainly contains only one —C≡N frequency (2,175 cm^{-1}) whereas the Raman spectrum contains two (2,225 and 2,175 cm^{-1}), and that the infra-red band is coincident with the depolarized Raman line. This pattern of —C≡N frequencies is, in itself, strong evidence for the planar structure.

We thank Dr. W. O. George and Dr. D. Steele for help in obtaining infra-red spectra. One of us (R. A. G. C.) acknowledges leave of absence from Shell Research, Thornton, to work at Swansea.

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Reactions of Sulphoxides with Phosphorus Compounds and Reactive Organic Halides

Dimethyl sulphoxide is known as an oxidizing agent for organic halogen derivatives[1] and certain aromatic methyl compounds[2]. Reactions of sulphoxides with acid anhydrides give carboxylic acids and acyl derivatives of sulphides[3]. Organic sulphoxides have attracted attention as ligands in co-ordination compounds; but silicon halides and diphenyl sulphoxide give halogenated diphenyl sulphides and silicon oxychlorides and/or silica[4]. Lappert and Smith[5] investigated addition compounds of sulphoxides with boron, silicon, and tin halides, and reaction with boron trichloride or silicon tetrachloride gave a boron oxychloride-chloromethyl methyl sulphide addition compound, or silica and chloro-substituted sulphides.

Our work with phosphorus compounds and organic halides is summarized in equations (1–6).

$$Ph_3P + Me_2SO \rightarrow Ph_3PO + Me_2S \quad (1)$$

$$Ph_2PCl + 2Me_2SO \rightarrow Ph_2PO_2H + Me_2S + MeSCH_2Cl \quad (2)$$

$$PhPCl_2 + 3Me_2SO \rightarrow PhPO(OH)_2 + Me_2S + 2MeSCH_2Cl \quad (3)$$

$$N_3P_3Cl_6 + 6Me_2SO \rightarrow N_3P_3O_6H_6, MeSCH_2Cl + 5MeSCH_2Cl \quad (4)$$

$$N_3C_3Cl_3 + 3Me_2SO \rightarrow N_3C_3O_3H_3 + 3MeSCH_2Cl \quad (5)$$

$$PhCOCl + Me_2SO \rightarrow PhCO_2H + MeSCH_2Cl \quad (6)$$

Dimethyl sulphoxide oxidizes certain tervalent phosphorus compounds to the quinquevalent state, when the by-product is dimethyl sulphide (1), or causes replacement of chlorine by hydroxyl in quinquevalent compounds, when chloromethyl methyl sulphide is formed (4). A combination of these processes is observed with diphenylchlorophosphine (2) and phenyldichlorophosphine (3). Hexachloro*cyclo*triphosphazatriene gives a moisture-sensitive chloromethyl methyl sulphide addition compound of the hydroxyoxophosphazane (4), and the product of a similar reaction with octachloro*cyclo*tetraphosphazatetraene gives the dihydrate of tetrakis(hydroxyoxo)*cyclo*tetraphosphazane[6] on mild treatment with water.

Cyanuric chloride (5) and benzoyl chloride (6) are converted to cyanuric acid and benzoic acid respectively. It seems likely that this reaction will have wide applications to inorganic and organic compounds containing reactive halogen atoms.

Diphenyl sulphoxide is less reactive than dimethyl sulphoxide but the same pattern of behaviour is observed.

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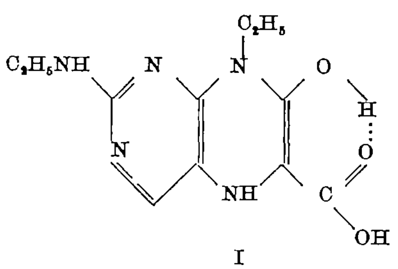
Structures of Sepiapterin and Isosepiapterin

Sepiapterin and isosepiapterin are yellow, yellow-fluorescent compounds which occur in *Drosophila melanogaster* and accumulate in the sepia mutant. They are believed to be closely related 2-amino-4-oxo-6-acyltetrahydropteridines, the acyl group in isosepiapterin being propionyl and in sepiapterin, lactyl. However, there has been some controversy concerning the location of the hydrogen atoms on the pyrazine part of the molecule. Forrest[1] favoured a distribution of the hydrogens on the 5 and 8 positions largely by analogy with the yellow 5,8-dihydro compounds (for example, I) synthesized by Pfleiderer and Taylor[2], and with the 5,8-dihydro structure assigned to the red dihydroquinoxaline (II) on the basis of its nuclear magnetic resonance spectrum by Barltrop, Richards and Russell[3].

Nawa[4], on the other hand, considered the 7,8-dihydro structure more probable because of his



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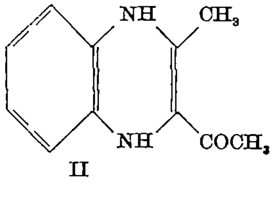

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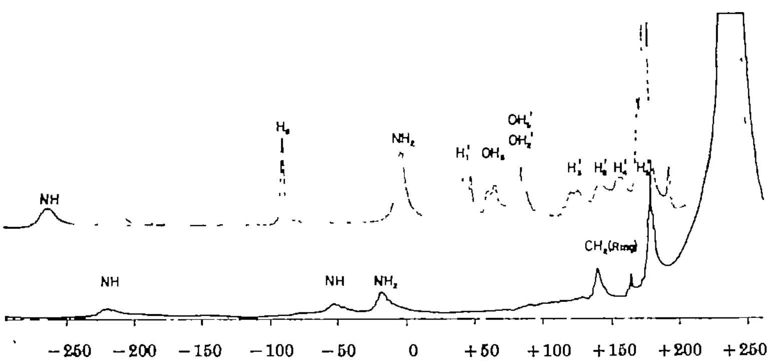
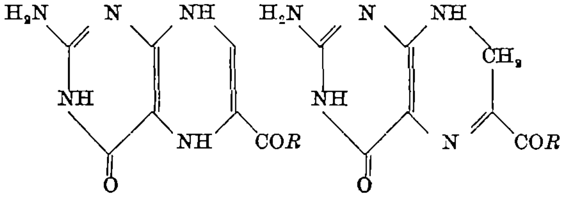


Fig. 1. The nuclear magnetic resonance spectra of isosepiapterin (——) and guanosine (ref. 6) (– – –) measured in dimethylsulphoxide, with toluene as an internal standard. Chemical shifts (c.p.s.) are converted to the figures quoted in the text by dividing by 60 and subtracting 6·86

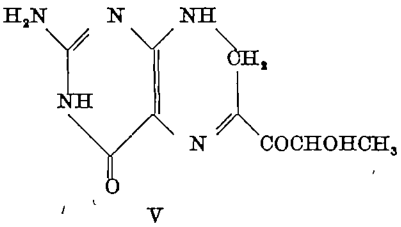
isolation of 7,8-dihydroxanthropterin as a degradation product from sepiapterin.

A direct test of these alternatives has now become possible using nuclear magnetic resonance spectroscopy. By inspection of the two possible formulæ (III and IV) it can be seen that III has one amino-group and three imino-groups, whereas IV has one amino-group and two imino-groups.



III IV

The nuclear magnetic resonance spectrum of isosepiapterin dissolved in dimethylsulphoxide (approx. 0·5 M; solution is supersaturated (Fig. 1)) reveals the presence of 3 peaks which can be ascribed to protons attached to nitrogen. These can be further resolved by comparison with the spectrum of guanosine[5,6], in which the pyrimidine ring is chemically identical with that of isosepiapterin. Thus, the peak at − 7·16 p.p.m. from tetramethylsilane (calc.) corresponds to the amino-group and that at − 10·56 p.p.m. from tetramethylsilane corresponds to the imino-group in the pyrimidine portion of the molecule. The third peak, which occurs at − 7·76 p.p.m. from tetramethylsilane (calc.), must then correspond to the imino-group or groups in the pyrazine ring. Since the area underneath this peak is approximately half the area below the amino-peak and about equal to that below the imino-peak assigned to the pyrimidine ring, the conclusion is inescapable that there is only one imino-group in the pyrazine ring. Hence, the structure of isosepiapterin must be IV (R = CH_2CH_3). In addition, the peak at − 4·53 p.p.m. from tetramethylsilane (calc.) may be ascribed to the ring methylene group present in the 7,8-dihydro formulation but absent from the 5,8-dihydro structure.



V

Although the peaks in these spectra are broad, presumably due to solvent interaction with the protons attached to nitrogen, and their height is not great due to solubility limitations, the observations are quite unambiguous. An advantage of this technique is that no chemical reactions are performed on the material under investigation; therefore, any possibility of isomerization is eliminated.

Since isosepiapterin and sepiapterin have very similar chemical properties and superimposable absorption spectra, the structure of sepiapterin, by analogy, must be 2-amino-4-oxo-6-lactyl-3,4,7,8-tetrahydropteridine (V).

We thank Mrs. Lila Gatlin and Dr. J. C. Davis for the nuclear magnetic resonance spectra and for advice on their interpretation.

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Ionization Potential of NO_2

THE ionization potential of NO_2 has been the subject of controversy for some time, and a satisfactory answer has not yet been given.

The spectroscopic and photo-ionization results lead to the following possible values: 9·78 eV[1], 11·3 eV[2], 12·3 eV[3]. Recently, Tanaka and Irsa proposed another higher value at 18·87 eV[4]. These different results probably correspond to different excited states of the ion.

On the other hand, electron impact results show a great disparity, varying from 9·91 eV to 13·98 eV (refs. 5–7), and a recent direct determination by Kiser[8] leads to 11·27 eV. I had previously[7] suggested that the indirect electron impact value 9·91 eV deduced from the appearance potential of NO_2^+ from nitromethane[5,7] corresponded to the ground ionic state of NO_2^+, which is linear, whereas the directly determined high value, 13·98 eV, would refer to the bent excited state, in accordance with the Franck–Condon nature of the ionization process.

If such is the case, one would expect the indirect value to be always the same, whatever the parent molecule. We therefore determined the appearance potential of NO_2^+ from ethyl-nitrate $C_2H_5ONO_2$. The mean result of three measurements, to which three different methods of estimating ionization potentials were applied (vanishing current, modified Warren's method, semilog method), was AP (NO_2^+) = 11·40 ± 0·12 eV.

Using $\Delta H_f(NO_2)_g$ = 8·03 kcal/m[9], $\Delta H_f(C_2H_5O)_g$ = − 8·5 kcal/m[10], and ΔH_f $(C_2H_5ONO_2)_g$ = − 36·8 kcal/m[11], one calculates D $(C_2H_5O - NO_2)_g$ = 36·33 kcal/m. This result may be combined with the appearance potential of NO_2^+ according to the usual way:

$$AP(NO_2^+) \geqslant D(C_2H_5O - NO_2) + IP\,(NO_2)$$

and leads to the result $IP(NO_2) \leqslant 9{\cdot}93$ eV.

This is exactly the same value as obtained from nitromethane. Although the perfect agreement probably is fortuitous, it may be considered as a further confirmation of the value of the lowest ionization potential of NO_2, in agreement with the lowest photoionization result, as well as with our previous interpretation.

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BIOCHEMISTRY

Purification of Streptolysin O

Among the numerous extracellular enzymes and toxins elaborated by the different strains of groups *A*, *C* and *G Streptococci*, streptolysin *O* has been investigated very extensively. A number of reports[1–3] have suggested the close relation existing between streptolysin *O* and the damage occasioned by *Streptococcus* in several infections especially in rheumatic fever. The different stages of discovery and study of biological and physico-chemical properties of streptolysin *O* have been reviewed thoroughly by Rammelkamp and Dingle[4], Herbert and Todd[5] and Bernheimer[6].

In the past two decades, many attempts have been made to purify streptolysin *O*, and more or less partially purified preparations were obtained[7–12]. In this communication a new method of purification is outlined. The details will be published elsewhere[13]. A preparation of very high specific activity has been obtained showing a main single diffusion line against anti-streptolysin *O* sera and an apparently homogeneous behaviour in the ultracentrifuge.

The crude streptolysin *O* is obtained after 24-h culture of the strain *A* 78 (Lancefield group *A* Griffith type 3, Institut Pasteur Collection) grown on the medium described by Wahl and Meyer[14]. The titre obtained was 80–160 minimum hæmolytic dose per ml., or 5–10 combining units per ml. The crude streptolysin *O* is concentrated ten times either by ultra-filtration through a parlodion membrane, as described by van Ramshorst[15], or by successive freezing and thawing. The concentrated dark-coloured fractions of streptolysin *O* which thaw before water show a very high toxic titre.

We add solid sodium chloride (20 g/100 ml. to the concentrated streptolysin *O*) and the toxin is cooled overnight at −10° C. Cooled trichloroacetic acid 2 M is added slowly at −10°. When *p*H 4 is reached, a dark and sticky precipitate is obtained. 2 h later the suspension is centrifuged at 0° C, the supernatant is discarded and the precipitate dissolved in the minimal volume of 0·01 M KH_2PO_4–K_2HPO_4 buffer (*p*H = 6·4) and dialysed two days against the same buffer. The solution obtained (fraction P_1) is precipitated by solid ammonium sulphate up to 68 per cent saturation at 0° C. The supernatant is discarded, the precipitate is dissolved in the buffer and after dialysis the solution (P_2) obtained is adsorbed on DEAE – cellulose DEAE – cellulose 5 per cent + cellulose 10 per cent in 0·02 M phosphate buffer). The brown pigments are firmly adsorbed and after centrifugation and several washings with the buffer and recentrifugations the pooled clear-yellow supernatant (P_3) is precipitated by solid ammonium sulphate up to 68 per cent saturation at 0° C. The precipitate is dissolved in 0·01 M buffer and dialysed two days. The solution P_4 obtained is passed through a DEAE – cellulose column (2 cm × 30 cm) and eluted by solutions of KH_2PO_4/K_2HPO_4 ranging from 0·003 to 0·1 M.

Table 1

Fraction	Specific activity (combining units/mg N)	Yield (per cent)	No. of antigenic constituents*
Crude toxin	8·3	100	9
Concentrated toxin	5·0	99	9
P_1	123	84	8
P_2	411	55	6
P_3	—	40	—
P_4	784	30	5
P_5	3,300	15	2 (+1)
P_6	—	6	1 (+1)†
P_7	6,400	3	1 (+1)†

* Estimated by immunodiffusion against a serum prepared by immunization of horses with crude formol detoxified streptococcal toxin.

† The two last preparations P_6 and P_7 contain one major antigenic constituent and a minor one (+1), only visible when a very large excess of antigen is used in the immunodiffusion test.

— Not done.

The purest fraction obtained (P_5) is eluted at 0·01 M and is precipitated by solid ammonium sulphate (55 per cent saturation). The precipitate is dissolved in a 0·02 M phosphate buffer and the solution (P_6) is refractioned by ammonium sulphate. The 'purest' fraction, P_7, is obtained by precipitation between 45 and 55 per cent ammonium sulphate (see Table 1 for the purification method). The P_7 preparation is a colourless limpid solution with a specific

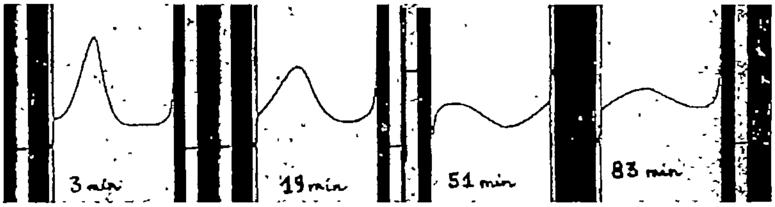

Fig. 1. Ultracentrifugation of streptolysin *O* (preparation P_7). 59,780 r.p.m. 'Spinco' analytical centrifuge photographs taken after 3, 19, 51 and 83 min (synthetic boundary cell)

activity (when expressed in combining units/mg nitrogen) 2,130 times higher than the crude streptolysin *O*. The ultra-violet absorption spectrum presents a maximum at 2750 Å and a minimum at 2550 Å. The ratio of optical densities at these wave-lengths is low (1·17). The fraction of P_7 has not yet been ultracentrifuged for the determination of its sedimentation characteristics. However, the sedimentation in phosphate buffer at 20° of fraction P_8 (1,000 times purified, showing two major lines in gel precipitation) presents an almost single symmetrical peak in the ultracentrifuge (synthetic boundary cell). Fig. 1 illustrates the sedimentation pattern, which shows a very strong diffusion of the preparation and a sedimentation coefficient lower than 1. This result implies a molecular weight of streptolysin O certainly lower than 10,000. More precise experiments are now being undertaken to determine with more accuracy the molecular weight of purified fraction P_7.

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Quantitative Extraction of Acid-soluble Human Skin Collagen with Age

It has been shown that the acid-extractable collagen from the skin decreases with age (see review by Harkness[1]), but no quantitative data are available in man or animals which permit correlation of extractable collagen with growth, development, and age over the entire life-span. This communication presents and discusses such findings in man.

Specimens of skin were obtained at autopsy within 8 h of death from accident cases or from patients suffering from non-debilitating diseases. Total body skin was removed from fœtuses and new-borns. In the remainder, strips were excised from the abdomen. In no specimen was gross or microscopic evidence of decomposition present. Each specimen was treated individually and extraction was carried out immediately. The epidermis and subcutaneous fat were removed by scraping with a sharp blade and the wet weight determined. The weight of the dermis was never less than 3 g, usually 10–80 g. The extraction method was that used by Gallop[2] and Boedtker and Doty[3] on ichthyocol, but the citric acid extract was dialysed against water instead of dibasic sodium phosphate. All extractions were limited to a 48-h time-interval as such solutions have been shown by several investigators to contain a homogeneous molecular species[2–4]. After Kjeldahl digestion, the concentration of soluble collagen nitrogen was determined by the Conway diffusion technique[5]. To calculate the dry weight of collagen, a value of 18·2 per cent nitrogen previously obtained from the amino-acid data of human skin fœtal collagen[4] was used. Reproducibility on duplicate determinations by the Conway procedure was ± 2 per cent. Results were expressed as milligrams of soluble collagen per gram of wet dermis. It has been shown[6] that the percentage of water in the skin of human infants of age 3–6 months is similar to that of adults. During the preparations, the solutions were never exposed to temperatures above 5° C.

The extracted molecule has been characterized previously for the human fœtus and new-born[4]. Similar values for the sedimentation coefficient, 3·10*S*, limiting viscosity number (1,350) and temperature of denaturation, 36° C, established for the new-born were also found in representative samples in the older age-group.

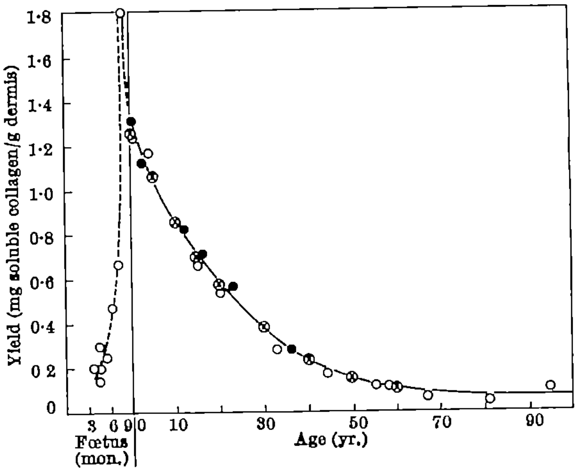

Fig. 1. Yield of citric acid-soluble human skin collagen with age. ●, Specimens obtained from accident cases; ○, specimens obtained from patients with non-debilitating diseases; ⊗, calculated values using equation 1

Fig. 1 shows the yield of citric acid-soluble human skin collagen with age. The yield is very low in the young fœtuses. This may only be relative, reflecting the increased cellularity of the dermis, which contains more fibroblasts and relatively less collagen in the young fœtuses than in the older specimens. As the cellularity decreases during fœtal development, the yield increases and reaches a maximum several weeks prior to birth and then falls precipitously. The extractable collagen decreases during successive decades to the age of 60, and thereafter is essentially constant. The following relationships of age and extractable collagen are apparent: puberty (age 13) slightly more than one-half that of the new-born; menopause (age 45), approximately one-seventh; age 60 and over, approximately one-fifteenth.

The dependence of extractable collagen on age was found to follow a two-parameter relationship in the interval 0–60 years:

$$N = N_0 e^{-A/k} \qquad (1)$$

where N is the extractable collagen (mg) per gram of wet dermis; N_0 is a constant of 1·3 mg per gram of wet dermis which is the extractable collagen at zero age; A is the age of the patient in years; k is a constant equal to 23·5 years. The value of N at age k is N_0e^{-1}, where e is the base of the natural logarithm.

Equation (1) expresses N as a function of A. By use of the inverse function, age may be estimated by measuring N. In Fig. 1, the theoretical curve is superimposed on the curve drawn from the experimental data and the deviation of the theoretical from the experimental curve does not exceed 5 per cent.

Equation (1) describes the course of a process analogous to typical first-order reactions of chemical physics, and this equation may be derived statistically. The *in vivo* transition of citrate soluble collagen to the insoluble form through the formation of intermolecular cross-links follows a similar exponential course in the interval between 0 and 60 years, and may be a statistical process independent of known rate-regulating mechanism.

The relationship (equation 1) is probably characteristic of all collagen regardless of source, and the inherent 'ageing' parameters are embodied in the constants N_0 and k. Exceptions to this exponential law have been observed in patients with chronic liver disease and other prolonged debilitating disorders and also in patients treated with steroids. These exceptions are now being investigated. The physiological implications of this first-order process during growth, development and ageing are unknown. Further quantitative studies of collagen are necessary to find out if different physiological characteristics of collagen follow this exponential relationship as a function of age.

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Ultra-violet)Absorption Spectra of Simple Aliphatic Guanidines

ACCORDING to Gillam and Stern[1], light absorption of the azomethin bond (=C=N—) possessing a nitrogen chromophore has so far not been examined adequately in the aliphatic series. In the case of amino-acids, the significance of the region below wave-length 220 mμ, which had been neglected, was pointed out by Saidel *et al.*[2].

Recently, on examining the infra-red and ultra-violet spectra of primycin[3], an antituberculotic antibiotic of Sakaguchi-positive nature, the presence of the azomethine group was shown by the ultra-violet maxima at 205–210 mμ of the basic chromogenes. We pointed out also the effect of the applied solvent systems on absorption values. The guanidine derivatives of biologically important effect (arginine, creatine, glycocyamine, etc.) are known to possess at most only an end absorption. Therefore, it seemed interesting to develop further the experimental conditions on the basis of which aliphatic guanidines can directly be determined at their absorption maxima (Fig. 1). The pre-requisites of this determination are as follows. (1) No changes should occur in the wave-lengths position of the maxima (λ_{max}) established in three different concentrations of the tested compounds. (2) The relative error (± Δ per cent) of the 'E-values' of the dilution series measured at the constant maxima should range within defined error limits, that is, these values should obey Beer's law. On choosing the solvent system in a satisfactory way, these conditions could be fulfilled in each case.

The data of absorption were established with a Beckman *DU* spectrophotometer (model 2400), by the method described earlier[3]. The absorption spectra were plotted as logarithmic values of the molecular extinction coefficient, $\log \varepsilon = \log\left(E\times\frac{M}{c\times l}\right)$, where $E = \log I_0/I$; c = g/l.; and l = 1 cm/silica cuvette.

External factors, such as solvents, errors due to dilution, stray radiation of the region of wave-length below 205 mμ, and further certain inner factors such as the molecular state of strongly dissociated guanidine bases and of their derivatives of amphoteric nature, respectively, interfere with and affect, respectively, the determinations to a marked extent. Arginine, in an aqueous alcoholic solution (*BEW*) with λ_{max} at 201 mμ, did not obey Beer's law. Therefore, our further investigations were adjusted to a so-called 'arginine test'. In the development of the absorption maximum of arginine, the H⁺ ion concentration of the solvent played an appreciable part. In our measurements, standard aqueous buffer solutions of *p*H values controlled by a *p*H-meter (up to ± 0·05 *p*H) or preferably aqueous alcoholic buffer solutions (prepared from BuOH : EtOH : H_2O in a ratio of 1 : 1 : 2) denoted briefly as *BEW*-buffer were used[3]. The maximum values and the absorption intensity values of both arginine and other guanidines

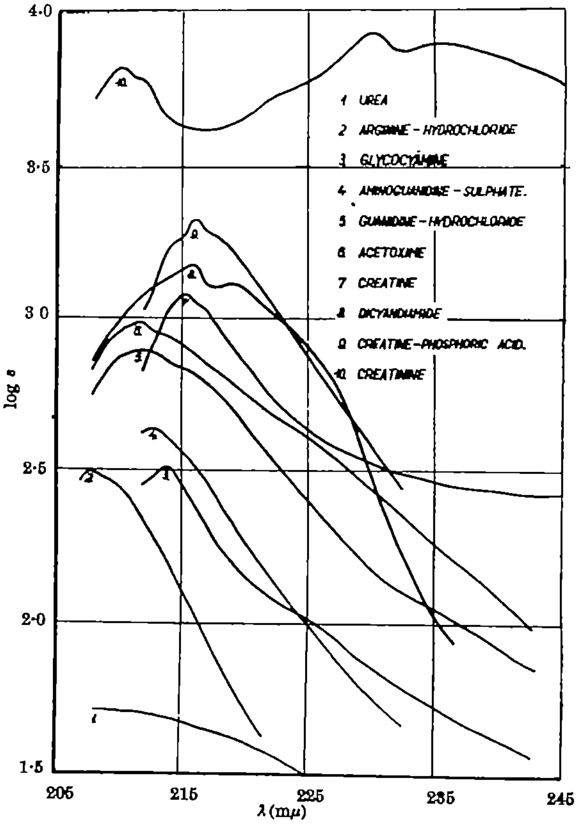

Fig. 1

proved to vary rather sensitively with *p*H values. In aqueous buffer solvents, arginine showed only at the *p*H values 7·0 and 10·5 maxima of high intensity but over 10 per cent error limit, at wave-lengths below 205 mμ. A similar case was also observed with the system's alcoholic *BEW*-buffer, however, with the difference that at *p*H value 8·05 it was possible to observe the highest position of constant maximum of arginine experienced, at 208 mμ as well. The values of the relative error of dilution sequences measured at 208 mμ ranged within 1–3 per cent, in a fairly reproducible way.

On examining the buffer capacity of the solvents, it was found that, similarly to the observations made at the amino-acids[3] : "the choice of a suitable concentration of a particular buffer was important". The spectrum of arginine (208 mμ) established in a *BEW*-buffer (*p*H 8·05; 0·025 M) served as a basis for comparison. On applying the solutions of the same buffer substance of gradually increasing concentration (0·003–0·3 M) as solvents, the spectra and the dilution series were repeatedly established. On raising the concentration of buffer solution, the wave-length values of the maxima 204–208 mμ disclosed a 'bathochromic', while those of absorption intensity after an initial rise a 'hypochromic', shift. Also the relative error of the dilution series diminished. On taking the necessary precautions, the absorption spectra (Fig. 1) of 8 aliphatic guanidine derivatives were established by us at three different dilutions (c = 0·01–0·1). In a 0·025-M *BEW*-buffer (of *p*H 8·05), the following constant selective maxima were observed, λ_{max} (ε_{max}) : arginine, hydrochloride 208 mμ (331); guanidine, hydrochloride 212 mμ (790); dicyandiamide 216 mμ (1,495); creatinine 210 and 230 mμ (6,330 and 8,020). At the same time, urea showed an end absorption, while acetoxime disclosed at 212 mμ (950) values characteristic of its azomethine group. In a 0·1-M *BEW*-buffer, in turn (of *p*H 8·05), the following measurable constant maxima appeared: aminoguanidine, sulphate 213 mμ (289); glycocyamine 214 mμ (310); creatine 215 mμ (1,192); creatine phosphate 216 mμ (2,065). The dilution series of the above-mentioned compounds obeyed Beer's law within an error limit of 2–5 per cent.

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Isolation of Aspartic Acid Activating Enzyme from Bakers' Yeast

IN the investigation of amino-acid activating enzymes in various tissues, aspartic acid activating enzyme has been found[1,2]. Although several other activating enzymes have been purified[3-5], the aspartic enzyme has not so far been isolated. This communication describes a simple procedure for isolating the enzyme from bakers' yeast.

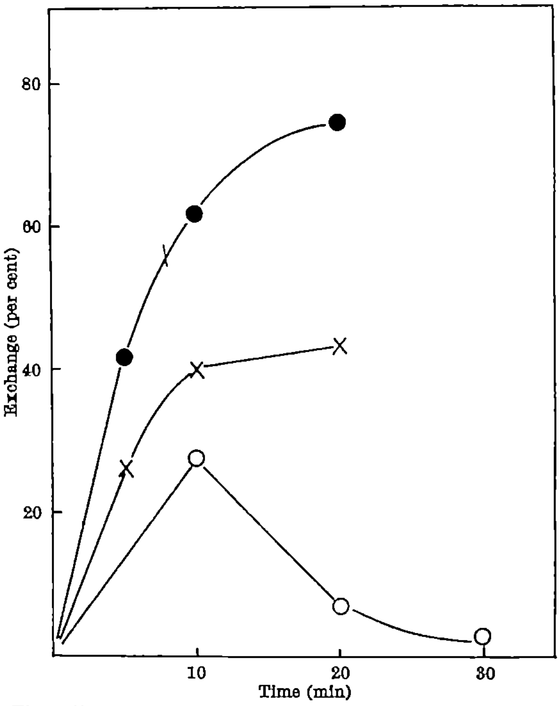

Fig. 1. ^{32}P ATP-PP exchange catalysed by aspartic acid activating enzyme from bakers' yeast. ○, 4 8–5·1 *p*H fraction (2 mg enzyme/ml. reaction mixture), ×, 4·8–5·1 *p*H fraction (2 mg enzyme/ml. reaction mixture) + 10 μmole/ml. *l*-threonine; ●, 60–70 per cent saturation ammonium sulphate fraction (1·2 mg enzyme/ml. reaction mixture)

The enzyme activity was determined by phosphorus-32 PP–adenosine triphosphate exchange[3]. The reaction mixture contained (per ml.) 100 μmole *tris* *p*H 7·1, 10 μmole magnesium chloride, 4·5 μmole adenosine triphosphate, 4·5 μmole ^{32}P-sodium pyrophosphate (80,000 c.p.m.), 8 μmole amino-acid, 10 μmole sodium fluoride and the enzyme. The mixture was incubated with and without amino-acid at 37° and the results are expressed as per cent phosphate exchange[3].

An extract was prepared from bakers' yeast according to Van de Ven *et al.*[6]. This extract was fractionated by drop-wise addition of 0·2 M acetic acid at 3° and the fraction precipitated between *p*H 4·8 and 5·1 was collected, washed briefly with cold 0·01 M acetate buffer *p*H 4·8 containing 0·15 M potassium chloride, and then stirred for 1 h in sucrose buffer as used by Zamecnik *et al.*[7]. This fraction, on testing by pyrophosphate exchange in the presence of a complete range of amino-acids, showed slight activity in the presence of alanine and tryptophan, and a marked exchange in the presence of aspartic acid. However, the rapid exchange in the first 5 min was followed by a decline in AT^{32}P concentration (Fig. 1). The fall in the rate of phosphate exchange appeared to be due to the effect of the enzyme aspartokinase[8], which in the presence of aspartic acid and adenosine triphosphate catalysed the formation of adenosine diphosphate and aspartyl phosphate. When *l*-threonine, which inhibits the enzyme aspartokinase[9], was added to the reaction mixture there was no decline in the phosphate exchange (Fig. 1).

If the enzyme fraction was subjected to further purification by ammonium sulphate fractionation, the fraction precipitated between 60 and 70 per cent saturation was found to be free from the residual alanine and tryptophan activating enzymes, and also free from aspartokinase. In the presence of aspartic acid normal phosphate exchange took place even in the absence of *l*-threonine (Fig. 1). There was no exchange caused by *l*-threonine alone in either of the two fractions. The reaction is dependent on the concentration of aspartic acid, and the Michaelis constant is of the order of $3\cdot1 \times 10^{-4}$ M. The *p*H optimum is 6·8–7·1.

The activating enzyme prepared in this work is free from a proteinase which was found in the crude yeast extract and which caused difficulty in determining enzyme activity as it caused high blank reading.

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PHYSIOLOGY

Repetitive Activation of Motor Nerve Endings

It has been suggested that after repetitive activation motor nerve endings become hyperpolarized and that consequently the spike potential in the endings is larger in amplitude and more effective in transmission[1]. An alternative suggestion is that activation causes a mobilization of transmitter in the terminals so that more is available for release[2]. If the spike potential could be recorded in the terminals, it would give information about the relative importance of these two hypotheses. Hitherto this has been impossible in a mammalian preparation because there was no reliable method of locating the microscopically small endings. This difficulty can, however, be overcome by using as a criterion the ability to record extracellular miniature end-plate potentials, for it is generally accepted that this is only possible within a few micra of junctional regions[3].

4 M sodium chloride filled glass microelectrodes with a tip diameter of 1·5–3μ and a resistance of 1–3 MΩ were used to record extracellular miniature end-plate potentials in the rat diaphragm–phrenic nerve preparation *in vitro*. The mounting of the preparation, the recording technique and the bathing solutions have been previously described[4]. Neuromuscular transmission was blocked in all experiments by raising the magnesium chloride concentration of the bathing solution to between 11–13 m.moles/l.

When recorded by a microelectrode located as described the extracellular end-plate potentials were always preceded by a diphasic (positive–negative) spike potential (Fig. 1*A*), which is shown at higher amplification and on a faster time base in Fig. 1*B*. As this spike potential was all or nothing in character and had the same threshold as the following end-plate potential, we assume that it was recorded from the nervous element of the junction at which the miniature end-plate potentials and extracellular end plate potentials were generated. The effect of repetitive phrenic nerve stimulation on spike and end-plate potential was examined by photographically superposing 30–60 faint traces. End-plate potential and spike were recorded simultaneously at two gains, the lower gain (*A*) being used for later measurement of end-plate potentials and the higher gain (*B*) for measurement of spikes. The graph in Fig. 1*C* of end-plate potential and spike amplitudes at frequencies of 1–320/sec, and the sample records of end-plate potentials (*A*) and spikes (*B*) show that, as the frequency of stimulation was increased above 10/sec, there was a parallel increase in the amplitudes of end-plate potentials and spikes (measured between positive and negative peaks). After stimulation ceased the end-plate potential and spike amplitudes returned to the control size with a similar time course. The logarithm of the increased end-plate potential amplitudes was found in a large series of experiments, to be linearly proportional to the increased spike amplitudes.

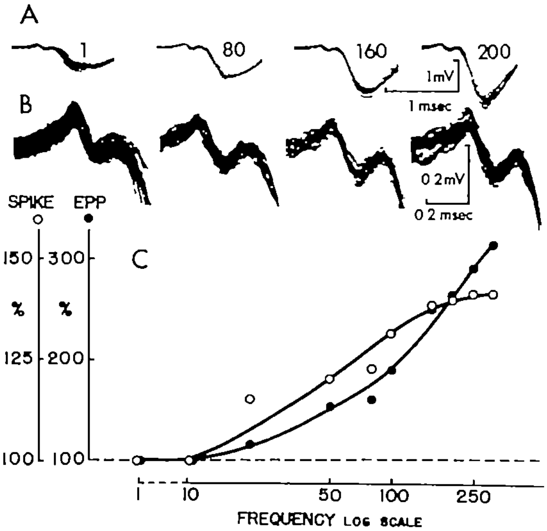

Fig. 1. Extracellular records of end-plate potentials and spike potentials at a neuromuscular junction during repetitive stimulation. *A*, Extracellular end-plate potentials at stated frequencies. Records were produced by superposition of many faint traces. *B*, as *A* but at a higher gain to show the presynaptic spike potential. *C*, End-plate potential and spike potential amplitudes during repetitive stimulation at frequencies of 1–320/sec. The amplitudes were measured from the records shown in *A* and *B* and similar records at other frequencies. The spike and end-plate potential amplitudes found during 1/sec stimulation were taken as 100 per cent

Further experiments showed that the end-plate potential potentiation which can be detected after a single impulse or a few impulses at high frequency was not accompanied by an increase in spike amplitude. In these experiments paired stimuli 4 msec apart were delivered to the phrenic nerve at 2-sec intervals for about 200 trials. The average second end-plate potential response was, as expected at such a close interval[5], larger than the first by some 20–30 per cent. The second spike potential, however, was on the average always smaller than the first, as would be predicted if it was generated when the terminals were depolarized by the negative after-potential following the first spike[6]. It must be concluded, therefore, that the potentiation after a single impulse is of a different character from that during and following prolonged tetanic stimulation, and could well be due to mobilization of transmitter. This conclusion has recently been supported by the finding that hyperpolarizing currents which are thought to mobilize transmitter can increase the potentiation found after a single impulse or a few impulses at high frequency[7].

Thus impulses in nerve terminals appear to increase transmitter release by at least two mechanisms. The first—mobilization—is the primary mechanism since it is produced by a single impulse. It is thought to be a spatial reorganization of transmitter so that there is an increased amount in close relationship to sites of release[2]. The second mechanism—an increase in spike amplitude—needs repetitive stimulation for its development because it is produced by the cumulative hyperpolarization of nerve terminals which follows such stimulation[6].

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Essential Absence of Glucose in the Fœtal Fluids of the Sheep

Barklay, Haas, Huggett, King and Rowley[1] estimated the fœtal fluids of the sheep between the 49–140th day of gestation for total reducing substances by copper reduction and for fructose by resorcinol. The glucose concentration was taken as the difference between these two values. The mean values were 61 mg and 150 mg/100 ml. for the amniotic and allantoic fluids respectively; for fructose both allantoic and amniotic fluids had mean values of 195 mg/100 ml. Re-examination of the fœtal fluids using the specific glucose oxidase method of Huggett and Nixon[2] has revealed that, in fact, the concentration of glucose is extremely small and in some cases absent.

Determinations were made on Welsh mountain sheep of known gestational age over the age-range 90–145 days; in this breed term is at 148 days. Under procaine spinal anæsthesia the uterus was exposed and samples of the amniotic and allantoic fluids withdrawn by syringe from the respective fluid compartments. The samples were deproteinized with sodium hydroxide–zinc sulphate. In addition to glucose, the concentrations of total reducing substances and fructose were also measured using the methods of Somogyi[3] and Chinard *et al.*[4] respectively. The mean and range of the values obtained are shown in Table 1. No correlation of concentrations of the substances with fœtal age was discernible.

Table 1. MEAN AND RANGE OF GLUCOSE, FRUCTOSE AND TOTAL REDUCING SUBSTANCES IN SHEEP FŒTAL FLUIDS (MG/100 ML.)

	Amniotic fluid		Allantoic fluid	
Glucose	2·8	(0–11) *26*	3·1	(0–11) *26*
Fructose	252	(45–625) *26*	172	(50–630) *26*
Total reducing substances	380	(170–725) *18*	338	(135–720) *26*

No. of observations in italics.

These results indicate that both fluids contain appreciable concentrations of reducing substances other than glucose and fructose.

It is suggested that the essential absence of glucose in the fœtal fluids is in part due to the entry of urine low in glucose into the fœtal fluid compartments and reflects the active renal tubular reabsorption of glucose. This conclusion is supported by plasma clearance studies on the sheep fœtal kidney (Alexander and Nixon, submitted for publication).

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Importance of Calcium for Acetylcholine-evoked Salivary Secretion

ONE of the functions of the chemical transmitter acetylcholine is to evoke secretion. How it does this is unknown; but recent evidence[1] indicates that, at one of its sites of action, the adrenal medulla, acetylcholine stimulates secretion through some calcium-dependent process. Thus, the stimulant effect of acetylcholine on catecholamine release was found to vary with the extracellular calcium concentration over a wide range, and calcium itself in certain circumstances proved an adequate stimulus for secretion. These observations considered along with the known ability of acetylcholine to increase membrane permeability at various sites led to the suggestion that acetylcholine evokes catecholamine secretion by promoting the influx of calcium into the chromaffin cells[1]. This idea gains support from experiments showing that acetylcholine increases the uptake of calcium-45 by the adrenal medulla[2].

The adrenal medulla is unique among the organs secreting in response to acetylcholine: first, the chromaffin cells are developmentally related to neurones; second, the acetylcholine receptors at this 'nicotinic' site are readily distinguishable pharmacologically from those at the other, 'muscarinic', sites of secretion, the exocrine glands; and finally, the secretory products of the chromaffin cells, the catecholamines, are chemically unrelated to the principal constituents of exocrine secretions, water, electrolytes and protein. Nevertheless, certain morphological features of exocrine secretion are similar to those of catecholamine secretion: protein appears to be released from exocrine gland cells in much the same way as catecholamines are released from the chromaffin cells of the adrenal medulla, that is, by the process that has been described as 'vesiculation and membrane flow'[3,4]. The question therefore arises whether calcium may also be involved in the secretory response to acetylcholine at muscarinic sites. The present experiments have revealed that calcium has an important role at one such site, the submaxillary salivary gland.

Our experiments have been carried out on the submaxillary glands of cats perfused *in situ* with Locke's solution at about 25° C. Under such conditions the

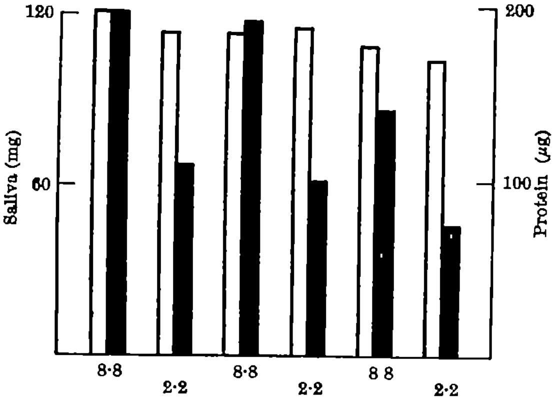

Fig. 1. Effect of excess calcium on secretion from the submaxillary gland in response to acetylcholine (10^{-4} g/ml. for 30 sec). The responses were obtained at 20 min intervals and each represents collection over 5 min. White, total weight of saliva (mg); black, protein content of saliva (μg)

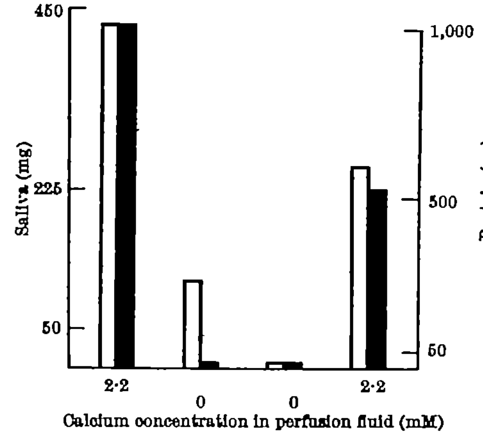

Fig. 2. Effect of calcium deprivation on secretion from the submaxillary gland in response to acetylcholine (10^{-5} g/ml. for 2 min). The responses were obtained at 20-min intervals and each represents collection over 5 min. White, total weight of saliva (mg); black, protein content of saliva (μg)

output of water and electrolytes (sodium, potassium and chloride) in response to acetylcholine (10^{-5} g/ml.), given for 30 sec–2 min, was reproducible for 2 h or more when repeated at 20-min intervals: the output of protein declined in successive tests but this decline was regular and did not obscure experimentally induced changes of the magnitude that we observed.

In a first group of experiments it was found that the amount of protein secreted in response to acetylcholine was increased when the calcium content of the perfusion fluid was raised fourfold. This effect is illustrated in Fig. 1, which shows that the increase in protein output was not accompanied by any change in the total amount of saliva. The high calcium medium was also without effect on the amount of sodium, potassium or chloride secreted in response to acetylcholine. A second group of experiments showed that the secretory response to acetylcholine was profoundly reduced when calcium was absent from the perfusion fluid (Fig. 2). In such experiments, however, the effect was not confined to the protein: the secretion of water and electrolytes was also depressed.

It is clear from these experiments that calcium has some important part to play in the secretory response to acetylcholine at a typical muscarinic site of action, the submaxillary salivary gland. The profound effect of calcium deprivation on the acetylcholine-evoked secretion of water and electrolytes is reminiscent of existing evidence that in the absence of calcium there is a severe reduction in sodium and water transport in the rat intestine[5,6]. The variation in protein output with calcium concentrations above as well as below the normal is paralleled by the similar effect of calcium on catecholamine secretion[1]. In view of this—and because of the similarities in the morphological aspects of protein and catecholamine secretion—the possibility arises that acetylcholine effects the release of protein in the same way as it seems to effect the release of catecholamines, that is, by causing an influx of calcium ions into the secretory cells. Calcium indeed may be involved generally in secretory processes associated with vesiculation and membrane flow.

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Effect of Tri-iodothyronine on Ethanol Metabolism in the Dog

Kalant, Sereny and Charlebois[1] have recently reviewed the literature pertaining to the effectiveness of tri-iodothyronine in the treatment of acute alcoholic intoxication. Contrary to several earlier studies by other investigators, Kalant *et al.* observed no effect in patients in either the rate of disappearance of blood alcohol or in percentage improvement in the performance of a letter-marking test following treatment with tri-iodothyronine. Since these investigations on man yielded results similar to those which we had obtained in dogs it seemed of interest to report our findings in intact dogs and in rat liver homogenates.

Mongrel male dogs, 15·8–19·6 kg fasted overnight, were injected intravenously with 20 per cent ethanol in physiological saline in volume sufficient to give 4 g/kg of ethanol. All injections were completed by 10 a.m. The animals were divided into control and experimental groups. Blood samples were obtained at 12, 1, 2, 3, 4.30, 6 and 8 p.m. Immediately following venepuncture at 1 p.m., experimental animals were injected intravenously with 0·333 mg/kg of 1-tri-iodothyronine, dissolved in 0·01 N sodium hydroxide, and control dogs were similarly injected with the equivalent volume of 0·01 N sodium hydroxide alone. The concentration of blood ethanol was determined in each blood sample. In order that the potency of tri-iodothyronine might be proved, sodium hydroxide solutions of this substance were injected intraperitoneally into fasted rats. In every case increased oxygen consumption was demonstrated. Control rats, receiving sodium hydroxide alone, failed to exhibit increases in oxygen consumption.

Additional experiments were designed to determine the effect of tri-iodothyronine on hepatic alcohol dehydrogenase activity. Buffer solution containing glycine, sodium hydroxide, semicarbazide and ethylenediamine tetraacetate was adjusted to *p*H 9·54. In

Table 1. RATE OF DECLINE IN BLOOD ETHANOL IN DOGS

	1.00–2.00	2.00–3.00	3.00–4.30	4.30–6.00	6.00–8.00 p.m
Controls (10 dogs)	*468–445	445–425	425–400	400–371	371–331
	†23 ± 3·7	23 ± 2·9	17 ± 2·0	19 ± 1·8	20 ± 2·2
Exp. (10 dogs)	*451–427	427–410	410–382	382–352	352–308
	†24 ± 3·9	17 ± 2·7	19 ± 1·8	20 ± 1·7	22 ± 2·1

* Range in blood levels, mg/100 ml.

† Mean rate of decline, mg/100 ml./h.

Statistical evaluation: no significant difference between the various groups even at the 0·05-level.

a portion of this buffer, tri-iodothyronine, 0·333 mg/l· was dissolved. Specimens of rat liver were homogenized in either: (*a*) buffer alone, or (*b*) buffer-tri-iodothyronine. In each case the homogenate was diluted 1 : 400 with (*a*) or (*b*). Diphosphopyridine nucleotide (DPN) was added to a portion of the liver homogenate–buffer mixture, incubated for 20 min at room temperature, and set at 100 per cent transmission in a Beckman *DU* spectrophotometer at wavelength 340. DPN was added to cuvettes containing homogenate and buffer or homogenate–buffer–tri-iodothyronine and incubated 20 min. Ethanol was then added to each cuvette, except the one set at 100 per cent transmission, and changes in optical density were determined at 2-min intervals for 12 min. Statistical evaluation of the results: the 6·2 per cent lower activity when tri-iodothyronine is present is significant at the 0·05-level but not at the 0·01-level.

The results may be summarized as follows:

(1) Tri-iodothyronine failed to accelerate the rate of ethanol metabolism in intact dogs.

(2) Alcohol dehydrogenase activity in liver homogenate of rats was to some extent depressed when tri-iodothyronine was present in the homogenate. Increased activity did not occur.

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Variability of Twitch Potentiation in Frog Skeletal Muscle

TWITCH responses of frog skeletal muscle can be potentiated without any increase in peak tetanus responses, by many agents, for example, lyotropic anions[1-3]. The magnitude of twitch potentiation in these investigations varied considerably; but according to Hill and Macpherson (ref. 3, p. 88) "this cannot be avoided since the effect varies from muscle to muscle". Recently, zinc also has been shown to act as a potentiator[4-6]. In these experiments, the potentiation (increment of twitch tension expressed as a percentage of the initial twitch tension) varied from 60 to 490 per cent. A major source of this variability is herein identified, and a control procedure is presented that should aid future potentiation studies.

Isometric contractions of frog (*Rana pipiens*) sartorius muscles were recorded at room temperature (average 23° C, range 21°–25° C) using an *RCA* 5734 transducer tube. After excision, with the pelvic bone attached, the muscles were equilibrated for at least 1 h in 100 ml. of normal frog Ringer's solution, and then mounted by firmly clamping the pelvic bone. A metal chain[7] was attached to the tibial tendon either by tying with waxed cotton thread directly to the tendon, or by engaging a hook at the end of the chain to a loop of cotton thread tied on to the tibial tendon. The chain was then hooked to an isometric lever contacting the plate pin of the transducer. Muscles were routinely mounted under a constant resting tension of approximately 2 g. Slightly supramaximal stimuli of 0·3 msec were used. Fused tetanus responses were obtained using a train duration of 200 msec and a stimulation frequency of approximately 120 shocks/sec.

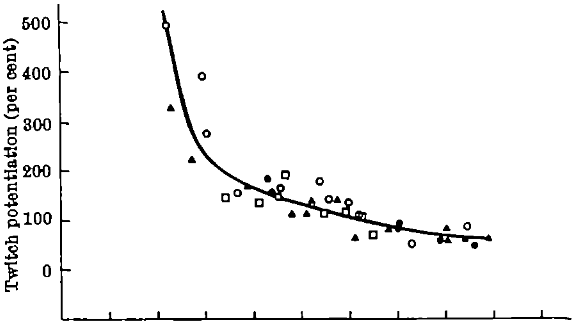

Fig. 1. Percentage of twitch potentiation obtained in 38 muscles treated with 0·05, 0·1 or 1 mM zinc plotted against the corresponding normal values of $(P/P_0)_t$. Concentration of zinc (mM/l.): ▲, 1; ○, 0·1; ●, 0·05; □, 0·1 (final concentration)

Analysis of the experiments shows zinc potentiation varies inversely with the ratio, $(P/P_0)_t$, of the normal tension of the twitch, P, to that of the tetanus, P_0. Fig. 1 shows the maximal potentiation obtained in 38 muscles, treated with 0·05, 0·1, or 1 mM zinc, plotted against the corresponding normal values of $(P/P_0)_t$. All these zinc concentrations produce the full potentiation effect of zinc[6]. Similar results have been obtained by Ramsey and Street[8] for post-tetanic potentiation and by A. Sandow (personal communication) with nitrate and quinine potentiation. These results, in general and particularly those presented in Fig. 1, clearly demonstrate that the degree of potentiation is an inverse function of the value of $(P/P_0)_t$, and thus they especially suggest that abnormally high degrees of potentiation may not be owing to an intrinsic property of the agent causing the potentiation, but to the presence of an abnormally low value of $(P/P_0)_t$.

Therefore, one can deduce from Fig. 1 how important it is to use muscles with similar value of $(P/P_0)_t$ in determining how twitch tension varies with, for example, the concentration of a potentiator. Alternatively, one may prefer to examine the concentration dependence by exposing a single muscle to successively increasing concentrations of the potentiator[5,6]. Perhaps Hill and Macpherson's (ref. 3, p. 88) conclusion "there is no 'threshold' to the nitrate or iodide effect—it increases continually with concentration" would be modified by consideration of the effect of $(P/P_0)_t$ on potentiation.

Variation in $(P/P_0)_t$ may be in part an intrinsic property of muscle, perhaps reflecting a range of zinc content in normal muscle. But another and much greater source for variation in $(P/P_0)_t$ appears to arise from the compliance added in a series when a muscle is mounted for isometric recording. The variability of $(P/P_0)_t$ decreased as we more uniformly connected a muscle's tibial tendon to the recording-lever. The variations in added compliance that occur with some common linkages have been emphasized recently by Jewell and Wilkie[9]. They note that the total series compliance of isometrically mounted muscles has varied widely in different investigations from 10 per cent muscle length/tetanus tension to 2 per cent muscle length/tetanus tension. From Hill's[10] experiments in which springs of known compliance were added in series with an isometrically mounted muscle one can infer that $(P/P_0)_t$ should vary inversely with added series compliance.

Several investigators[11,12] have reported the related observation that the degree of apparent potentiation can be increased by deliberately adding extra compliance in series with a muscle. We simply wish to emphasize that unintentional variations in added compliance can largely determine the magnitude of potentiation and suggest that the use of muscles with similar values of $(P/P_0)_t$ will help minimize the variability of potentiation data.

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I thank Dr. A. Sandow for his advice.

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PHARMACOLOGY

Pharmacologically Active Extracts from Oak Gall

FELDBERG *et al.*[1,2] have shown that guinea pigs are protected to some degree against a lethal histamine aerosol by injection of extracts of Hungarian oak gall. As well as confirming these results, we have shown that the extracts exert some protection against other agonists. However, the work reported raises the question of the mode of action of these extracts.

Dried alcoholic extracts of Hungarian oak galls (*Andricus quercus-tozas*, Bose) were obtained by the method of Feldberg and Kovacs[1] and solutions were prepared in 0·9 per cent saline for injection. Guinea pigs were randomly divided into two equal groups. One group, normally six animals, received saline intraperitoneally and the other group received 40–50 mg powdered oak gall extract in 2 ml. saline by the same route. After 1 h, the pigs were exposed to an aerosol of the agonist and the time of exposure before convulsions and/or collapse was recorded. Table 1 gives typical results obtained.

Table 1. PROTECTION OF GUINEA PIGS AGAINST AEROSOLS OF VARIOUS AGONISTS

Aerosol	Percentage concentration of agonist (w/v in water)	Mean time to convulsions and/or collapse (sec) Treated	Controls	Statistical significance
Histamine acid phosphate	0·8	450	125	$0·01 > P > 0·005$
Acetylcholine bromide	8·0	173	107	Not significant
5-hydroxytryptamine creatinine sulphate	0·6	266	58	$0·01 > P > 0·005$

A further investigation was made using the technique of Konzett and Rössler[3] as modified by Holgate and Warner[4] to measure the pulmonary resistance in guinea pigs exposed to intravenous injections of histamine, acetylcholine or 5-hydroxytryptamine before and after intraperitoneal or intravenous injection of extract. Intraperitoneal injection of doses of extract which protected animals exposed to histamine aerosol failed to change significantly the responses to any of the foregoing bronchoconstrictors.

The maximum tolerated dose of extract by the intravenous route was ~ 10 mg/kg. In one experiment with this dose of extract, the weight of histamine had to be increased four times to maintain its response. No evidence of antagonism to histamine was obtained with an intravenous dose of 5 mg/kg extract. Neither acetylcholine nor 5-hydroxytryptamine could be antagonized.

The results confirm Feldberg and Kovacs's findings that the extracts confer some degree of protection against a histamine aerosol[1]. Furthermore, they show that a similar protection is afforded against 5-hydroxytryptamine; the less obvious effect against acetylcholine is not statistically significant.

Standard antagonists lead to a reduction in the degree of bronchial resistance as measured by the Konzett–Rössler technique in addition to protecting against an aerosol. In contrast, while affording protection against aerosols, the oak gall extract shows only a slight effect on the degree of bronchial resistance. Although limitations are imposed by the crude nature of the extracts used, it appears possible that the protection afforded may not be achieved by blockade at the drug receptors in the bronchioles, but by some other mechanism.

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Effects of Thiols on Oxytocin and Vasopressin Receptors

IT was shown by van Dyke *et al.*[1] that thioglycollate inactivates oxytocin and vasopressin presumably by reducing the S–S bond in these polypeptides. This effect has been widely used to detect the presence of posterior pituitary hormones in tissue extracts by incubating them with thioglycollate and testing whether their pharmacological activity is afterwards abolished. We have confirmed that thioglycollate inactivates oxytocin and vasopressin, but find that this is a slow process and that the lack of activity of S–S polypeptides in the presence of thioglycollate is largely due to a pharmacological antagonism at the receptor level. Another thiol, α-thioglycerol, also exhibits this antagonism, although its ability to destroy S–S polypeptides is negligible.

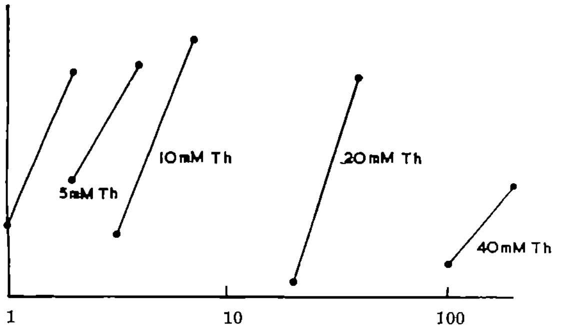

Fig. 1. Displacement of log dose-response curve of oxytocin by thioglycollate. Rat uterus in de Jalon solution at 30°

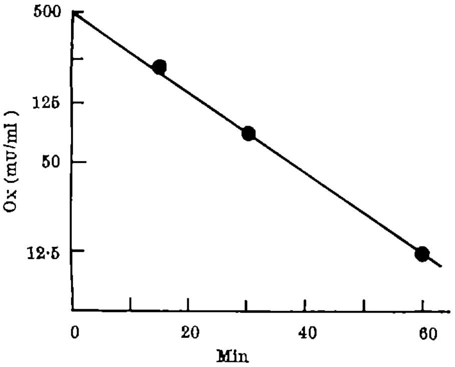

Fig. 2. Rate of destruction of oxytocin when incubated with 20 mM thioglycollate at 20° and pH 7·6

Fig. 1 shows the antagonism of oxytocin by neutralized thioglycollate on the rat uterus. The antagonistic effect can be overcome by increasing the dose of oxytocin; the dose ratio[2] varies from 1·6 for 5 mM to 125 for 40 mM thioglycollate. This antagonism is reversible. Similar effects were obtained on the guinea pig and rabbit uterus. Since in these experiments oxytocin was injected into a bath containing thioglycollate, the question arises whether the antagonism could be due to its rapid destruction as soon as it is added to the bath. To decide this point we tried to measure the destruction-rate of oxytocin incubated with thioglycollate *in vitro*, but this was complicated by interference of thioglycollate contained in the incubation mixtures with the assays. It was found eventually that the destruction-rate is exponential (Fig. 2) so that a valid estimate of the rate constant can be obtained from incubation experiments with relatively high concentrations of oxytocin which can be sufficiently diluted for assay to eliminate interference by thioglycollate.

Fig. 2 shows that the half-life of oxytocin in 20 mM thioglycollate at 20° is 10 min, which is too slow to account for the observed antagonism. At this temperature the dose ratio is 12; assuming that at 20° the uterus takes 4 min to reach its maximum response after adding oxytocin. the calculated destruction in the bath is only about 25 per cent, which gives a dose ratio of 1·3 and not 12. Lys^8-vasopressin is less active on the uterus than oxytocin, but when used in equi-active amounts it is equally antagonized by thioglycollate. This antagonism likewise cannot be due to chemical destruction since the rate of destruction of vasopressin by thioglycollate *in vitro* is similar to that of oxytocin. The uterus contracting effect of bradykinin—a polypeptide which contains no S–S bonds—is not appreciably antagonized by thioglycollate. Further evidence that thiols antagonize independently of destruction is provided by the effects of α-thioglycerol, which antagonizes oxytocin and vasopressin on the uterus, but does not inactivate them appreciably even in 50 mM concentration; α-thioglycerol could thus be used to differentiate posterior pituitary hormones from other substances by an action depending solely on pharmacological antagonism.

It was of interest to find out whether other effects of oxytocin and vasopressin are also antagonized by thioglycollate. So far the isolated mammary strip and perfused hind limb had been investigated. In the mammary strip of the rat[3] 20 mM thioglycollate antagonized the contractile effect of oxytocin but not that of acetylcholine. In the perfused hind limb of the rat[4] no antagonism of vasopressin by thioglycollate was found. These findings suggest that the thiol-sensitive receptors for S–S polypeptides on uterus and myoepithelial cells are different from the vasopressin receptors on blood vessels.

The antagonistic effect of thiols may help to shed light on the receptors on which S–S polypeptides act. One possibility is that the receptor surface itself contains an S–S group which can be inactivated reversibly by thiols, so that the reaction between S–S polypeptide and tissue receptor would be of the nature of an interaction between disulphides, perhaps a disulphide exchange[5] or a chelation involving Mg^{++} or Ca^{++}. An antagonism based on the reversible inactivation of a receptor would be expected to be different from a competitive antagonism such as might be produced by an oxytocin analogue[6]. That this is so is indicated by the log dose-effect curves in Fig. 1, which are displaced to a greater extent by increasing concentrations of thioglycollate than would be expected from a simple competitive antagonism.

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PATHOLOGY

Rate of Cell Proliferation in a Mouse Squamous Cell Carcinoma

Tumours apparently grow faster than normal tissues. It seems to be a widely accepted belief that this always depends on the rapid rate of cell proliferation in malignant tumours. Growth, however, may be defined as the net gain of renewal over loss. If the 'rate of cell renewal', the input, and the 'rate of cell death', the output, are two continuous functions, $F(t)$ and $G(t)$ respectively, then at a moment t the tumour mass (M) is:

$$M = \int_0^t F(t)\mathrm{d}t - \int_0^t G(t)\mathrm{d}t$$

According to this definition, growth is dependent not on the rate of cell proliferation alone, but equally on the rate of cell loss, that is, the life-time (or maturing time) of the cells.

The assumption of a rapid rate of cell proliferation in tumours is based on the high mitotic count observed in many malignant tumours, and on the high number of labelled cells often observed in tumours with the tritiated thymidine technique. It is, however, self-evident that the mitotic count is fundamentally dependent on the mitotic duration. A long mitotic duration will give rise to a high mitotic count without saying anything of the rate of cell proliferation.

The old dogma of a constant mitotic duration for each cell type in a given species may be open for re-evaluation, at least concerning rapidly changing cell populations as epidermal cells. It has been demonstrated[1] that both mitotic duration and DNA-synthesizing time may vary considerably in mouse epidermis after application of carcinogenic substances. It has also been demonstrated that[2] the well-known daily rhythm in mitotic count in the epidermis of the mouse may be explained by variation in the mitotic duration without any significant change in the mitotic rate. Setälä[3], and Baserga *et al.*[4] have indicated that some tumours do not necessarily proliferate faster than the normal tissues of origin.

We have observed the rate of cell proliferation in an epidermoid tumour of the skin of a hairless mouse. The tumour was induced by five paintings with a 1 per cent solution of 3-methylcholanthrene in benzene and was growing rapidly when the observations were made. Histology showed a typical squamous cell carcinoma. This animal was injected with 10 μc. tritiated thymidine intraperitoneally half an hour before a biopsy of the tumour was taken. Immediately after biopsy, the animal was injected intraperitoneally with 0·1 mg 'Colcemid'. The animal was in a rather poor condition after the ether narcosis, but recovered and was finally killed 5 h after the 'Colcemid' injection. The skin with the tumour was stripped off and immediately immersed in a 4 per cent solution of neutralized formalin and afterwards embedded in paraffin. After cutting sections of the specimens, autoradiography was performed and the number of mitoses, labelled cells and labelled mitoses were registered among 30,000 tumour cells.

The results were as follows: Mean count of mitotic figures in the biopsy specimen obtained before the 'Colcemid' injections: 1·75 per cent of all cells present. The percentage of labelled cells was 8·38. Mean count of mitotic figures 5 h after the 'Colcemid' injection was 2·16 per cent of all cells present.

When, from the results shown here, we calculate the mitotic duration after the method described by Leblond and Stevens[5], the mitotic duration is estimated to about 4 h. If proportionality between mitotic count/mitotic duration, and number of labelled cells/DNA-synthesizing time exists, the DNA-synthesizing time can be calculated to about 20 h.

The number of labelled mitoses 5·5 h after the injection of tritiated thymidine was found to be 2·5 per cent of all mitoses, a value which is rather low in comparison with normal values from the epidermis. This may indicate that even the premitotic resting phase, G_2, is prolonged.

The normal mitotic duration in the epidermis of this strain of hairless mice at this hour of the day is found to be about 1 h, and the normal DNA-synthesizing time about 5 h[1]. The mitotic rate in normal epidermis of these mice is about 0·6 per cent of all nucleated epidermal cells per hour[1]. If the mitotic rate is calculated in the epidermoid carcinoma described here, it is found to be 0·4 per cent per hour.

It is fully recognized that observations from only one tumour is scarce information from which to form a definite conclusion. This tumour, however, seems to be characterized by a low mitotic rate, a high mitotic count, a long mitotic duration and a long DNA-synthesizing time, that is, a slow process of cell proliferation.

The rapid growth of this tumour may be a result of a prolonged life-time of the cells, which, instead of being normally differentiated to horny material and lost, remain relatively undifferentiated.

The routine use of a high mitotic count as a sign of rapid proliferation rate in many tumours may be disputed.

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IMMUNOLOGY

A Screen for Agents Inhibiting the Immune Response and the Growth of Tumours

IN a previous communication[1] it was shown that there is a correlation between the ability of an agent to inhibit antibody production and its ability to inhibit tumour growth. Single doses of agent and T.A.B. vaccine were given simultaneously, and sera were titrated 9 days later. Under these conditions 5 of 12 compounds known to be active inhibitors of tumours in man or laboratory animals were also found to depress antibody production to a moderate or a marked extent. Of 12 compounds which were not tumour-inhibitory, none did so, even in lethal doses.

It is well known that the effect of radiation on immune processes is markedly dependent on timing; irradiation 1–2 days before administering antigen greatly inhibits antibody production, but even lethal doses of radiation are relatively ineffective if given after the antigen[2]. It was found that dependence on timing also characterized the activity of the alkylating agents and antimetabolites[3]. However, it was surprising to find that the time for most effective administration of most agents was not before injection of antigen, as it is with radiation, but 1–2 days afterwards. 'Myleran' was the only agent that resembled radiation in this respect. It seemed likely that, if dependence on time were taken into account, it might be possible to improve the correlation between the tumour-inhibiting capacity of an agent and its power to depress antibody production.

Male *A2G* mice, weighing 15–21 g, were given 0·2 ml. T.A.B. vaccine intraperitoneally and, 2 days later, a single dose of agent subcutaneously. The dose was such as to kill about 10–60 per cent of mice during the following week. Nine days after injection of vaccine, equal volumes of sera from groups of 3–5 survivors were pooled, sufficient mice being used to

microanalysis

Oh, thee!
I would not thee mislead
Who canst the smallest particle impel
To tell
Its inmost secret thought
So tell I thee indeed
Within the labyrinth of myself is caught
Of sulphur a one millionth part
For every one of me

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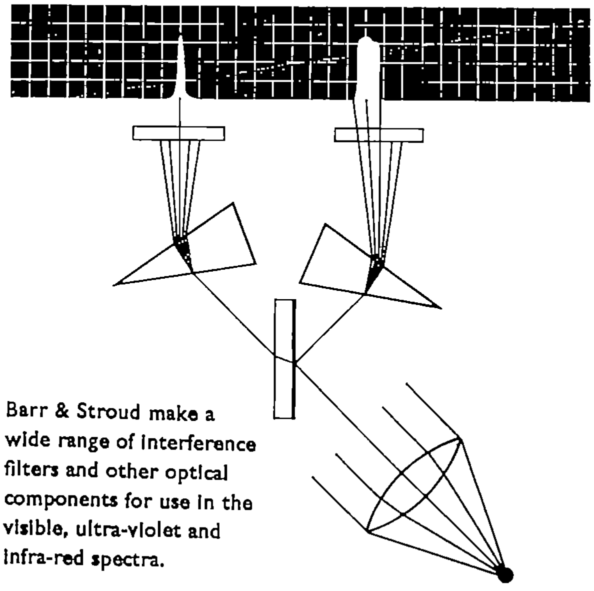

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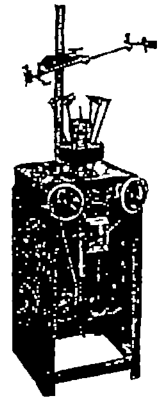

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Table 1. EFFECT OF TOXIC DOSES OF VARIOUS AGENTS ON ANTIBODY PRODUCTION. (EACH POINT REPRESENTS A SERUM POOL FROM A GROUP OF 3–5 MICE)

Agent	Dose (mg/kg)	Survivors	Titre						
			<2	2	3	4	5	6	7
						← Normal range →			
MISCELLANEOUS AGENTS									
Alloxan	600	10/12						⁝	
Bacitracin	1,050	12/21					:	.	
Benzimidazole	550	15/20						⁝	
Boric acid	3,600	9/24					:	.	
Caffeine	290	10/12					.	:	
Desoxypyridoxine	250	14/20					.	⁝	
Erythromycin	5,000	10/12						⁝	
D-Glucosamine	8,000	10/12						:	.
Griseofulvin (i.v.)	250	11/15					:		.
Neomycin	300	17/22					⁝		
Polymyxin '*B*'	50	13/36					:	.	
Potassium cyanide	8	11/30					:	.	
Sodium fluoracetate	10	9/12					:	⁝	
Sodium nitrite	185	19/34					:	⁝	
Streptomycin	750	17/20					:	⁝	.
Terramycin	550	14/18					.	:	.
ANTI-TUMOUR AGENTS									
Urethane	1,650	21/36						⁝	:
Chlorambucil	40	12/15		.		.	.		
Vincaleukoblastine	8	20/24	: :	.			.		
Mannomustine	100	10/12		.	:				
Thiotepa	18	10/12	.		:				
Nitrogen mustard	2·6	11/12	.	.	.				
5-Fluorouracil	250	13/24	⁝		.				
Triethylene melamine	2·5	9/20	.	:					
Amethopterin	10	9/12	.	:					
Melphalan	12	8/18	:						
Cyclophosphamide	350	10/12	⁝						
6-Mercaptopurine	300	11/12	⁝						
6-Thioguanine	90	13/24	⁝						

provide 2–6 such serum pools, which were then titrated for *S. typhi H* agglutinins by the Widal technique.

The response of normal mice was measured in 84 groups of 4 or 5 mice. The geometric mean titre was $1/2^{5\cdot49} \pm {}^{0\cdot67}$ (range $1/2^4$–$1/2^7$). Occasional batches of vaccine which apparently lacked potency, giving titres below this range, were not used.

Twenty-nine compounds were tested as already described. Of these, 16 were miscellaneous agents without anti-tumour activity and 13 were agents useful in treating neoplasia in man. None of the miscellaneous compounds depressed antibody production; no sera from treated mice had titres of less than $1/2^5$. In contrast, of the 13 anti-tumour agents, 12 inhibited antibody production to a significant extent and, with 10 of them, no sera from treated mice had titres in the normal range. 'Chlorambucil' was marginally effective, one serum pool out of three having a titre well below the normal lower limit. Urethane was the only agent tested that was immunologically ineffective under the conditions of this experiment (Table 1).

This test therefore appears to be useful in screening agents for ability to suppress the immune response and to inhibit the growth of tumours, except for agents such as radiation and 'Myleran', which are effective only if given before the antigen. Indeed, its efficiency in selecting tumour-inhibitory compounds is not inferior to that of many screening methods in which the effect of the agent on tumours is measured directly[4].

I thank Prof. F. Bergel of the Chester Beatty Research Institute, the Wellcome Research Laboratories, Lederle Laboratories, Roche Products, Ltd., and Leda Chemicals for gifts of chemicals.

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Kidney Specific Antigen and its Species Distribution

THE cells of a normal organ or tissue contain characteristic material which distinguishes them from other cells. Such organ-specific material is abundant in cell microsomal fractions which contain endoplasmic reticulum[1], and these fractions are used as antigens for the preparation of organ-specific antisera.

Anti-kidney sera were produced in rabbits by immunization with microsomal material from fresh human and hamster kidneys; cell fractionation and general immunological methods were the same as those used in previous studies of organ-specificity[2,3]. The anti-human-kidney serum was made kidney-specific by absorption with homogenates of human lung and liver; the anti-hamster-kidney serum was absorbed with hamster tissue and required additional absorption with sheep red cells to remove Forssman antibody.

The reactions with human and hamster tissues of the antisera before and after absorption were studied by immunofluorescence, gel diffusion, and complement fixation, and are summarized in Tables 1 and 2. Immunofluorescence staining of fresh frozen sections of kidneys and other organs was carried out by the sandwich method using goat anti-rabbit-globulin conjugated with lissamine rhodamine *B* (*RB* 200)[4].

Specific staining of human and hamster kidney was stronger when the kidney was treated with the antiserum against the corresponding species. With unabsorbed antiserum, staining of an unsystematic character but mainly endothelial in distribution was also obtained in other organs (liver, heart, testis and epididymis), but after absorption of the serum with homologous liver and lung homogenates, there was virtually no reaction with any organ other than kidney. The staining in kidney by such absorbed antisera was found mainly in the cell membranes of the nephron, particularly in the brush border of the proximal convoluted tubules. There was also some intracellular staining much the same as that reported previously for the hamster[2] but more diffuse in the tubular cell cytoplasm of the human kidney. Glomer-

Table 1. REACTIONS OF UNABSORBED AND ABSORBED ANTI-HUMAN-KIDNEY SERUM

		Immune serum				Normal (pre-immune) serum	
Absorptions by human tissue homogenates		Nil	Liver	Liver + lung	Kidney	Nil	Liver
Immunofluorescence staining							
Kidney	Human	+ + +	+ + +	+ +	–	(–)	–
	Hamster	+ +	+ +	+	–	(+)	–
Liver Heart Testis Epididymis	Human	+	(+)	(–)	–	(–)	–
	Hamster	(+)	–	–	–	(+)	–
Gel diffusion lines against human kidney		3	2	1	0	0	0
Complement fixation against kidney antigen (8γN)	Human	1/160	1/160	1/40	< 1/5	0	..
	Hamster	1/10	1/10	1/10	< 1/5	0	..

(+) Slight unsystematic staining. (–) Virtually no staining.

Table 2. REACTIONS OF UNABSORBED AND ABSORBED ANTI-HAMSTER-KIDNEY SERUM

		Immune serum				Normal (pre-immune) serum	
Absorptions with hamster tissue homogenates		Nil	Lung + sheep red cells	Lung + sheep red cells + Liver	Lung + sheep red cells + Kidney	Nil	Lung + liver
Immunofluorescence staining							
Kidney	Hamster	+ + + +	+ + +	+ +	(–)	–	–
	Human	+ +	+	+	–	(+)	–
Liver Heart Testis Epididymis	Hamster	+ +	+	(–)	(–)	(–)	–
	Human	–	–	–	–	–	–
Gel diffusion lines against hamster kidney		2	2 (1 Faint)	1 (Faint)	0	0	0
Complement fixation against kidney antigen (8γN)	Hamster	1/80	1/40	1/10	< 1/5	0	.
	Human	0	0	0	0	0	..

ular staining, although detectable, was not conspicuous. There was no specific staining with pre-immune sera or with either antiserum absorbed with human or hamster kidney.

Gel diffusion and complement-fixation corroborated the organ-specificity of the sera: antibody activity persisted despite repeated serum absorptions by lung and liver homogenates but was virtually abolished by absorption with kidney homogenate. These serological methods were less sensitive than immunofluorescence for detecting species cross-reactions though some cross-reactivity between hamster kidney and anti-human-kidney serum was demonstrated by complement fixation; this activity was abolished by absorbing the serum with human kidney homogenate.

Table 3. RELATIVE FLUORESCENT STAINING BY KIDNEY-SPECIFIC ANTISERA OF KIDNEYS FROM VARIOUS ANIMAL SPECIES

	Anti-human-kidney serum	Anti-hamster-kidney serum
Human	Very intense	Intense
Hamster	Intense	Very intense
Other mammals (cat, rodents)	Intense	Intense
Birds (hen, pigeon)	Moderate	Moderate
Reptiles (chameleon, lizard)	Nil	Nil
Amphibians (frog, toad)	Nil	Nil
Fish—marine (cod, plaice, saithe)	Faint	Faint
Fish—freshwater (brown trout, salmon, goldfish)	Very intense	Intense

The results of immunofluorescence staining of various other animal kidneys by each of the kidney-specific antisera are summarized in Table 3. Kidney-specific antigen was found in all the mammals and birds studied, but the intensity of staining in the latter was not strong; the histological distribution was also substantially the same as already described in that cell membrane staining was always found. No reaction could be demonstrated with reptile or amphibian kidneys, but the marine fish kidneys showed faint staining of the cell membranes lining the lumen of some of the tubules. In contrast, the kidneys of the freshwater fish examined, in particular the brown trout, showed brilliant staining of parts of the tubules (Fig. 1). The kidney-specific material was more abundant than in any other species and its histological localization was different: it was confined to a broad intracellular band adjacent to the tubular lumen and giving an appearance remarkably reminiscent of a glandular secretion. In none of the species examined was any specific staining demonstrated in organs other than kidney.

The implications of these variations in kidney-specific material among the species could scarcely be surmised without more exhaustive comparative zoological studies, but its abundance in the freshwater fish might conceivably be related to special renal functional demands. Such a correlation with function would have a parallel in the behaviour of thyroid microsomal antigen, which is increased in hyperthyroidism[5].

One outcome of these findings is the suggestion that immunofluorescence may be of considerable value in providing a simple method of investigating hitherto unexplored histochemical similarities and fine differences between species.

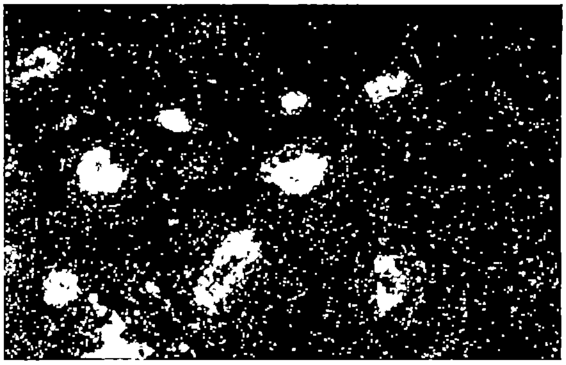

Fig. 1. Fresh frozen section of brown trout kidney, fixed in absolute ethanol at –76° C and treated first with rabbit anti-human-kidney serum absorbed with human liver and lung homogenates, and then with *RB* 200-conjugated goat anti-rabbit-globulin. Bright fluorescent staining, indicating kidney-specific antigen, is conspicuous at the inner border of the cells of several of the tubules. The dim tubular fluorescence is solely autofluorescence and the black patches, bottom left, are the normal pigment of fish kidney

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BIOLOGY

Malic Enzyme Activity in Apple Fruit

The capacity of apple fruit to decarboxylate malic acid oxidatively (the malate effect) is evident early in apple fruit ontogeny[1], as well as in the stages characterized by the climacteric and post-climacteric phases of fruit respiration[2]. The stoichiometric characteristics of the malate decarboxylation reaction[1] and the symmetrical distribution of carbon-14 in malic acid resulting from $^{14}CO_2$ fixation in apple fruit[3] suggested the malic enzyme may function in both reactions. This investigation was initiated to determine if malic enzyme is responsible for malate decarboxylation and carbon dioxide fixation into malic acid in apple fruit.

McIntosh fruit were harvested at 2- or 3-day intervals, beginning approximately one week prior to the autogenous respiratory climacteric and continued into the post-climacteric stage. Procedures for preparation of peel tissue disks and manometric determination of malate decarboxylation as described by Neal and Hulme[2] were used. The assay conditions are given in the legend to Fig. 1. The malic enzyme was extracted from cortical tissue of pre- and post-climacteric McIntosh fruit. The diced cortical tissue was vacuum infiltrated with 0·2 M potassium carbonate to neutralize the acids and protect the enzyme against denaturation[4], disintegrated at 0° C with an equal weight of neutral 0·01 M cysteine, and filtered through a milk filter pad. Adaptation of 'Carbowax 4,000' fractionation procedure 1 (ref. 5), through step 3, resulted in sufficient purification of the malic enzyme for the purpose of this investigation. Glycylglycine was substituted for phosphate buffer in view of interference with manganese in the enzyme assay used. Malic enzyme activity was determined spectrophotometrically at 340 mμ subsequent to the reduction of triphosphopyridine nucleotide (TPN) by the enzyme in the presence of L-malate. The conditions of the assay are given in the footnote of Table 1.

Table 1. ACTIVITY OF MALIC ENZYME OBTAINED FROM McINTOSH APPLE FRUIT AT DIFFERENT STAGES OF MATURITY*

Stage of fruit maturity	Specific activity units (mg protein)
Immature (small green)	12
Mature (pre-climacteric)	15
Over-ripe (post-climacteric)	29

* The assay consisted of: glycylglycine, 75 μM; $MnSO_4$, 0·18 μM; TPN, 0·24 μM; L-malate, 9 μM; and enzyme in a total volume of 3·0 ml. at *p*H 6·4 and 20° C.

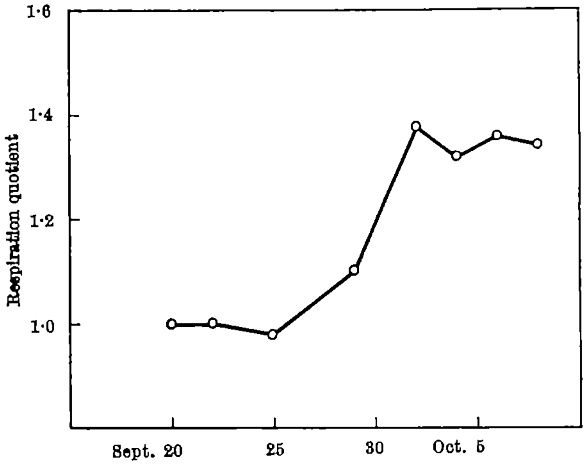

Fig. 1. Respiratory quotient for malate respiration of peel disks from McIntosh fruit gathered at various stages of maturity. The reaction mixture contained 0·5 g peel disks in 0·067 M phosphate buffer, *p*H 4·0 and 0·1 M L-malate in a Warburg vessel in the dark at 30° C with air as the gas phase

Protein content of the enzyme preparation was determined spectrophotometrically[6]. Enzyme activity is expressed in the unit defined by Ochoa[7].

The onset of the malate effect in McIntosh peel tissue was found to coincide with the beginning of the respiratory climacteric as reported for Bramley's Seedling[2] and Cox's Orange Pippin[1]. The respiratory quotient for malate respiration (Fig. 1) was close to unity prior to the onset of the climacteric and reaches a plateau value between 1·3 and 1·4 within one week. Maximal rate of malate decarboxylation was attained at approximately 0·02 M L-malate (Fig. 2). According to Neal and Hulme[2], the malate concentration within the peel tissue is approximately one-tenth the ambient concentration[2]. On this basis the malate concentration at half-maximal velocity (the apparent K_S for L-malate) would be approximately 1×10^{-3} M at *p*H

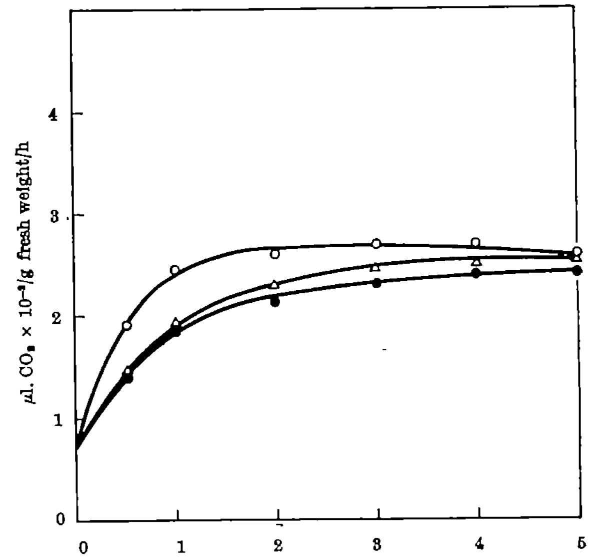

Fig. 2. Influence of L-malate concentration alone (○) and in the presence of 0·02 M malonate (Δ) or D-malate (●) on malate decarboxylation by apple peel disks. Malonate and D-malate were added after 1 h in the presence of L-malate

4·0 and 30° C. The reaction is specific for L-malate. In contrast, D-malate and malonate at 0·02 M competitively inhibited L-malate decarboxylation.

McIntosh apple fruit cortical tissue was found to contain approximately 50 units of malic enzyme activity per gram fresh weight on the basis of the assay used. This amounts to the equivalent of 336 µl. carbon dioxide per hour calculated from the extinction coefficient of TPNH ($6·22 \times 10^6$ cm^2 mole^{-1}) and the stoichiometric equivalence of TPNH and CO_2 in the malic enzyme reaction. This is the same order of magnitude as the malate decarboxylation-rate of the peel disks. Fractionation with 'Carbowax' resulted in approximately 8-fold purification with recovery of more than 90 per cent of the enzyme activity obtained in the initial extraction. The specific activity of the initial extract averaged about 5, while that of the second 'Carbowax' precipitate varied from 30 to 40 in post-climacteric fruit. The specific activity (enzyme units/mg protein) of malic enzyme was higher in post-climacteric than in pre-climacteric McIntosh fruit (Table 1). The increase in specific activity is interesting in view of the net protein synthesis observed to occur during the course of the climacteric[9]. However, a more exhaustive study will be required to establish the significance of this observation in relation to the malate effect[2].

The pH optimum of apple fruit malic enzyme was dependent on L-malate concentration[8], but not on the ionic strength of the assay medium. Activity was dependent on the presence of a divalent cation[7], the order of effectiveness being Mn > Co ≫ Mg. The enzyme was specific for TPN and L-malate; neither DPN nor D-malate would substitute. Dihydroxymaleate and dihydroxytartarate at 1×10^{-3} M were 50 and 75 per cent inhibitory, respectively; while malonate, maleic, and meso-tartarate were 10–15 per cent inhibitory at the same concentration.

The general properties of the fruit enzyme are similar to those reported for wheat germ[7] and pigeon liver[7,8]. The equilibrium constant (L-malate) (TPN^+)/(pyruvate)(TPNH)(carbon dioxide) = $19·6^{-1}$ M at pH 7·4 and 22° C[10] is favourable in the direction of fixation of carbon dioxide in a closed system. The partial pressure of carbon dioxide within the fruit and the ratio of TPN/TPNH may be important considerations in determining the direction of the reaction. A conservative estimate would place the malic acid concentration at 0·03 M in the mature apple fruit and hence would not be limiting to the decarboxylation reaction providing this concentration is available to the enzyme. However, Hulme[11] has induced a pre-climacteric malate effect by injection of malic acid into the fruit which may indicate a concentration gradient of malic acid between active (cytoplasmic) and inactive (vacuolar) pools[1]. Non-washed fruit peel tissue, with an endogenous reserve of malic acid, decarboxylates added malate, indicating that a sub-maximal concentration of endogenous malate is present in the cytoplasm.

The demonstration of malic enzyme activity in apple fruit supports its postulated participation in oxidative decarboxylation of malate[2] and the fixation of carbon dioxide into malic acid[3]. Evidence for malic enzyme activity in pre-climacteric fruit support the occurrence of the malate effect early in the ontogeny of fruit[1]. It is postulated that the malate effect is a reflexion of metabolic processes concerned with pyruvic acid utilization and is perhaps regulated by the availability of oxidized pyridine nucleotide. The quantity of malic enzyme is not considered to be the limiting factor in malate decarboxylation in matur apple fruit tissue.

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Separation of the Enzymes present in the Mitochondrial Fraction from Apple Peel

As a result of investigations of the respiration of disks of apple peel, it was suggested[1] that the extra carbon dioxide evolved during the period of the respiration climacteric in apple fruits may be due, at least in part, to the operation of a malic enzyme system. Dilley[2] has recently isolated a malic enzyme, dependent on TPN and Mn^{++}, from American apples. Using Dilley's method of isolation we have confirmed the existence of a similar enzyme in the pulp of the Cox's Orange Pippin apple. We have, however, also found a similar enzyme in mitochondria isolated from the peel of the same apples[3]. At this stage it should be emphasized that by 'mitochondria' is meant the centrifugate obtained under standard conditions and that, while all the evidence suggests[3] that the preparation contains a high proportion of intact mitochondria, other particles and agglomerates having the same size/density characteristics could be present. For example, the preparations contain variable amounts of polyphenolic material[4].

The activity of the malic enzyme from various preparations obtained from apple fruits are shown in Table 1. (In Table 1 the following abbreviations are used: C.O.P., Cox's Orange Pippin Apples; p.c., postclimacteric condition; C.A., storage in a controlled atmosphere (3 per cent oxygen and 5 per cent carbon dioxide; this is often referred to as 'gas storage' in Great Britain).)

In all cases the specific activity was determined by the spectrophotometric method of Ochoa[5], except that with our preparations the protein content was measured as the nitrogen insoluble in trichloroacetic acid, multiplied by 6·25. It was impossible to obtain an accurate measure of the activity of the malic enzyme in our mitochondria by the manometric

Table 1. ACTIVITY OF MALIC ENZYME OF APPLES OBTAINED FROM VARIOUS SOURCES

Source	Specific activity	Total activity (per 2·5g original tissue)
Cortical tissue of p.c. McIntosh apples (Dilley, preceding communication)	29	—
Cortical tissue of p.c. C.O.P. apples	23	—
Mitochondria of peel of cold stored p.c. C.O.P. apples	12	30
Mitochondria of peel of C.A. stored p.c. C.O.P. apples	8	—
S.N. fraction of sonicated mitochondria from cold stored p.c. C.O.P. apples (10 sec)	58	21
(50 sec)	41	34

determination of the oxygen absorbed and the carbon dioxide evolved because, as will be seen later, they contained, in addition to DPN and the usual DPN-dependent malic dehydrogenase, a very active carboxylase dependent on Mn^{++}. The result given in Table 1 for the supernatant (S.N.) fraction of sonicated mitochondria refers to suspensions of mitochondria treated in a sonic vibrator (25,000 cycles, 60 W) and then centrifuged at 25,000*g* for 15 min in a refrigerated centrifuge.

It remains for future work to decide whether the 'soluble' malic enzyme obtained by Dr. Dilley and by ourselves from the apple tissue arises from the breakdown of mitochondria during the isolation procedure, or whether the malic enzyme found in the mitochondrial preparations is merely 'soluble' enzyme absorbed on the mitochondria. Rutter and Lardy[6] found a malic enzyme of low specific activity in mitochondrial preparations from pigeon liver. The relatively low specific activity of the enzyme in our own mitochondrial preparations is not surprising since much of the protein in the preparations will consist of the enzymes operating the Krebs cycle. In addition, we know that the preparations contain an appreciable amount of inactive protein brougnt down by the tannin present in the material even when polyvinylpyrrolidone is used in its preparation[4].

The mitochondrial fraction also contains an active carboxylase. Some co-carboxylase must also be present, since addition of thiamine pyrophosphate increases the carbon dioxide liberated from pyruvate by only about 30 per cent. The carboxylase activity of the mitochondrial suspensions and of the supernatant solutions following high-speed centrifugation (as for malic enzyme, here) of sonicated particles is shown in Table 2. Manganese is necessary for the action of the enzyme; magnesium, at the same concentration, is only about 60 per cent as effective as manganese. The optimum *p*H of the enzyme is 6·2. The activities given in Table 2 were measured in a Warburg respirometer, the contents of each flask being as follows: 40 µmoles pyruvic acid, 400 µmoles sucrose, 18·7 µmoles potassium dihydrogen phosphate, 10 µmoles manganese sulphate, 0·1 µmole co-carboxylase, 0·25 ml. mitochondrial suspension and water to make to 2 ml.

Table 2. CARBOXYLASE ACTIVITY (AT *p*H 6·2) OF PREPARATIONS FROM THE PEEL OF MATURE C.O.P. APPLES

Source	Specific activity*	Total activity (per 2·5 g original tissue)
Whole mitochondria	11	27
S.N. fractions of sonicated mitochondria		
10 sec	27	11
50 sec	14	12

* One enzyme unit = amount of enzyme required to produce 1 µl. carbon dioxide in 5 min at 25° C under conditions described in text. Specific activity = units per mg protein.

The activities of these crude preparations are, of course, only very approximate and no allowance has been made for the inactivation due to the accumulating acetaldehyde. Nevertheless, the relatively high carboxylase activity could explain the large carbon dioxide evolution and the formation of acetaldehyde in the 'malate effect' described by Neal and Hulme[1] for peel tissue from postclimacteric apples.

The problem now arises of the separation and purification of these enzymes associated with the mitochondrial fraction, especially in order to study changes occurring during the respiration climacteric and the subsequent senescence of the fruit.

Sonication (as here) causes an obvious physical breakdown of the mitochondrial particles and, on high-speed centrifugation, a green supernatant solution is obtained which contains Krebs cycle enzymes of relatively high specific activity (malic dehydrogenase 50 per cent higher after 40 sec sonication than in the original unsonicated suspension; diaphorase, 30 per cent higher). The activity of these Krebs cycle enzymes increases with time of sonication between 10 and 100 sec, whereas the malic enzyme and the carboxylase tend to have a higher activity at the lower sonication times. This suggests that the latter two enzymes may be less tightly bound to the structure of the particles. Sonication of the preparations will clearly be a most useful first step in the isolation of the enzymes present in the mitochondrial fraction.

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Effects of Kinetin and Other Plant Growth Regulators on Starch Degradation

IT has been known since the work of Hayashi[1] that gibberellins stimulate the activity of amylase in germinating barley and wheat grains. This work has been extended by Paleg[2-4], who has reported an increase in the activity of both α- and β-amylases in barley endosperm following treatment with gibberellic acid (GA_3). So far as we are aware this communication is the first to suggest that kinetin may also play a part in starch degradation. This effect of kinetin was first suspected when young excised wheat coleoptiles treated with solutions of gibberellic acid and kinetin[5] were stained with iodine. Starch was found to be present in the control coleoptiles whereas in those treated with the two growth regulators no starch was detectable. Estimations of reducing sugars in extracts of these coleoptiles, after treatment, also demonstrated that the growth response promoted by these two growth regulators was accompanied by starch breakdown (Table 1). When excised coleoptiles were grown in D-glucose solution alone the starch remained and yet the maximal growth response was as great as with kinetin. These results thus indicated that the growth responses induced by GA_3 and kinetin in young coleoptiles could be explained, at least in part, simply in terms of the liberation of D-glucose.

Table 1. AVERAGE OF THREE REPLICATE TREATMENTS OF 20 EXCISED DAY-OLD COLEOPTILES AFTER 24 H AT 25° C IN DARKNESS IN A KLINOSTAT (1 R.P.M.)

Treatment	Elongation as percentage above original length	Coleoptiles stained for starch with iodine	Reducing sugar content of extracts from 20 coleoptiles: Mg equiv. D-glucose	Reducing sugar content of extracts from 20 coleoptiles: Significant difference from controls
GA_3 10^{-4} M	56·9	negative	0·214	$P = 0.02$
K 10^{-4} M	41·7	negative	0·182	$P = 0.01$
IAA 10^{-4} M	11·1	positive	0·146	$P = 0.05$
C water	10·7	positive	0·157	
D(+) glucose 10^{-1} M	45·0	positive	—	

GA_3, Gibberellic acid; IAA, indolyl-3-acetic acid; K, kinetin.

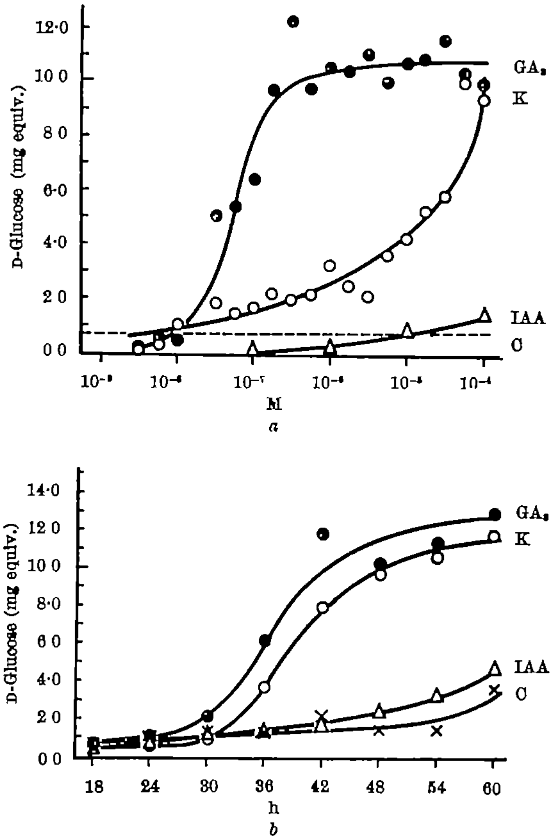

Fig. 1. Reducing sugar released from seed halves by treatment with gibberellic acid (GA$_3$), kinetin (K) and indolyl-3-acetic acid (IAA) at 26° C in darkness. *a*, Dosage response curves obtained after 36-h treatment, *b*, the effects of 10^{-4} M solutions after incubation for different periods of time

Indolyl-3-acetic acid produced no measurable effect on growth or starch breakdown (Table 1).

In order to overcome the complicating factor of growth, experiments similar to those of Paleg[2] were carried out using wheat instead of barley endosperm. Working under sterile conditions, wheat seeds were cut in half longitudinally and the embryo halves removed and discarded. Ten seed halves were placed on filter paper in Petri dishes containing 8 ml. of solution, and the reducing sugars liberated into the medium after various periods at 26° C were determined by Nelson's colorimetric modification of Somogyi's method[6]. The results were expressed as mg equivalents of D-glucose.

Dosage response curves were obtained for gibberellic acid, kinetin and indolyl-3-acetic acid after treatment of the seed halves for 36 h at 26° C in darkness, and these are shown in Fig. 1*a*. The maximum release of reducing sugars by gibberellic acid was given by a 5×10^{-6} M solution, whereas with kinetin a 10^{-4} M solution was required and even at this concentration the amount of reducing sugar released into the medium was less than with gibberellic acid. The results also indicated that indolyl-3-acetic acid caused little, if any, breakdown of starch. In another experiment the effects of 10^{-4} M solutions of gibberellic acid, kinetin and indolyl-3-acetic acid on seed halves incubated for different periods of time from 18 to 60 h were investigated and the data are given in Fig. 1(*b*). This experiment again demonstrated the similarity of kinetin and gibberellic acid, although kinetin was again less efficient than gibberellic acid in liberating reducing sugars. Preliminary experiments using paper chromatography to analyse starch degradation sugars suggest that kinetin, like gibberellic acid, affects amylases rather than the phosphorylase degradation pathway. The differences in the dosage response curves for gibberellic acid and kinetin could be explained if these two substances differ in: (*a*) their rates of movement to their site(s) of action at cell level; or (*b*) the basic mechanism by which they operate; possibilities here include the removal of an amylase inhibitor[7-9], their effects on the properties of the cell membranes, or even, perhaps, their promotion of enzyme synthesis.

Further work is in progress to find out which, if any, of these possibilities could account for the differences observed.

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Growth of *Nardus stricta* on a Calcareous Soil

Recent work[1] has indicated that the addition of phosphate enables many grass species to grow successfully on a well-drained, highly calcareous soil which, otherwise, would not support their growth. The work recorded here was carried out to determine whether the addition of phosphate would enable a true calcifuge species[2], *Nardus stricta*, to grow on highly calcareous soils.

The form of the experiment and the results obtained are summarized in Table 1. The *Nardus* seedlings were transferred to boxes containing the soils immediately on germination and were harvested after twelve weeks. Each treatment, which included eight plants, was replicated three times.

The results show that *Nardus stricta* tolerates and indeed makes excellent growth on the calcareous soils where phosphate has been added as $CaHPO_4.2H_2O$; in such instances the plants showed no trace of chlorosis and inflorescences were developed. Comparison of columns *a* and *b* in Table 1 indicates that the beneficial effect of adding $CaHPO_4.2H_2O$ is not due to any lowering of the soil *p*H value. No beneficial effect was brought about by the addition of phosphate in the form of the potassium salts to the calcareous soils, and the death of plants in such cases is probably due to the harmful influence of the exceptionally high *p*H values, which arise as a consequence of adding large amounts of potassium salts to calcareous soils.

It has been suggested[3,4] that inability to obtain sufficient phosphate may be an important factor in restricting the growth of calcifuge plants on calcareous soils. Results from the above experiment

Table 1. MEAN SHOOT DRY WEIGHT (G) OF *Nardus stricta*. FIGURES IN BRACKETS ARE MEAN SOIL *p*H VALUES

	(*a*) —		(*b*) +2 per cent $CaHPO_4.2H_2O$		(*c*) +2 per cent KH_2PO_4		(*d*) +2 per cent K_3PO_4		(*e*) +2 per cent K_2SO_4	
John Innes No 1 potting compost	0·28	(6·5)	0·44	(7·0)	0·56	(7·3)	*	(8·7)	0·29	(8·0)
John Innes No. 1 potting compost + 2·5 per cent $Ca(OH)_2$	0·005	(8·5)	0·24	(8·7)	*	(10·0)	*	(10·2)	*	(9·1
John Innes No. 1 potting compost + 4·0 per cent $Ca(OH)_2$	0·005	(8·6)	0·17	(8·8)	*	(9·8)	*	(10·0)	*	(8·8)

* Seedlings died within a week of planting. *L.S.D.* at 5 per cent for plant weights, 0·07 g. *L.S.D.* at 5 per cent for *p*H values, 0·80.

Table 2. MICROGRAMS ALUMINIUM AVAILABLE PER GRAM OF AIR-DRIED SOIL

	—	+2 per cent $CaHPO_4.2H_2O$	+2 per cent KH_2PO_4	+2 per cent K_3PO_4	+2 per cent K_2SO
Jonn Innes No. 1 potting compost	6·5	6·0	8·0	30·3	5·0
John Innes No. 1 potting compost + 2·5 per cent $Ca(OH)_2$	27·3	5·0	25·0	28·0	35·1
John Innes No. 1 potting compost + 4·0 per cent $Ca(OH)_2$	32·3	4·5	29·1	30·0	45·0

L.S.D. at 5 per cent, 10·1 μg aluminium.

showed, however, that $CaHPO_4.2H_2O$ may act, not only directly by supplying extra phosphate to the plant, but also indirectly by reducing the amount of available aluminium in the soil. Table 2 gives the data on the available aluminium content, estimated from water extracts[5] of the soils at the completion of growth.

It will be seen that there is throughout a strong negative correlation between the growth made and the available aluminium content of the soils. It is well known that aluminium is often toxic to plants, especially under very acid soil conditions where aluminium would exist in solution mainly as Al^{+++}. In soils with high *p*H values aluminium would come into solution as the aluminate ion[6], but the influence of the aluminate ion, as such, on plant growth is as yet unknown.

At present, it is not possible to distinguish between the direct and indirect effects of the extra phosphate added as $CaHPO_4.2H_2O$. There is, however, no doubt that it enables a typical calcifuge species to tolerate, and grow successfully on, highly calcareous soils despite both their high calcium content and their high *p*H.

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Dyes as Artificial Hatching Agents for Beet Eelworm, *Heterodera schachtii* Schm.

ARTIFICIAL hatching agents for *Heterodera schachtii* have often been sought[1], but only two substances, 3,9-diamino 7-ethoxyacridine (rivanol) and 9-amino-acridine, have been reported to give hatches approaching that obtained in sugar-beet root diffusate[2].

I have tested 70 dyes from 16 dye-groups on replicated batches of 100 cysts of *H. schachtii* and found large differences between their ability to affect hatching. Solutions of several dyes give hatches as great as obtained in a standard beet root diffusate; others stimulate hatch less, but give a significantly larger one than is obtained in water, and many are inactive (Table 1). Tests with the most active dyes at different dilutions gave hatching curves resembling those obtained with root diffusates at different dilutions (Fig. 1).

The widely different constitutions of the dyes, their complexity and uncertainties about the structure of some, make it impossible to relate structure with hatching activity, but hydrophylic radicals, such as sulphonate or hydroxyl groups, attached to the benzene nucleus seem to lessen activity. Examples of this are the series (*a*) Janus green, Janus black and Janus blue, and (*b*) methyl red alcohol soluble, methyl red water soluble, methyl orange and orange *G*.

Little is known of the constitution of natural hatching factors for *H. schachtii*, but purified products from rape (*Brassica napus* L. var. *arvensis* (Lam.) Thellung) have several properties similar to the

Table 1. HATCHING ACTIVITY FOR *H. schachtii* OF SOME DYES* EXPRESSED AS A PERCENTAGE OF THE HATCH IN UNDILUTED STANDARD SUGAR-BEET ROOT DIFFUSATE. BASED ON TOTAL HATCHES AFTER THREE WEEKS FROM FOUR BATCHES OF 100 CYSTS

Dye	Hatch rating	Dye	Hatch rating
Methyl red alcohol soluble	121	Rivanol	110
Janus green	114	Auramine *O*	110
Picric acid	113	New blue *R*	100
Sugar-beet root diffusate	***100***		
Nile blue sulphate	91	Brilliant green	91

Significantly lower than root diffusate $P = 0·1$ per cent but significantly higher than water

Dye	Hatch rating	Dye	Hatch rating
Carminic acid	87	Rhodamine *B*	47
Carmine lithium	82	Brilliant cresyl blue	46
Bismarck brown *Y*	79	Primuline	46
Methyl orange	78	Cresyl fast violet	43
Methyl red water soluble	68	Gallamine blue hydrochloride	43
Rhodamine 6*G*	66	Xylene red *B*	40
Acriflavine	66	Janus black	38
Coriphosphine *O*	61	Chlorophosphine	34
Aurophosphine	57	Gallocyanin hydrochloride	34
Gentian violet	54	Xylene cyanol *FF*	33
Crystal violet	52	Eosin *Y* extra	32
Atebrin	52	Celestin blue	29
Rose Bengal *G*	50		

Not significantly higher than water. $P = 0·1$ per cent

Dye	Hatch rating	Dye	Hatch rating
Malachite green	26	Deltapurpurin	23
Lacmoid	25	Brilliant sulphaflavine	22
Rosolic acid	24	Soluble blue 8*B*	22
Water	***20***		
Acridine orange *R*	19	Gallamine blue bisulphite	9
Safranin *O*	18	Brilliant delphine blue	8
Janus blue	17	Orange *G*	7
Acridine yellow *GR*	11	Chrysoidin *Y*	5
Gallocyanin bisulphite	10		

Other inactive dyes

Phosphine
Victoria blue
Phenol red
Neutral red chloride
Fluorescein
Vital red
Trypan blue
Vital new red
Evans blue
Azo blue
Danil blue
Trypan red
Pontamine sky blue 5*BX*
Pontamine sky blue 6*BX*
Chlorazol fast red *F*
Thioflavine *S*
Thionin blue
Methylene blue
Methylene green
Toluidine blue

* Nomenclature from Gurr (ref. 4)

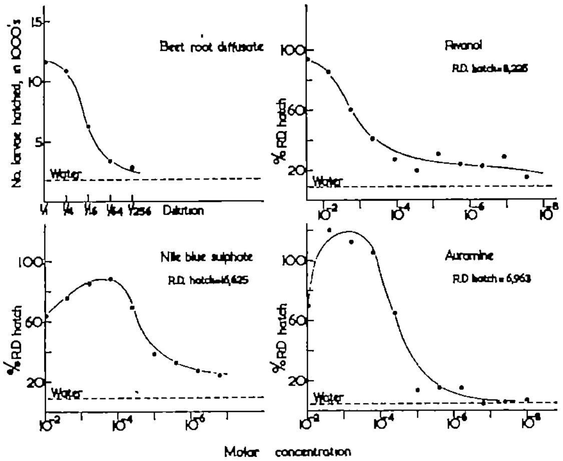

Fig. 1. Hatch-dilution curves of some dyes and of sugar-beet root diffusate. Abscissae, molar concentration of the dyes; ordinates, hatch expressed as a percentage of that in a standard beet root diffusate in the same test. *RD* = total hatch in three weeks, mean of four batches of 100 cysts, in undiluted root diffusate

H. rostochiensis factor from potato[3]. The latter is a monobasic carboxylic acid with an equivalent weight of about 220 and a formula approximating to $C_{11}H_{16}O_4$ (ref. 3), which, unlike the dyes, contains no benzene rings. The range of concentrations over which potato factor is effective has not been determined precisely, but in hatch-dilution tests using partially purified factor, activity approaches the water hatch at between 10^{-8} and 10^{-9}. The threshold concentration of auramine and nile blue sulphate (Fig. 1) lies between 10^{-6} and 10^{-7} molar, the respective absolute concentrations being 3×10^{-7} or 10^{-8} for auramine and 7×10^{-7} or 10^{-8} for nile blue sulphate. Rivanol is less effective.

Like natural hatching factors, these substances show some specificity. None stimulated *H. gottingiana* cysts and only celestin blue and methylene green stimulated *H. rostochiensis*, and then did so only slightly.

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Urinary 3-Methoxy-4-Hydroxymandelic Acid Excretion and Serum Electrophoretic Patterns in the Normal and Bloated Bovine

WORK has been in progress here since 1956 to determine if a metabolic difference exists between normal bovines and those subject to tympanites (bloat). Serum epinephrine was first measured on normal and bloated animals by the method of Weil-Malherbe and Bone[1], since a difference in rate of metabolism was suspected. Although the trend for normal cattle to have a higher level of epinephrine was apparent, the results were largely inconclusive, owing to excessive variation among animals. The epinephrine averaged 2·35 and 2·03 µg/100 ml. whole blood for 27 normal and 13 bloated cattle, respectively.

Differences in excitement of the animals during taking a sample induced variable results. Because of these differences, a metabolite of epinephrine and norepinephrine (3-methoxy-4-hydroxymandelic acid) was studied using the method of Sunderman *et al.*[2]. The mandelic acid averaged 1·76 µg for 8 samples from 3 normal steers and 1·10 µg/ml. urine for 14 samples from 4 bloated steers. These samples were taken at random times throughout the day and from different treatments. These results on concentration of mandelic acid obviously did not reflect total excretion since the volume of urine was not considered. Consequently, subsequent data were expressed as a ratio between mandelic acid and creatinine[3] excretion. Table 1 shows the relationship of mandelic acid to creatinine of a normal and bloated steer with and without stress by an electric prod.

The results indicate a greater total secretion of epinephrine and/or norepinephrine (as measured by the output of mandelic acid). Furthermore, the bloated animal did not respond to stress as quickly as did the normal animal. Stress was measured by peak in mandelic acid excretion.

Table 1. AVERAGE µG MANDELIC ACID/MG CREATININE EXCRETION IN THE NORMAL AND BLOATED BOVINE*

	No stress Average of 9 hourly collections over a 12-h period	Stress† Average of 11 hourly collections over a 12-h period after stress
Normal	1·86	2·43
Bloated	1·60	1·68

* Total urine volume for the 12-h period was not obtained.
† Stressed with 10 shocks from an electric prod.

The lag in excretion prompted another collection to determine if the lag were chiefly due to a slower metabolism of epinephrine. One normal and one bloated animal were given 0·75 mg epinephrine/kg body-weight and samples collected hourly over the next 23 h. The normal animal excreted a total of 12·211 mg of mandelic acid during this period with an average of 3·14 µg mandelic acid/mg creatinine. The bloated animal excreted 5·039 mg of mandelic acid with an average of 1·90 µg mandelic acid/mg creatinine during the same period. A maximum hourly excretion of mandelic acid (3·330 mg or 9·59 µg mandelic acid/mg creatinine) was reached 6 h after administering epinephrine to the normal animal whereas the peak excretion (763 µg or 2·74 µg mandelic acid/mg creatinine) in the bloated animal was not observed until 13 h after administration of epinephrine. Thus, it appears that the total excretion of epinephrine and its subsequent metabolism to mandelic acid is impaired in the bloating bovine.

A consistent difference was found in the electrophoretic patterns[4] of serum and urine proteins from normal and bloated animals. In serum, these differences were observed in the β- and γ-globulin fractions and may represent either abnormal amounts of certain protein components, that is, lipoproteins, or variation in the γ-globulin fraction itself.

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VIROLOGY

Viral Interference induced in Mice by Acute or Persistent Infection with the Virus of Lymphocytic Choriomeningitis

CONGENITAL or neonatal infection of mice with the virus of lymphocytic choriomeningitis (LCM) is often characterized by prolonged survival of the host, persistence of virus in the tissues, chronic viræmia and failure to develop specific antibodies[1]. This 'persistent tolerated infection' has been likened to the phenomenon of immunological tolerance[2]. Despite inability to form neutralizing antibody, adult mice previously infected at or before birth with LCM virus also exhibit the capacity to withstand challenge with homologous virus in a dose that would readily kill uninfected adult mice[3]. It has been postulated[3] that this resistance to superinfection (tolerant immunity) is related to lack of an allergic tissue response to LCM virus in the immunologically unresponsive host. Another possible explanation is that LCM virus in the tissues of the persistently infected mouse acts as an interfering agent[4] in much the same way that LCM virus interferes with poliovirus in the monkey[5].

The LCM virus used in these experiments was the partially attenuated *UBC* strain[3] obtained from Dr. John Hotchin of the Division of Laboratories and Research, New York State Department of Health. Stock virus prepared as a 10 per cent suspension of infected mouse brain had a LD_{50} titre of $10^{6\cdot 8}$/0·03 ml. when tested by intracerebral injection of weanling mice (12–14 g, 3 weeks old) but caused only sporadic deaths in weanling or new-born mice infected by intraperitoneal or intranasal routes. The interfering action of LCM virus was tested by challenging mice with immunologically unrelated Eastern equine encephalomyelitis (EEE) virus.

Experiments were first designed to determine the capacity of adult mice infected with LCM virus at 3–4 weeks of age to withstand subsequent challenge with EEE virus. In one such study 230 weanling mice in groups of 20–30 were infected intranasally with LCM virus at chosen intervals; 40 mice injected with saline diluent served as controls. All but 20 of the LCM infected mice were challenged intraperitoneally with a dose of EEE virus estimated to kill 90 per cent of control mice. Additional mice were similarly infected in order to determine the content of LCM virus in lung and brain at the time of challenge with EEE virus. As noted in Table 1, appreciable resistance to EEE virus developed by the second day; at this time the titre of LCM virus in lung had risen to a significant level, but none could be detected in brain. Immunity to challenge with EEE virus persisted for 8 days after LCM infection and disappeared thereafter. Titres of LCM virus in the tissues also declined as susceptibility to EEE virus returned.

Table 1. RESISTANCE TO INTRAPERITONEAL CHALLENGE WITH EEE VIRUS IN WEANLING MICE PREVIOUSLY INFECTED INTRANASALLY WITH LCM VIRUS

Interval between *LCM and EEE* Days	Mortality (%)	$\log_{10}$ LCM titres* in 10 cage-mates at time of EEE challenge	
		Lung	Brain
1	75 (15/20)	2·6	<0 5
2	40 (8/20)	3·5	<0·5
3	30 (6/20)	3·4	<0·5
5	40 (8/20)	4 8	1·5
6	30 (6/20)	4 5	3·8
7	35 (7/20)	4·0	0·5
8	40 (8/20)	2·8	1·5
9	70 (14/20)	2·2	0·5
10	95 (19/20)	2·2	<0·5
28	87 (26/30)		
No LCM	90 (36/40)		
No EEE	15 (3/20)		

* LCM virus titres in tissues determined by intracerebral injection of weanling mice.

It seemed reasonable to expect a more durable immunity to EEE virus in mice infected intracerebrally or intranasally with LCM virus a few hours after birth. Despite all precautions, most of the new-born mice injected intracerebrally died of LCM infection. However, those that survived into adulthood usually developed chronic LCM infection which lasted at least 7 months. Quantitative estimates of the degree of viræmia were made periodically and showed that 10^4 LD_{50} was not an unusual amount of LCM virus to be present in 0·1 ml. of blood several months after infection. Two separate experiments shown in Table 2 demonstrate the response of mice to intraperitoneal challenge with EEE virus 32 days after LCM infection was established at birth. In the first experiment the challenge inoculum of 1,000 LD_{50} of EEE virus was somewhat over-ambitious; but the persistently infected mice were clearly resistant as compared with uninfected controls reared from identical litters. The second experiment shows that LCM virus administered to new-born mice either intracerebrally or intranasally induced comparable degrees of protection to subsequent challenge with an LD_{50} of EEE virus.

Table 2. RESISTANCE TO INTRAPERITONEAL CHALLENGE WITH EEE VIRUS IN 32-DAY-OLD MICE INFECTED AT BIRTH WITH LCM VIRUS

Exp. No.	Interfering agent	Mortality	*Day of death,* ± S.E.	*LCM* Viræmia*
1	Controls	30/30	4·07 ± 1·1	—
	LCM (intracerebral)	9/13	7·7 ± 0·4	13/13
2	Controls	30/37	—	—
	LCM (intracerebral)	0/17	—	12/17
	LCM (intranasal)	3/10	—	10/10

* At time of challenge with EEE virus.

The results reported by Traub[4] were confirmed by studies of congenitally acquired LCM infection. In these experiments, mice born to chronically infected mothers were tested for the presence of LCM virus in blood and challenged intraperitoneally with EEE virus. These mice resisted challenge with EEE virus to about the same degree as those in which a persistent LCM infection was initiated shortly after birth. It is of interest that one experiment failed because third-generation litters of 'control' mice were found to have been inadvertently infected with LCM virus, thus rendering them resistant to EEE virus.

Many types of heterologous interference, of which the LCM–EEE system is undoubtedly an example, have been shown to be mediated by production of interferon[6]. Therefore, numerous attempts were made to demonstrate interferon in tissues of mice infected as adults or at birth with LCM virus. Infected tissue suspensions were prepared from lung or brain, dialysed at *p*H 2, concentrated tenfold by pervaporation, and assayed in *L* cells or primary mouse embryo cultures for the presence of interferon by the plaque inhibition method[7], using vesicular stomatitis as the test virus. In short, none of these tissues showed any interferon activity. These results are perhaps not too surprising in view of the difficulties sometimes encountered in detecting interferon in tissues of intact animals[7].

An attempt was also made to reproduce a model of the LCM interference system in tissue culture. It was found that LCM virus persisted in cultures of strain *L* cells, a stable line of mouse fibroblasts, for 27 passages over a period of 154 days. No consistent cytopathic changes could be demonstrated in these chronically infected cells despite the presence, on occasion, of 10^4 LD_{50} of LCM virus. The carrier cultures exhibited moderately increased resistance to superinfection with vesicular stomatitis virus. However, at no time could a non-viral resistance-promoting factor (interferon) be demonstrated in the culture fluids.

The results of these studies indicate that LCM virus acts as an interfering agent in acutely or chronically infected mice. No experimental support could be obtained for the hypothesis that resistance to superinfection is mediated by interferon produced in tissues of mice with acute or persistent LCM infection.

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CYTOLOGY

Method for Distinguishing between Human and Mouse Cells in Tissue Cultures

IN recent years it has become apparent that a cell strain grown *in vitro* can very easily become contaminated with, and in time perhaps overgrown by, cells of another strain. It is possible to determine at least the species from which the cells have originated; but the available procedures are elaborate and time-consuming.

The purpose of this communication is to propose a simple procedure by which human cells can be distinguished from mouse cells in a few minutes even by an untrained person.

This procedure depends on the presence in human serum of a cytotoxic system[1] consisting of a natural antibody, complement and divalent cations[2] and on sensitivity of mouse, but not human cells, to the effects of the system.

The difference in sensitivity has been shown in experiments in which human serum (1 part to 4 parts BSS) was added to 1×10^6 cells of the strains listed in Table 1. By this treatment, cells of all four mouse strains were killed after 1 h of incubation at 37° C, whereas cells of all six human strains remained alive even after 24 h of incubation.

These observations were further elucidated in experiments to determine the ability of mouse and human cells to adsorb the antibody from serum. The cells were shaken gently with the serum for 18 h at 4° C, then centrifuged, and the clear supernatant serum was tested for its cytotoxic effect on mouse cells. Mouse cells of all four strains adsorbed the antibody and there was a relationship between the number of cells used for adsorption and the number o mouse cells surviving the effect of 'adsorbed serum (Table 1).

Table 1. ADSORPTION BY CELLS OF NATURAL CYTOTOXIC ANTIBOD FROM HUMAN SERUM

Cells used for adsorption	No. of strain *L* cells per microscopi field 24 h after addition of serur treated with following No. of cell $(\times 10^6$/ml.)					
	0	0·25	0 5	1	2	4
Mouse cells:						
Subcutaneous connective tissue (strain *L*) (ref. 3)	0	0	47	415	450	58
Lymphosarcoma (*6C₃HED*) (ref. 4)	0	1	5	201	440	52
Mammary carcinoma (TA_3) (ref. 5)	0	0	109	231	396	41
Carcinoma (Ehrlich) (ref. 5)	0	0	0	5	346	41
Human cells:						
Intestine (Henle) (ref. 6)	0	0	0	0	0	
Liver (Chang) (ref. 7)	0	0	0	0	0	
Kidney (Chang) (ref. 7)	0	0	0	0	0	
Cells from synovial fluid (McCoy) (ref. 8)	0	0	0	0	0	
Cervical carcinoma (HeLa) (ref. 9)	0	0	0	0	0	
Cells from muscle biopsy (Auersperg) (ref. 10)	0	0	0	0	0	

On the other hand, the human cells did not adsor the cytotoxic antibody, and consequently, regardles of how many human cells were used for adsorption they did not detoxify the serum (Table 1). Th ability to adsorb cytotoxic antibody was not limite to mouse cells grown in cultures: cells isolate directly from the spleen, kidney and liver of norma mice could adsorb it likewise (Table 2).

Table 2. ADSORPTION OF NATURAL CYTOTOXIC ANTIBODY FRO HUMAN SERUM BY FRESHLY ISOLATED (NOT CULTURED) MOUSE CELL

Type of mouse cells used to adsorb antibody	Strain *L* cells incubated with	Percentage of eosin-stained strai *L* cells* after 10 min incubatio at 37° C with following dil tions of serum:				
		1 : 6	1:12	1:24	1:48	0
Liver $(3 \times 10^7$ cells/ml.)	Untreated serum	96	64	19	13	1
	Adsorbed serum	26	13	17	14	1
	Adsorbed serum + cytotoxic antibody	95	80	47	13	1
Kidney $(2 \times 10^7$ cells/ml.)	Untreated serum	96	64	19	13	1
	Adsorbed serum	41	14	11	11	1
	Adsorbed serum + cytotoxic antibody	82	80	47	14	1
Spleen $(9 \times 10^7$ cells/ml.)	Untreated serum	96	64	19	18	1
	Adsorbed serum	14	12	12	12	1
	Adsorbed serum + cytotoxic antibody	92	66	37	18	1

* Injured or dead cells

These results were confirmed by an experiment o different design in which cultures of mouse cells o cells obtained directly from spleens, livers an kidneys of normal mice were incubated with heate human serum and then mixed with mouse erythro cytes. Mixed agglutination occurred in every case There was no agglutination however, when huma cells, after incubation with heated serum, were mixe with mouse erythrocytes, indicating that huma cells did not have receptor sites to bind the antibody

It was concluded that the varying sensitivities o cells to the cytotoxic system are related to th presence or absence of receptor sites for the antibod on the cells. Hence, mouse cells are killed by huma serum whereas human cells are not.

It should be pointed out that the cytotoxic reactio is not limited to mouse cells but that cells of some other species are also killed by human serum. Furthermore, human cells are not the only ones unaffecte by human serum[11]. Nevertheless, the difference i the sensitivity to human serum between human and mouse cells can be effectively utilized in some situations for confirmation and, in some cases, eve identification of species origin of the cells. If on considers that most cell strains now used in tissu culture laboratories are either of human or mouse origin and that cell contaminations are usually either by HeLa cells (human) or *L* cells (mouse), then it is possible that human serum might well be used in a routine for screening cells.

Human serum may also be useful as a cytotoxic agent in cases in which human cell cultures have become contaminated by a few mouse cells, because when added to such cell cultures it will kill the contaminant mouse cells in a few minutes, leaving the human cells unaffected.

In the application of human serum as a cell screening agent or as a cytotoxic agent, several points should be considered: (1) The cell cultures should not be too densely populated, in order that the cytotoxic antibody can attach itself to the surfaces of all, or at least most, of the cells. (2) Fresh serum should be used, because death of the cell depends on the presence of human complement which is very labile and can be easily lost on handling or storage. (3) In this reaction, human complement cannot be replaced by guinea pig complement[1,2]. (4) In case of a negative reaction (no cell death) it would be advisable to repeat the procedure using serum from other humans because in some individuals the titre of cytotoxic antibody may be too low.

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Fine Structure of Chromosomes in the Mitotic Prophase of Ascites Tumour Cells

A COMPLEX, linear structure along the axis of certain meiotic prophase chromosomes has been observed in several animal spermatocytes[1–5]. Such structure has never been detected in mitosis or other stages of meiosis. The present communication is concerned with an electron microscope study of prophase chromosomes of ascites tumour cells, in which an axial structure of the chromosomes has been revealed. Ascites tumour cells, Ehrlich ascites tumour cells and Yoshida sarcoma cells have been examined by the electron microscope following conventional osmium fixation and methacrylate or epoxy resin embedding.

Fig. 1 shows a low-power electron micrograph of the nucleus of Yoshida sarcoma cell, which is believed to be in very early prophase since it shows a disorganization of the nucleolonemata[6] with a helical structure[7]. At the lower part of the disorganized nucleolus, two parallel linear densities are visible. They are interpreted as a chromosomal axis from which lateral chromosomal elements are going to develop. Fig. 2 shows that the chromosomal axis originates

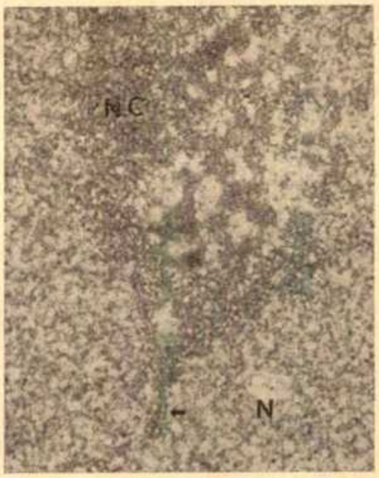

Fig. 1. The early prophase nucleus (*N*) of Yoshida sarcoma cell, showing the disorganized nucleolus (*NC*) and parallel dense lines (arrow). (× *c*. 22,400)

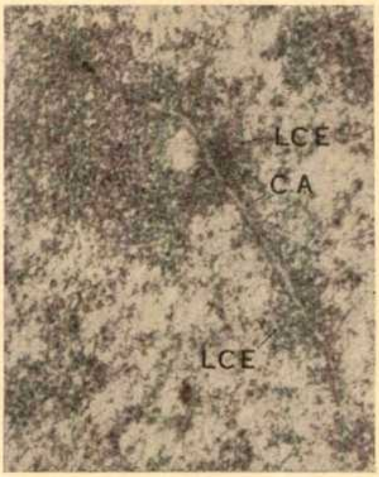

Fig. 2. A part of the prophase nucleus of Ehrlich ascites tumour cell, showing a chromosomal axis (*CA*) in a longitudinal section, lateral chromosomal elements (*LCE*) and a pair of dense dots (arrow). (× *c*. 30,400)

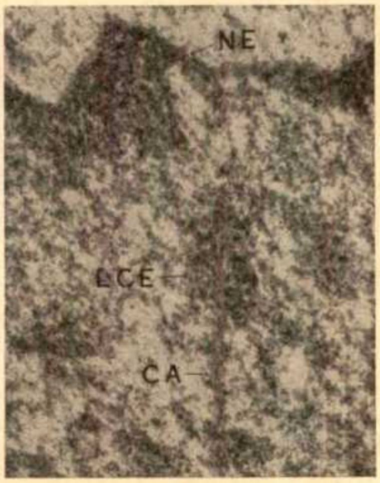

Fig. 3. A part of the prophase nucleus of Ehrlich ascites tumour cell, showing a chromosomal axis (*CA*), lateral chromosomal elements (*LCE*) and nuclear envelope (*NE*). (× *c*. 27,300)

from the nucleolus and lies in the plane of the section for a considerable distance. Two dense lines run parallel to each other, keeping a certain distance. Dense chromosomal elements seem to be reorganized almost symmetrically at both sides of the chromosomal axis. The dense elements appear to be granular at many points, but at a few points fibrillar. A cross-section through the chromosomal axis (Fig. 2) always shows a pair of dense dots, each of which has a core surrounded by a dense membrane. The mitotic chromosomal axis develops towards the nuclear envelope and finally comes to a close association with the nuclear envelope. Such structure has been confirmed with the aid of serial sections, one of which can be seen in Fig. 3.

The hypothesis of the conversion of RNA into DNA is not on a sufficiently sound basis, but the present investigation points to the nucleolus, composed of RNP functions, as the centre of the formation of early chromosomes. The association between the formation of early chromosomes and the nucleolus is of considerable interest, but has been described only briefly here. More detailed reports on this and related problems will be published elsewhere.

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Preliminary Studies on the Basis of Cytoplasmic Inheritance in Amœbæ

USING the technique of nuclear transfer[1], it has been shown that many of the cellular properties of the large, uninucleate amœbæ, *Amoeba proteus* and *A. discoides*, are influenced by self-replicating cytoplasmic hereditary determinants. The characters determined in this way are those which depend on the way in which macromolecules, the specificity of which has been determined by nuclear genes, are organized into functional units[2].

The amœbæ used in the present investigations were *A. proteus*, $_{\mathrm{Tl}}P$ and *A. discoides*, $_{\mathrm{Tl}}D$. In addition to characters determined by nuclear factors $_{\mathrm{Tl}}P$ differs from $_{\mathrm{Tl}}D$ in the following cytoplasmically influenced characters: higher mean nuclear diameter, greater streptomycin sensitivity and streptomycin uptake, form when moving and shape in anti-sera. Nuclear diameter[3] and streptomycin sensitivity[4] have been used as markers in our present investigations, which are designed to determine the cellular basis of these examples of cytoplasmic inheritance.

The properties of amœbæ which make them suitable material for nuclear transfer also make possible micro-injection of cytoplasm. In the present experiments the effects of $_{\mathrm{Tl}}D$ cytoplasm injected into whole $_{\mathrm{Tl}}P$ amœbæ, and vice versa, are being investigated. 25 per cent of the receptor cells survive injections of cytoplasm equivalent to 6–10 per cent of their original volume and form flourishing mass cultures. Attempts to increase the ratio of donor to receptor cytoplasm by withdrawing cytoplasm from the receptor before injection resulted in lower survival-rate.

Measurements of mean nuclear diameter a streptomycin sensitivity of heterotransfer clon obtained from nuclear transfer experiments a shown in Table 1.

Table 1

	Amœbæ			
Character	$_{\mathrm{Tl}}P$	$_{\mathrm{Tl}}P_n \rightarrow {}_{\mathrm{Tl}}D_c$	$_{\mathrm{Tl}}D_n \rightarrow {}_{\mathrm{Tl}}P_c$	$_{\mathrm{Tl}}D$
Mean nuclear diameter (μ)	$47 \cdot 84 \pm 0 \cdot 62$	$41 \cdot 80 \pm 0 \cdot 6$	$46 \cdot 60 \pm 0 \cdot 59$	$39 \cdot 56 \pm 0$
Streptomycin sensitivity *	10·5	5·0	4·8	4·2

* Expressed as mean generation time in days in 0·36 μg strep mycin/ml.

We have found that injection of $_{\mathrm{Tl}}D$ cytoplasm into whole $_{\mathrm{Tl}}P$ amœbæ results, in approximatel 20 per cent of clones formed from amœbæ whic survive the operation, in a significant decrease i mean nuclear diameter, such that this is not significantly different from that of the $_{\mathrm{Tl}}P_n \rightarrow {}_{\mathrm{Tl}}D$ heterotransfer. When the streptomycin sensitivity of these clones was measured, it was found that this marker characteristic had been similarly changed In clones of normal amœbæ, mean nuclear diameter and level of streptomycin sensitivity are not associated.

The reverse procedure, that is, $_{\mathrm{Tl}}P$ cytoplasm injected into whole $_{\mathrm{Tl}}D$ amœbæ, gives a similar result in that 30 per cent of the surviving clones show a mean nuclear diameter not significantly different from the $_{\mathrm{Tl}}D_n \rightarrow {}_{\mathrm{Tl}}P_c$ heterotransfer.

All these measurements have been carried out o amœbæ separated by at least 50 cell divisions fro the original post-operative cell, so that its constituents will have been diluted by a factor of 2 It therefore appears that a situation in which cyt plasmic hereditary determinants can be expresse and presumably replicate, results from the injecti and assimilation of a relatively small volume non-homologous cytoplasm.

We are at present attempting to extend the list of reliable and easily measured cytoplasmically determined markers and to find out if the examples of cytoplasmic inheritance discussed here are associated with a particular cytoplasmic fraction. This system is also being used to examine examples of incompatibility between nucleus and cytoplasm of different strains, which are similar to those met in nuclear transplantation experiments with other organisms, for example, Amphibia.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, October 29

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. C. E. Everard, Mr. R. J. Chorley and Mr. B. T. Bunting: "Slope Profiles".

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine. Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Prof. E. J. Richards: "Noise Control".

Institution of Electrical Engineers, Electronics Division joint meeting with the British Institution of Radio Engineers Medical Electronics Group, at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Data Processing in Medicine".

Institution of Mechanical Engineers, Process Engineering, Refrigeration, Ventilation and Vacuum Plant Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Reducing the Cost of Maintenance".

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. B. F. Skinner (Harvard): "The Future of Teaching Machines".*

Society of Chemical Industry, London Section—(at 14 Belgrave Square, London, S.W.1), at 6 30 p.m.—Mr. G. Sykes: "Horizons in Disinfection and Antisepsis" (Jubilee Memorial Lecture).

Tuesday, October 30

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C.1), at 1.15 p.m.—Dr. G. Fielder: "The Moon's Surface".*

Institution of Chemical Engineers (at Church House, Westminster, London, S.W.1), at 2.30 p.m.—Symposium on "The Economic Life of Process Plant".

Faraday Society (in the Lecture Room, Physical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford), at 5 p.m.—Dr. J. H. van der Waals (Amsterdam): "Clathrates as Elementary Solutions" (Bourke Lecture).*

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. J. L. Monteith: "Exploring the Air Near the Ground" (Sixth Form Lecture; to be repeated on October 31 and November 1.)

Institution of Electrical Engineers, Science and General Division P.G.S8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof M. W. Humphrey Davies: "Fundamental Problems in Engineering Education" (Group Chairman's Address.).

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. W. Klyne: "The Shapes of Molecules".* (Fifth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Prof. B. F. Skinner (Harvard): "Recent Advances in the Experimental Analysis of Behaviour".*

Society for Analytical Chemistry, Physical Methods Group (at the Chemical Society, Burlington House, Piccadilly, London; W.1), at 6.30 p.m.—Meeting on "Recent Work in Radiochemical Methods of Analysis".

Society of Instrument Technology, Systems Engineering Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. P. J. Thomas: "A Factory Data Collection System".

Wednesday, October 31

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Prof. W. J. H. Butterfield: "Diabetes and the Study of Carbohydrate Metabolism in Man".*

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m —Dr. J. G. Ramsay· "The Uses and Limitations of β-Diagrams and π-Diagrams in the Geometrical Analysis of Folds"; Mr. A. Simpson: "The Stratigraphy and Tectonics of the Manx Slate Series, Isle of Man".

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C 2), at 5.30 p m.—Dr. D.B. Irving: "Power and Society" (Chairman's Address).

Wednesday, October 31—Saturday, November 3

Radio Society of Great Britain (at Seymour Hall, Seymour Place, London, W.1)—International Radio Communications Exhibition.*

Thursday, November 1

Royal Institution, Library Circle (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr W. A. Dickie· "Printer and Paper Maker Pioneers. a Comparative Sketch of the Work of Caxton and Spilman".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. T. Symington: "The Pathological Physiology of the Human Adrenal Cortex and Its Relationship to Hypercorticalism".* (Sixth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Society of Chemical Industry, Microbiology Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. H. G Pereira· "Structure and Antigenic Constitution of Adenoviruses"; Dr. J. S. Porterfield: "Some Problems of Viruses found in Warmer Climates".

Royal Institute of Chemistry, London Section (joint meeting with the Brunel College of Technology Chemical Society, at Brunel College of Technology, Woodlands Road, London, W.3), at 7 p.m.—Dr. E. F. G. Herington: "Zone Refining".

Thursday, November 1—Friday, November 2

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2)—Symposium on "Plastic Insulated Mains Cable Systems.

Friday, November 2

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. V. Petrow "Steroid Progress 1962".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. W. Ditchburn, F.R.S.: "Some Experiments on the Way We See".

Saturday, November 3

University of London (in the William Beveridge Hall, Senate House, London, W.C.1) from 10 a.m. to 4.30 p.m.—"The Structure and Organization of the University of London"—Conference of Members of Convocation. Main Speakers: Sir Douglas Logan and Sir John Lockwood.

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Prof. Eta Harich-Schneider· "Old Court Dances in Japan".

Monday, November 5

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. D. E. Broadbent: "Theories of Short-Term Memory".

University of London (in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1), at 5 30 p.m —Prof. C. de Jager (University of Utrecht): "The Emission of Gas by the Sun".*

Institution of Mechanical Engineers, Steam Plant Group (at Neville Hall, Westgate Road, Newcastle upon Tyne), at 6 p.m.—Meeting on "Some Design Problems arising in the Development of Very Large High Speed Turbines".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—Mr. H. Gibb: "Rituals of Rice" (Colour films with commentary).

Royal Institute of Chemistry, London Section (joint meeting with the Woolwich Polytechnic Chemical Society, at the Woolwich Polytechnic, Thomas Street, London, S.E.18), at 7.30 p.m.—Prof. R. N. Haszeldine: "Double Bonds that are Different".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Lecturer, Grade B (graduate, preferably with experience of microbiology) in Botany in the Department of Biology and Geology—The Principal, L.C.C. Norwood Technical College, Knight's Hill, London, S.E.27, quoting Ref HE.4/B/2374/10 (November 3).

Lecturer or Assistant Lecturer, Grade B (preferably with experience at postgraduate level in microbial genetics or helminthology) in either Microbiology or Parasitology—The Secretary, Royal College of Advanced Technology, Salford 5, Lancs, quoting Ref. CH/7 (November 5).

Personal Technical Assistant to Prof. Bernal, for research into the structure of liquids—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (November 5).

Research Assistant (suitably qualified graduate) in the Naval Architecture Department and in the Computing Laboratory, to prepare and run programmes for routine ship calculations—The Secretary of University Court, The University, Glasgow (November 7).

Assistant Lecturer in Numerical Analysis in the Department of Mathematics, for duties which will include using and teaching the use of the Atlas Computing Machine (previous experience of large computers is not essential)—The Registrar, The University, Manchester (November 10)

Assistant Lecturer or Lecturer in Zoology—The Registrar, The University, Sheffield (November 10).

Scientific Assistant (graduate, pass or honours) in biochemistry, physiology or chemistry; or non-graduate with research experience) for investigation of metabolism of C^{14}-steroids—Prof. A. A. Harper, Physiology Department, The Medical School, King's College (University of Durham), Newcastle upon Tyne (November 10).

Lecturer (with an honours degree in electrical or electronic engineering, and experience in some branch of electrical engineering) in the Department of Electrical Engineering—The Registrar, The University, Leeds 2 (November 12).

Lecturer (preferably with medical qualifications or interests) in the Department of Biochemistry—The Secretary, University College, Gower Street, London, W.C.1 (November 16).

Senior Lecturer or Lecturer in Bacteriology; and a Senior Lecturer or Lecturer in Pathology—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 18).

Senior Lecturer or Lecturer in Mathematics; and a Senior Lecturer or Lecturer in Physics at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 18).

Senior Technician/Technician in Veterinary Physiology and Biochemistry; a Senior Technician/Technician in Veterinary Anatomy and Histology; and a Senior Technician/Technician in Animal Husbandry at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 18).

Lecturer or Assistant Lecturer (with an interest in some branch of solid-state physics or of radiospectroscopy) in Physics—The Registrar, University College of North Wales, Bangor, North Wales (November 19).

Lecturer or Assistant Lecturer in the Department of Pure Mathematics—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (November 20).

Director of the Fisheries Biochemical Research Unit—The Secretary, The University, Aberdeen (November 21).

Lecturer in Physics at Fourah Bay College, The University College of Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 28).

Lecturer (permanent or temporary) in the Department of Physics at the University of the Witwatersrand, Johannesburg—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, November 30).

Senior Lecturer or Lecturer in Physics; and Teaching Fellows and Senior Teaching Fellows in Physics—The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia; or The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia November 30).

Chair of Anthropology in the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 3).

Assistant Lecturer (with a good honours degree) in Microbiology—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1.

Biochemist (Basic grade) (preferably with experience in routine medical biochemistry)—Group Pathologist, The Area Laboratory, West Park Hospital, Epsom, Surrey.

Experimental Officer or Assistant Experimental Officer (preferably F.I.M.L.T. or H.E.C. Biology, with experience in virology and tissue culture methods)—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey.

Hospital Physicist (Basic Grade) (honours physics graduate)—Dr. B. Levin, Pathology Department, Queen Elizabeth Hospital for Children, Hackney Road, London, E.2.

Radiation Biologist (Assistant Professor or Associate Professor) in the Department of Biology, to develop a research programme in some field of radiation biology—Prof. M. Shaw, Head, Department of Biology, University of Saskatchewan, Saskatoon, Canada.

Research Assistant (preferably with previous laboratory experience, and with G.C.E. "A" level or higher qualification in chemistry) in the Chemistry Department, Chester Beatty Research Institute, Fulham Road, London, S.W.3, to assist in analytical or biochemical studies of enzymology applied to cancer research—The Secretary, Institute of Cancer Research—Royal Cancer Hospital, 34 Sumner Place, London, S.W.7, quoting Ref. 301/B/115.

Scientific Information Officer (Abstractor) (with a degree in agriculture or natural science and able to translate from German)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire.

Technical Officer (age under 40, with H.N.C. or university degree), to take charge of the new Radiochemical Laboratory for the separation of short-lived radioisotopes produced on the medical cyclotron—The Director, Cyclotron Unit, Medical Research Council, Hammersmith Hospital, Ducane Road, London, W.12.

Technician in the Department of Microbiology—The Assistant Bursar, The University, Reading.

Zoologist (with an honours degree in zoology and research or other experience in parasitology, or a higher degree in zoology) in the Veterinary Department, Kenya, for the supervision of routine helminthological diagnosis, and research on problems of helminthiases of livestock, especially the bionomics of nematode and trematode infestations—The Director of Recruitment, Department of Technical Co-operation, 8 Sanctuary Buildings, London, S.W.1, quoting Ref. RC.217/80/06.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Huntingdon Research Centre, (Nutritional Research Unit). Report 1952–1961. Pp. iv+59. (Huntingdon: Huntingdon Research Centre, (Nutritional Research Unit), 1962.) [129

The Institution of Metallurgists. Handbook No. 3: Associateship and Licentiateship Examinations—Examination Regulations and New Syllabuses, with Specimen Examination Question Papers. Pp. 30. (London: Institution of Metallurgists, 1961.) [129

Astronomical Contributions from the University of Manchester. Series III, No. 90: Problems of Lunar Topology—Proceedings of a Conference held at Bagnères-de-Bigorre between April 19–23, 1960. Edited by Z. Kopal and Ellen B. Finaly. Pp. 96. (Manchester: The University, 1962.) [129

Modernising School Mathematics: a Review of New Trends in Mathematical Education at the Secondary Stage. By M. G. Hughes. Pp. 48. (London: G. Bell and Sons, Ltd., 1962.) 2*s*. 6*d*. net. [129

Department of Scientific and Industrial Research: Road Researc Laboratory. Road Research Technical Paper No. 54: The Compactio of Concrete Road Slabs. By Dr. R. H. Kirkham and M. G. Whit Pp. vi+61+2 plates. (London: H.M. Stationery Office, 1962.) 4*s*. 6 net. [12

Planning, Vol. 28, No. 464, (27 August, 1962): Cartel Policy and th Common Market. Pp. 199–298. (London: Political and Econom Planning, 1962.) 7*s*. 6*d*. [12

Library Association. Special Subject List No. 38: Plastics. B E. R. Yescombe. Pp. 40. (London. Library Association, 1962 8*s*. (L. A. members 6*s*.) [12

Forestry Commission. Report on Forest Research for the year ende March, 1961. Pp. ix+209+12 plates. (London: H.M. Stationer Office, 1962.) 14*s*. net. [12

Other Countries

Proceedings of the California Academy of Sciences, Fourth Serie Vol. 30, No. 5 (July 31, 1962): A Preliminary Report on the Hesperiida of Baja California (Lepidoptera). By C. Don MacNeill. Pp. 91–11 Vol. 30, No. 6 (July 31, 1962): A History of Explorations for Vertebrates on Cerralvo Island, Baja California. By Richard C. Bank Pp. 117–125. (San Francisco: California Academy of Science 1962.) [12

Canada: Department of Mines and Technical Surveys. Geologica Survey of Canada. Geological Maps—Preliminary Series. Map 42-1961. Quesnel Lake, British Columbia (Sheet 93, East Half). Ma 2–1962: McLeod Lake, British Columbia, (Sheet 93J). Map 5–1962 Andrew Gordon Bay-Cory Bay, Baffin Island, District of Franklin Map 18–1962: Basler Lake, District of Mackenzie. (Ottawa: Director Geological Survey of Canada, 1962.) [12

Commonwealth of Australia. Commonwealth Scientific and Indus trial Research Organization—Division of Forest Products. Annua Report, 1961–62. Pp. iii+44. (South Melbourne: Forest Product Laboratory, C.S.I.R.O., 1962.) [129

Australia. Department of Agriculture, N.S.W. Science Bulletin No. 77: Root and Foot Rot Diseases of Wheat. By F. C. Butler. Pp. 98. (Wagga Wagga, N.S.W.: Agricultural Research Institute, 1962.) [129

Organization for Economic Co-operation and Development—European Nuclear Energy Agency. 1962–1963 Catalogue of Courses on Nuclear Energy in the European Countries of O.E.C.D. Pp. 184. (Paris: Organization for Economic Co-operation and Development—European Nuclear Energy Agency, 1962.) [129

New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March 1962. Pp. 87. (H.34). (Wellington: Government Printer, 1962.) [129

New Zealand. Air Department: New Zealand Meteorological Service. Misc. Pub. 114 (3): Summaries of Upper Wind Observations at Selected Stations—Nandi, Fiji, 1956–1960. Pp. 8. (Wellington: Director, New Zealand Meteorological Service, 1962.) [129

United States Department of the Interior: Fish and Wildlife Service. Bureau of Commercial Fisheries. Fishery Bulletin No. 187: Abundance and Distribution of Eggs and Larvae and Survival of Larvae of Jack Mackerel (*Trachurus symmetricus*). By David A. Farris. Pp. iv+247–279. 30 cents. Fishery Bulletin No. 194: Influence of Early Maturing Females on Reproductive Potential of Columbia River Blueback Salmon (*Oncorhynchus nerka*). By Richard L. Major and Donovan R. Craddock. Pp. iv+429–438. 15 cents. Fishery Bulletin No. 195: Determining Age of Young Haddock from their Scales. By Albert C. Jensen and John P. Wise. Pp. 439–450. 15 cents. Fishery Bulletin No. 196: Development, Distribution, and Comparison in Rudder Fishes *Kyphosus sectatrix* (Linnaeus) and *K. incisor* (Cuvier) in the Western North Atlantic. By Donald Moore. Pp. iv+451–480. 30 cents. Fishery Bulletin No. 197. Raft Culture of Oysters in Massachusetts. By Williams N. Shaw. Pp. iv+481–496. 20 cents. Fishery Bulletin No. 198: Rearing Tilapia for Tuna Bait. By Thomas S. Hida, Joseph R. Harada and Joseph E. King. Pp. iv+1—20. 20 cents. (Washington, D.C.: Government Printing Office, 1961 and 1962.) [129

Indian Forest Records, (New Series). Forest Pathology. Vol. 2, No. 6: Indian Timber and Its Decay. By Dr. K. Bagchee. Pp. 79–130+17 plates. (Delhi: Manager of Publications, 1960.) Rs. 14.50 np., 22*s*. 6*d*. [129

United States Department of the Interior: Geological Survey. Bulletin 1031-D: Geology of Waterpower Sites on Crater Lake, Long Lake and Speel River near Juneau, Alaska. By John Charles Miller. Pp. iii+71–101+plate 7. Bulletin 1063-C: Geologic Environment of an Oxidized Uranium Deposit in the Black Hills, South Dakota. By N. P. Cuppels. Pp. iv+61–83+plates 7–10. Bulletin 1107-D: Uranium Occurrences in Sedimentary Rocks of Pennsylvania. By Harry Klemic. Pp. iv+243–288+plate 2. Bulletin 1107-E: Uranium and other Trace Elements in Devonian and Mississippian Black Shales in the Central Midcontinent Area. By E. R. Landis. Pp. iv+289–336+plates 3–5. Bulletin 1142-F: Geology of the Magnesite Belt of Stevens County, Washington. By Ian Campbell and John S. Loofbourow, Jr. Pp. iv+53+2 plates. (Washington, D.C.: Government Printing Office, 1962.) [129

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SCIENCE AND THE EMERGENT COUNTRIES

THE reference in Sir John Cockcroft's presidential address to the British Association for the Advancement of Science (see *Nature*, 195, 875; 1962) to the importance of adequate technical assistance for the under-developed countries and especially to the opportunities which face the Overseas Research Council struck a recurrent note in the proceedings of the Manchester meeting. In his York Lecture on the following day, Prof. P. M. S. Blackett, himself a past president of the Association, dealt specifically with the theme of science and the developing countries. Pointing out that those countries were determined to become richer by the application of science and technology, and that one thing needed to achieve this was an educational system to supply the necessary number of scientists, engineers, administrators, technicians, doctors and other professional people, Prof. Blackett said that for some years these countries would need the help of trained men and women from the advanced countries. At present there is a real shortage of such trained people in the newly independent countries of Africa, for example, and while these territories were developing educational systems which would in due course meet this need from their own populations, British scientists, engineers and technicians could make a valuable contribution by going for a period of years to one of them.

Prof. Blackett, who was addressing an audience of young people, expressed the hope that university authorities and industry would encourage young graduates to take this step before 'settling down'. Here he was advocating something which goes considerably beyond the scope of Voluntary Service Overseas but which is fully in line with the policy of the Department of Technical Co-operation. It is already clear that Mr. Dennis Vosper has the right ideas, but it is very far from being clear that he and his Department have the full support of the Government or that adequate resources are being placed at his disposal. It is not even clear that the work he is doing has the interest and support it can legitimately claim from the Minister for Science, and on this probably no more convincing evidence or assurance could be forthcoming than some indication that the Overseas Research Council was being used extensively and imaginatively on lines such as Sir John Cockcroft suggested.

In the main it is from the younger scientists and technologists that the response to this appeal for personal service must come, though their seniors have a decisive part to play in seeing that the universities and professional institutions and industry provide the conditions and initiate the measures to facilitate secondment and service overseas. There is much room for such efforts, and, as Prof. Blackett pointed out on a previous occasion, there is no reason why the universities should wait on the Government's initiative. Indeed, a real lead by universities and professional institutions should strengthen the efforts of the Department of Technical Co-operation and could have a decisive influence on the Government.

Some indication of the situation in the under-developed areas and of specific opportunities was given in a paper by Mr. H. R. Mills on technical training in those areas presented to Section X of the British Association on September 3. Mr. Mills based his paper on a survey of technical training facilities in sixteen countries of south and south-east Asia which he made for the Colombo Plan Bureau in 1961. The main object of the latter was to ascertain what technical training facilities existed and the needs of each country, and to suggest ways in which these needs could be met by developing and using more fully the facilities available within the region and by sharing facilities between neighbouring countries as part of the Colombo Plan programme of technical co-operation. The survey was supported by a special grant from the Ford Foundation, and it showed that facilities for good training exist in south and south-east Asia in practically all the branches of technology which are needed and that all countries without exception were prepared to offer their own facilities to neighbouring countries in the region.

The survey should help to dispel the widespread ignorance of existing facilities which has led to the sending of technicians to Europe or the United States for ordinary technical subjects and thus reduce a demand which has seriously taxed technical institutions in Britain and elsewhere. Nevertheless, the actual shortage at the technician-level is very serious, much more serious than that of engineers and technologists, and for social reasons alone may take a generation to remove, and there is nothing in Mr. Mills's paper that weakens the force either of Prof. Blackett's appeal or of Voluntary Service Overseas, both of which are intended to alleviate an immediate scarcity which Mr. Mills's survey seeks to remove indigenously in the long term. Moreover, his paper, which recognizes the need for communication as well as co-operation between industry and technical education institutions and for planning technical training so as to meet the needs of industry when those needs have been properly assessed, should help the intending recruit to overseas service by the clear picture it presents of what is involved in the development of human resources in these countries.

Mr. Mills stressed particularly the need to plan technical education and to execute its development in step with social changes and the supply of adequate capital and material resources. Education departments and institutions, as well as the technical departments of Government, railways, public works and telecommunications, the private sector of industry and professional institutions were all concerned. A major problem in all the countries he visited was the poor communication between these agencies. Frequently, technical training was quite out of touch with industry and oblivious of the preference of industry for men trained in their own way in their own workshops. Furthermore, some developing countries sought to take technicians to higher academic standards than were actually required for the prospective occupation, and this was encouraged by insistence on a degree as minimum qualification for Government service. Again, there was an absence in technical colleges of staff with industrial or practical experience, and sometimes a prejudice against manual labour, particularly if it involved risk of soiling hands or clothes.

There are other factors in the social structure and pay structure which discourage recruitment to careers as technicians, and the excessive prestige of university education can be a serious factor, so much so that the gap between the technician and the engineer or technologist is a serious obstacle to development in south and south-east Asia. Language is also an obstacle to communication, and Mr. Mills strongly advocates retention of English technical terms in the national language by 'phonetic inclusion'. English is generally used as the medium of instruction for professional graduate engineers, but the technician usually hears sufficient English and is aware of the limitations of the craftsman's vocabulary so that there is no serious obstacle to communication.

From Mr. Mills's survey it would seem that purely by example the technician who serves a period overseas could contribute to break down some of the prejudice against manual labour or the wearing of overalls as well as facilitate communication between craftsman or technician and the technologist, quite apart from any help he may give in alleviating an extremely serious existing shortage. It is, of course, at the graduate-level and at rather more senior level that the technologist can help to supply practical experience in the technical colleges, and this is one point at which the co-operation of industry is essential. Further, the survey shows how invaluable could be the assistance of professional institutions in Britain, not only in working out effective secondment and placement schemes and giving them due publicity, but also perhaps even more in direct assistance to corresponding institutions in the developing countries by placing their own experience at the disposal of the younger institutions.

Technical education even in its broadest sense is a particular aspect of the economics of the under-developed countries which Mr. W. B. Reddaway discussed in his presidential address to the Economics Section also on September 3. Mr. Reddaway did not touch on technical education or even on education generally, although he recognized its importance. He was concerned essentially with the question why the level of income per head in these countries is so low, and what it is that prevents it from rising more rapidly. More positively he sought to show what should be done, and by whom, to accelerate the rise.

Mr. Reddaway began by reiterating a point he made in his book, *The Development of the Indian Economy*, that in countries with no public provision for the unemployed and a very low labourer's wage. the concept of unemployment is of limited value. Accordingly, he suggested, we should consider the problem in such countries not in terms of unemployment but rather in terms of the inadequate number of openings for really productive activity, and that development should be planned as if there were an inexhaustible supply of unemployed workers. Mr Reddaway fully recognizes, of course, that this proposition has to be qualified suitably by reference to the location of the work, the skills required and the speed of recruitment, nor does he overlook the difficulties that shortage of capital may lead to, and he points out that in an under-developed country the most important criterion of efficiency is usually output per unit of capital, not output per man.

Mr. Reddaway is obviously in favour of turning the vast labour potential in these countries to at least some account in making a good network of rural roads, and perhaps in other simple improvements, such as digging small wells and improving irrigation channels. He believes that the broad strategy of India's development planning makes sense and hopes that the developed countries will make the necessary aid available as well as the necessary trade facilities. On education, however, as already noted, and on food supply, he scarcely touches, and his address is a fitting counterpart to that on "Science and Hunger" which Earl de la Warr had delivered to Section M (Agriculture) on August 30.

Earl de la Warr set out to justify the assertion that immense as are the problems of world hunger and malnutrition, they are soluble if the knowledge that we already possess is used. The first point of attack, he insisted, must be education, not merely technical education but also general education that increased the demand for and the capacity to use technical advice. He stressed particularly the need to establish institutions for training indigenous advisers; his address clearly reminds us that, however important technical assistance may be to-day. in the long term the under-developed countries must depend on their own technical staff and advisers.

Earl de la Warr briefly reviewed some of the possibilities in this field before pointing out that the food surplus of the West was quite inadequate to make any effective contribution to the relief of shortages in under-developed areas for any significant period. They could not replace the need for self-support, and Britain's task was to help these people to help themselves and in such a way that they would become permanently independent. Surpluses could. of course, be used to build up the general wealth of countries that need assistance so that they can purchase the food that they either do not or cannot produce—but this means 'trade' not 'aid'. Earl de la Warr did not deny the importance of this method, and beyond this the distribution of food surpluses can contribute to the development of agriculture in such countries if they are used as a stock of capital to be drawn on while important agricultural reforms are undertaken, and to enable idle labour to be put to work without causing inflation. Indeed, a pledging session in New York on September 5 for the United Nations World Food Programme was aimed at raising a fund of 100 million dollars in surplus agricultural products and cash for development, and Earl de la Warr's address should remove any hesitation which the British Government had in pledging 5 million dollars to the programme.

Earl de la Warr concluded by expressing the hope that the United Kingdom would play a worthy part in this great international attack on hunger, which, as he observed, is essentially an extension into wider fields of work that has been done for generations by Britain's own Colonial agricultural and other Services in areas for which Britain has been responsible. The recurrent emphasis at successive British Association meetings in recent years on the interest, the opportunities and the responsibilities of the scientist in the under-developed territories is as welcome as it is timely. Its expression at these meetings should encourage scientists generally to make their full contribution to technical assistance, to overseas research and in educational work, for example, and not least when, in spite of the establishment of the Department of Technical Co-operation, Government support seems somewhat lukewarm. The British Association meeting has undoubtedly displayed some opportunities for specific action by scientists themselves, though it probably remains true that in the broadest sense the organization of overseas research generally and the revitalizing and effective use of the Overseas Research Council are still among the most important.

DIFFUSE X-RAY REFLEXIONS FROM CRYSTALS

Diffuse X-ray Reflections from Crystals

By Dr. W. A. Wooster. Pp. xi+200. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 35*s*. net.

WHEN Max von Laue suggested, 50 years ago, that X-rays might be diffracted by crystals, the physicists of his day were doubtful whether any observable pattern would be obtained because of the thermal vibrations of the atoms which might, they thought, smear out the diffraction spectra. However, the experiment was tried, and it succeeded so well that the question of thermal vibration effects became of secondary importance except in so far as it was interesting to study them through the temperature-sensitive diminution of Bragg intensity that they caused. The idea that there should be a definite pattern of diffuse scattering due to thermal vibration which would depend on crystal orientation (contrary to early Debye theory) was raised by Faxén and Waller in 1923, but so few crystallographers read these mathematical papers that even when the patterns were found on well-exposed Laue photographs taken with the whole radiation from copper targets (see, for example, *Proc. Roy. Soc.*, A, 125, plate 11, Fig. 6, opposite p. 674 (1929)) they were not recognized. They were in fact repeatedly seen and ignored until about 1938–39, when they were actively looked for by Laval in France and independently noted by Wadlund in the United States and Preston in Great Britain. The whole story, of which this is the briefest outline, is an amusing commentary on the 'two cultures' that can exist even within a most specialized corner of a specialized discipline when experimentalist and mathematician are unaware of each other's work.

Nowadays every X-ray crystallographer has to be familiar with the use of mathematical tools. The short statement on the inside paper cover of Dr. Wooster's book claims that "This book has been written with a minimum of mathematical analysis". Well, that may be so; but the "young research student" for whom the book is intended had better glance through it before he derives any comfort from that assurance. There is an index of symbols that runs to more than six pages with about thirty definitions on each page.

Dr. Wooster is an authority in the field and an experienced writer and teacher. Moreover, this is the first book of its kind, and it is bound, therefore, to find an honoured place in the crystallographer's library. But one cannot help regretting that the author has so deliberately restricted his treatment of the subject. He gives a general survey of the theoretical basis of diffuse scattering and of the experimental methods by which it has been observed; then adds a chapter on the determination of elastic constants from diffuse scattering, and one on the determination of the elastic frequency spectrum. Useful chapters follow on diffuse scattering due to structural imperfections and on that from molecular crystals, but there is no mention of zero-point energy, of diffuse effects due to radiation damage or of the ways in which these various effects may be sorted out experimentally. Electron and neutron diffraction effects have also been deliberately excluded, probably because the author would rather not deal with them unless he can do so exhaustively. But sometimes a brief outline may be better than nothing at all.

There are good indexes, but also several misprints and some errors. KATHLEEN LONSDALE

NUCLEAR REACTOR PHYSICS

Reactors

Vol. 2. Edited by H. R. McK. Ryder. (Progress in Nuclear Energy, Series 2.) Pp. vi+557+xii. (London and New York: Pergamon Press. 1961.) 105*s*. net.

SINCE the extensive declassification of work on the civil applications of nuclear power in the early 1950's, it has become difficult to keep up with the very large numbers of papers and reports being published in this field. The review series *Progress of Nuclear Energy* was therefore warmly welcomed when it was founded by Pergamon Press in 1955. The series is now divided into 12 sections, the volume under consideration being the second volume in the *Reactors* section.

The present book is based entirely on material presented at the 1958 United Nations Geneva Conference on the Peaceful Uses of Atomic Energy, and contains 16 papers of which ten are on reactor physics. and six are rather more general papers on specific reactor systems. Since some 2,000 papers were presented at the 1958 Geneva Conference, of which at least 250 were directly concerned with nuclear reactors, a review of the papers on reactors would have been of greater value. It is, however, to be regretted that the present work falls below the very high standards set by earlier volumes in the series, since instead of presenting reviews of the material presented at the 1958 Conference, it contains only reprints of a selection of the papers.

Although all these papers were published in the complete *Conference Proceedings* three years before the present work appeared, there is some value in having in a single compact volume a selection of papers dealing with one particular field, together with an index. However, any selection of 16 papers from so many is bound to be to some extent arbitrary, resulting in undue attention to detail in some areas at the expense of inadequate general coverage. For example, more than 150 pages are devoted to British, American and Russian work on physical measurements on fast reactor experimental assemblies, but the reader must refer to the full *Proceedings* to appreciate the relevance of such work to the design of a fast power reactor. Similarly, on a more general level, good descriptions are given of five different types of reactor system, but the reader will look in vain for any information on the design and operating experience of the two reactor systems first used for large-scale power production—the British gas-cooled graphite type and the United States pressurized water reactor.

It is easy to see that the editor and publishers have set themselves a very difficult task, but it is doubtful if this volume will meet the needs of the large body of readers who would have expected a more critical selection or review of the papers presented at Geneva. L. Rotherham

INDEX OF DIFFERENTIAL THERMAL ANALYSIS

Scifax (regd.) Differential Thermal Analysis Data Index, with Mineral, Inorganic and Organic Sections

Compiled by Dr. R. C. Mackenzie. Comprising 1630 punched cards (20 Introductory, 1012 Mineral, 287 Inorganic, 311 Organic); and Extra Code Card, 31 Guide Cards, Two 8 in. needles with handles, and Explanatory booklet. (London: Cleaver-Hume Press, Ltd., 1962.) £55.

This index consists of punched cards which are slotted to record the temperatures and sense of the principal and second differential thermal analysis peaks and whether these occur in an inert atmosphere. To code the peak temperatures, the range from less than $-100°$ C to greater than 1,800° C is divided into intervals (mainly 50° C) and to each is assigned a number from 1 to 27. The numbers are recorded on the card and also slotted on the upper edge, the other three edges remaining free. Each card is numbered and gives the name of the substance, its composition, and, if it is a mineral, a reference to Hey's *Chemical Index of Minerals*[1]. The various peaks that authors attribute to the substance, with reference to the literature as well as to other cards, are also recorded.

It is natural to compare the '*Scifax*' index with the well-known X-ray powder data card file (by A.S.T.M.). The A.S.T.M. file is available in several versions, including punched cards (but not slotted). The cards give the three principal *d* spacings and their relative intensities as well as the complete powder pattern of the substance and, if available, the experimental conditions, cell constants, system, space group, optical data and references. In comparison, the '*Scifax*' cards record only the bare minimum of information; obviously they would be more useful if the evidence of the purity of the listed samples, nature of the reactions occurring at differential thermal analysis peaks and experimental conditions were also shown. The A.S.T.M. file distinguishes data of high reliability, and this could with profit be followed in the '*Scifax*' index because there is unnecessary duplication of cards, and the proportion of questionable minerals is too high. The fault is more noticeable among the clay minerals where about 10 per cent are dubious (for example, monothermite, polynite, serpentine, hydrogoethite, beidellite, gumbrin, iron-magnesian parahalloysite, etc.).

The method of sorting cards is reasonably satisfactory, and it is usually possible to find cards from their coding without much ambiguity. The '*Scifax*' index is arranged alphabetically, but the compiler suggests resorting the cards either according to their coding (for diagnosis) or according to their chemical composition (for reference). For diagnosis, it would be necessary to code and slot two more edges with the substance reference number so as to be able to retrieve cards.

While the inorganic section consists in the main of data of well-defined compounds and is a welcome addition to the literature, the organic section includes a noticeable proportion of cards of poorly defined high-molecular weight compounds (for example, calcium resinate, humic acid, albumen, 'Amberlite' resin, fibrin, carbolignin, etc.). If a work of reference must record data of poorly defined materials it is better that they are put into a separate section.

The '*Scifax*' index is well produced and if used with caution it could be useful in identification, but its chief value lies in being a convenient source of information, especially if the data are supplemented by the user. R. Greene-Kelly

[1] Hey, M. H., *An Index of Mineral Species and Varieties* (British Museum (Natural History), London, 1950).

RECENT DEVELOPMENTS IN HIGH-PRESSURE TECHNIQUES

Modern Very High Pressure Techniques

Edited by Dr. R. H. Wentorf, Jr. Pp. xiv+233. (London: Butterworth and Co. (Publishers), Ltd., 1962.) 60*s*.

It is eighty-two years ago that J. R. Hannay probably first produced artificial diamonds by the application of high pressure and temperature. Since that period the interest in high pressure has increased enormously because of the fundamental importance of the properties of matter under the extremes of pressure and temperature, and the possibilities of permanently modifying the structure of common materials.

The practical difficulty of research in this field has always been the design and the materials of construction of the containing vessel and plunger. Bridgman made the first major step forward from gun design practice by using a cylinder which was subjected to progressive internal and external loading, and this enabled him consistently to reach pressures of 50 kilobars. Using a two-stage apparatus of this design he achieved pressures of up to 100 kilobars, but with the consequent penalty of a very big reduction in working volume. Bridgman probably achieved pressures of 200 kilobars in a very restricted volume in his anvil design by using what he termed "the principle of massive support". The reproducible and indeed commercial production of artificial diamonds in 1955 by a research team in the General Electric Co. of America was achieved using a 'belt' apparatus which was a development of the anvil principle. Since then there have been considerable developments and there are now several proved designs using the principle of massive support which can be used to subject samples of materials of worth-while size to pressures in excess of 100 kilobars at temperatures up to and above 2,000° C.

This book is edited by one of the original General Electric Co. team, and the authors of the various chapters have all made major contributions to this field of research. The book really forms a progress report on the present state of the art of carrying out various fundamental experiments under pressures in excess of 20 kilobars at temperatures from a few degrees above absolute zero to more than 2,000° C. Chapters are devoted to the general principles of high-pressure apparatus design, high-pressure optical and electrical measurements, Debye–Scherrer X-ray techniques for high-pressure studies, high-temperature methods at high pressure, high pressure at low temperature, etc.

The editor and contributors to this book are to be congratulated on having written a most valuable addition to the three or four books available on high-pressure techniques. The publishers are also to be complimented on their high standards in the production of this volume. B. CROSSLAND

METHODS IN ELECTROPHYSIOLOGY

Electrophysiological Methods in Biological Research

By Dr. Jan Bureš, Dr. Mojmir Petráň and Dr. Jozef Zachar. Pp. 515. (Prague: Publishing House of the Czechoslovak Academy of Sciences, 1960.) Cena vázaného vytisku Kčs 54.

THIS is an excellent book which will be of real use to neurophysiologists. It not only describes methods and equipment in general, but also sets out in detail how to perform a large number of particular experiments, from measuring the membrane potential of *Nitella* to making stereotaxic lesions in the brain of the cat. I myself know of no other book that does the same. The English translation is impeccable.

Chapter 1 is a first-class account of electrochemistry and membrane theory, with a better outline of the sodium theory of the nerve impulse than is to be found in most Western physiology textbooks. Chapter 2 deals with apparatus and technique. The electronics is sound but somewhat dated, without transistors; but the later sections on locating faults, getting rid of alternating-current hum, etc., and on how to make electrodes are highly practical. Chapters 3–9 introduce, and then describe, experiments on membrane potentials, the nerve impulse, muscle, the electroretinogram, the spinal cord, the cerebral cortex and subcortical structures—to mention the main headings. An appendix contains stereotaxic atlases of the brain of the cat, rabbit and rat.

The description of each experiment is divided into: problem, principle, apparatus, other requirements, object (animal preparation used), procedure, results and conclusions. The instructions are lucid and full; for example, detailed drawings are given of the dissection of nerves in the frog's leg. References are given throughout (the majority to papers in English), but in many instances the records illustrated do not appear to be those of the original papers; from which we may conclude that the authors have performed many of the experiments themselves. From this no doubt stems the high quality and freshness of their presentation. P. A. MERTON

LIPIDS AND LIFE

Lipide Metabolism

Edited by Prof. Konrad Bloch. Pp. xiii+411. (New York and London: John Wiley and Sons, Inc.. 1960.) 84*s*.

LIPIDS are biochemical entities of sometimes vague affiliations but most are derivatives of higher fatty acids. Until recently they were an unpopular and generally neglected subject for research, but the past decade or two has brought a striking change, reflected in the present-day wealth of research publications on lipid chemistry, metabolism and functions. The importance of lipids in the functioning of living tissue is no longer minimized.

Assessment of this advancing tide of knowledge is a matter for the specialist. The book in question contains eight monographs, all the work of acknowledged authorities and outstanding research workers in the fields they survey. The editor is likewise prominent among lipid chemists. As he comments in his preface, some important subjects had to be omitted to ensure prompt publication. One such is the biosynthesis of cholesterol, a field with which Prof. Bloch's name is closely associated. Perhaps he felt it unnecessary to include this topic, since a contemporary review on it was being prepared for publication elsewhere[1], but there is no reference in the present book to a source of information on cholesterol synthesis, for example, in sections where other aspects of cholesterol metabolism are discussed.

The subjects discussed are: (1) synthesis and oxidation of saturated fatty acids, by D. E. Green and S. J. Wakil, (2) metabolism of unsaturated fatty acids, by J. F. Mead, (3) metabolism and function of phosphatides, by R. J. Rossiter and K. P. Strickland, (4) metabolism of glycerides, by B. Borgström, (5) lipolytic enzymes, by M. Kates, (6) hormonal regulation of fatty acid metabolism, by R. G. Langdon, (7) formation and metabolism of bile acids, by S. Bergstrom, H. Danielsson and B. Samuelsson, (8) chemistry and metabolism of bacterial lipids, by F. Asselineau and E. Lederer. Some of these topics are more extensive than others, necessitating a more condensed appraisal, but all authors have succeeded in giving readable accounts of the present state of knowledge and of the steps by which it was reached. Sometimes elimination of all but a main theme gives greater cohesion; for example, Chapter 1 concentrates exclusively on 2-carbon addition or removal at the carboxyl end of the fatty acid chain, making no reference to acids with an odd number of carbon atoms or to ω-oxidation. This is not a criticism, since the authors at the outset define the limits of their subject and I myself share the editor's belief that "no worthwhile topic will remain overlooked for long". Allocation of more than 30 pages to the chemistry of bacterial lipids in a book on metabolism is justified by the unusual nature of all the compounds described.

I feel confident that no reader, whether himself a lipid specialist or not, will fail to find all the contributions stimulating and adequate within their specified fields. The consistent pattern of the whole book, its clarity of expression, and its virtually complete freedom from misprints are a tribute to the editor no less than to the authors. It has been a pleasure to read it; it is a pleasure to recommend it unreservedly.

J. A. LOVERN

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[1] Popják, G., and Cornforth, J. W., *Adv. Enzymol.*, **22**, 281 (1960).

SOUTHERN BRITAIN IN THE IRON AGE

Map of Southern Britain in the Iron Age

Scale 1 : 625,000 (About Ten Miles to One Inch). (Chessington, Surrey: Director-General of the Ordnance Survey, 1962.) Map and 56-page booklet, 17*s*. 6*d*. net; Map only, 7*s*. 6*d*.; Text only, 8*s*.

THE Ordnance Survey has recently issued a map of Iron Age sites in southern England. Along with John Betjeman's book of churches, the list of National Trust properties and the Automobile Association's *Handbook*, this publication should be in the dashboard cubbyhole of every interested motorist. It is, indeed, a very fine piece of work. Every Iron Age site of the 500 years before the coming of the Romans is categorized and mapped under various headings, each with its own symbol and colour to help distinguish the *A*, *B* and *C* and superimposed phases of the culture. No one can deny that the Iron Age in Britain was a very splendid period, and this work—it is much more than a mere map—gives at a glance an impression of its extent, importance and distribution.

The introduction describes the scope of the work, the way the map works with grid references, and gives an account of the characteristics of the things marked on it, for example, Belgic oppida, hill forts of various kinds, open settlements, lake villages, farms, fogous, shrines, cemeteries, barrows, burials, dykes, trackways and "Other Finds of Iron Age Material". It is unfortunate that no more than half a dozen lines could have been devoted to objects found at the sites or as isolated occurrences, especially as the introduction is followed by a splendid five-page article by D. F. Allen with two tables and eight full-page distribution maps devoted entirely to coin finds. While the Ordnance Survey keeps an index of small finds, it would have been most useful if even perhaps only the most important of these could have been mentioned under their sites in an extra column in the gazetteer. The Birdlip Mirror, for example, is a thing of great beauty—possibly one of the most beautiful works of art of its kind extant—but it and the many other beautifully and characteristically decorated objects that have been found are not mapped and barely referred to. When structures and coins are so well dealt with one cannot help regretting that the Celtic art—and art is the expression of culture—could not also have been catered for. On the cover of the bound edition some 30 characteristic objects are indeed incorporated, in colour, in a design, with a key to their provenance overleaf. But this is possibly misleading to the uninstructed amateur who may well think he will find information about them inside—it might have been more correctly indicative of the contents to have had drawings of hillforts, etc., or even a sketch of Maiden Castle, or a plan of Wandlebury Rings. But the specialist, of course, already knows all about Celtic art, and it is only the non-specialist who will suffer. This, after all, is a work of specialist importance. For students of the Iron Age and schoolteachers the splendid map is indispensable and O. G. S. Crawford would have rejoiced to see it.

M. C. BURKITT

THE CETACEANS

Whales

By Prof. E. J. Slijper. Translated by A. J. Pomerans. Pp. 475. (London: Hutchinson and Co. (Publishers), Ltd., 1962.) 63*s*. net.

IN this book, which is a revised translation of Prof. Slijper's *Walvissen* published in Holland in 1958, the author has concentrated on the physiology, functional anatomy and behaviour of the Cetacea. These are subjects in which he has long been interested and on which he has worked not only in the laboratory and in northern waters but also during an antarctic whaling expedition in one of the great modern pelagic whalers. His industry over a great many years has resulted in the production of a book which appears to omit nothing that is known of these aspects of whale biology. The work is not addressed to the zoologist alone, but also aims to interest "the widest possible circle of readers in the life of animals which by their size, their strange habits, and the adventurous methods by which they are caught, have for many centuries captivated the imagination of mankind".

After a historical introduction, Prof. Slijper deals with the subdivisions of his subject in separate chapters, showing that an immense amount of new information has been discovered about the biology of whales during the past fifty years; he also indicates the wide and fascinating field that awaits further study by zoologists. His concluding chapter deals with the future of whales and whaling, a matter that leads beyond the paths of zoology into the realms of economics, big business and even politics.

The author has been fortunate in his translator, A. J. Pomerans, who has performed his task most skilfully so that the text flows as smoothly as if it had been originally written in English; Prof. Slijper's own linguistic skill has no doubt contributed much to this happy result.

The book is profusely illustrated with photographs and with very beautiful, clear line-drawings which add much to its value and, probably, to its rather high price. It concludes with an extensive select bibliography grouped under the subjects of each chapter, an arrangement which is not entirely to be commended because it is not a list of references, and the works of some of the authors quoted in the text are not to be found in it. The book will undoubtedly succeed in its aim of interesting a wide circle of readers in addition to zoologists who have specialized in studying the Cetacea.

L. HARRISON MATTHEWS

EFFECT OF INSTRUMENT DEVELOPMENT ON THE PROGRESS OF CHEMISTRY*

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CHEMISTRY, very briefly, is concerned with making molecules from atoms or other molecules and in determining the structure of the molecules so produced. In the ultimate analysis both these matters are determined by interactions between atoms. However, the day is very far distant when chemistry would, in any event on paper, be a matter of being able to calculate from such atomic information the manner and the rate of chemical reactions and the kinds of molecules that will result from these interactions. Unlike physics, no fundamentally new principles have been discovered recently, but the ramifications of the subject are so large that it is necessary to be constantly on the alert for the development of new methods of doing basically simple things if progress is to be made at all. Quite a revolution in methods has occurred within the past fifteen years and there is every reason to believe that this will continue in the near future.

Systems of molecules come into a dynamic state of equilibrium once a chemical reaction ceases. The general description of equilibrium is the business of thermodynamics, to which is now added statistical mechanics, which in turn deals with energy states of molecules, and this in its turn is determined by atomic interactions. Thus, thermodynamic equilibrium depends ultimately on a precise knowledge of molecular structure. It is therefore logical to consider first the contribution of instrument development to the determination of molecules.

For convenience it may be best to deal with gases and solids separately. Liquids still constitute a very serious problem; it is not very clear whether to treat them as gases or solids, since approaches from both sides have their advantages and disadvantages. It is convenient also to consider the historical position in two parts. Let us call the period prior to 1940 'Phase 1'.

Phase 1 in solids had made enormous progress mainly due to X-ray diffraction studies. Provided the material is crystalline, then in principle no matter how complicated is the structure it can be sorted out with the position of atoms (except hydrogen) being defined with precision at least to within 10^{-3} Å. Electron diffraction has not made anything like the same progress owing to the low penetrating power of even high-voltage electrons. Maybe the disappointing feature is that electron diffraction does not really help at all in the determination of the surface layers of solids, and in particular in the monomolecular layers that are adsorbed on to the surfaces of solids. Similarly, X-ray diffraction did not contribute much to the determination of the structure of gaseous molecules or of molecules in liquids, and here there was a real lack of precise knowledge. Electron diffraction by gases was rather more successful, though the interpretation of the results was rather laborious.

With the interpretation of absorption spectra in the visible and ultra-violet regions of the spectrum, and to a very limited extent in the infra-red (instrumental difficulties here were enormous), some progress was made in the determination of the precise molecular structure of diatomic and some simple polyatomic molecules. Beyond this limit progress would seem to have come to a stop.

Even a basic measurement such as molecular weight was determined, not very precisely, by the classical methods of physical chemistry developed in the latter half of the nineteenth century. Molecular weights of more than a few hundred simply could not be determined, yet the science of high molecular chemistry was just beginning to develop. In the late 1930's the outstanding instrumental development was the oil-driven ultra-centrifuge, a combination of advanced mechanical engineering and precision optics that gave quantitatively complete evidence of the existence of large molecules with molecular weights of 10^6.

Phase 2: Modern physical methods have brought about a revolution in the matter, affecting both large and small molecules. With small molecules the introduction of a very high-precision mass spectrometer is the most significant advance by far. In this apparatus the substance is volatilized in the usual way in the ionization chamber and is ionized by electron bombardment so that only one electron is detached from the molecule. The mass of the positive ion is then measured directly. High precision is an absolute necessity because it is necessary to discriminate between molecules like N_2 and CO, both of which have almost exactly the same molecular weight, since not only is the weight of the molecule needed but also a clue to its structure.

In the case of large molecules one cannot use any method involving volatilization and so the molecules must be dissolved in a solvent, but the physical effect on a solvent is so small that exceedingly sensitive physical devices have been needed to measure the effect accurately. For example, the boiling-point of a solution is slightly greater than that of the pure solvent. This difference, which might be 10^{-4} °C, can only be detected by sensing elements such as a thermistor coupled to electronic amplifying devices of the highest sensitivity. Similarly, the osmotic pressure of such solutions may be equal to a head of a few millimetres of the solvent in question. Precise measurements can be made again only by optical and photoelectric amplifying devices. Provided certain conditions are fulfilled, solutions scatter light more than solvents. Although itself small, this effect increases with the increase in size of the solute molecule. Fortunately, even big molecules in solution are smaller than the wave-length of the visible light used, so that the theory of the method can be accurately worked out. Such solutions behave in a way which is far from ideal and hence the utmost dilution must be used. This cuts down the amount of scattered light until it is quite impossible to measure it visibly and difficult to measure it photographically. Fortunately, photomultiplier tubes have come in at the right time to provide the necessary accuracy and these enable molecules of molecular weight of up to 10^6 or even 10^7 to be determined accurately. The high sensitivity of the photomultiplier enables the scattering pattern to be determined and this gives information concerning the shape of the molecules in solution as well. This cannot be obtained absolutely in any other way, and therefore the method is invaluable in understanding the hydrodynamic properties of solutions of this kind.

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* Substance of the Thomson Lecture delivered before the Society of Instrument Technology at the Royal Institution, London, on October 4

Instrumental development led to another advance. Chemically, it is possible to attach to the end of molecules containing maybe 10^4 or even 10^6 atoms a specific atom or defined group of atoms, for example, bromine. If, therefore, this small number of atoms can be determined in a given material the weight of the molecule can be determined. Analytical chemical methods are completely useless for this purpose. However, if the terminal atom is radioactive, for example, carbon-14, or can be made radioactive by neutron bombardment, then accurate estimation of molecular weights can readily be made, but this depends on the means for making radioisotopes and on the counting equipment essential for techniques of this kind.

It might be thought that when molecules become large enough they should be capable of being seen in an electron microscope, but a molecule of molecular weight 10^6 is only about 20 Å in diameter if it is spherical in shape. While it is true that individual molecules can be identified the method is not practical for quantitative use in determining molecular weight in any accurate way.

Coming back to the question of molecular structure, the problem of structure determination for crystalline solids is solved, but again only if sufficiently small molecules or periodic arrays of ions are concerned. When the molecule becomes larger the problems increase enormously, since the diffraction pattern simply cannot be reduced by simple calculating machines. The large molecules of biological interest, for example, the vitamins, the proteins and the viruses, are complicated and progress recently has only been possible because of the availability of digital computers. Positions of the hydrogen atom cannot be determined because of the smallness of scattering of X-rays, but their position can be inferred pretty confidently. However, if the data must be obtained, neutron diffraction is a solution, although, unfortunately, the provision of a sufficiently intense stream of neutrons is not nearly so simple a proposition as that of the production of a beam of X-rays for structure determination.

In Phase 1 of this survey infra-red absorption measurements were extremely difficult to make, not because there was any special difficulty in making infra-red spectrometers but simply because the means of measuring the intensity of infra-red radiation was practically non-existent. The advances came from improved thermoelectrical devices; here it is possible to magnify the signals from such devices by electronic means and provide a signal of such a magnitude that it can operate robust recording devices. In fact, the information now provided is so voluminous that it cannot be completely interpreted, but the important point is that in the simpler polyatomic molecules one can determine with precision the shape and atomic distances, interatomic vibrations and rotational modes of motion.

Instrumental development has brought both infra-red and ultra-violet absorption spectrometry into virtually every laboratory. Structures of relatively simple molecules can then be completely determined, but much else is obtained as well—the effects of environment on the behaviour of molecules, for example, hydrogen bonding—the modification of vibration of molecules is affected by the nature of the environments of the solvent or absorption in solid surfaces.

However, to supplement these methods there are now others. Precision is always desirable and the infra-red methods sometimes are not suitable for determining accurately the moments of inertia of certain kinds of molecules. Fortunately, the development of means of measuring the absorption of microwaves for vapours and the very considerable precision of the frequencies of absorption that can be measured makes it possible to determine inter-atomic distances with accuracies down to about 10^{-3} Å. This method is, of course, restricted to substances having a sufficiently high vapour pressure and a sufficiently simple chemical constitution.

Perhaps the most extensive recent development, simply because it is so useful, is the technique of nuclear magnetic resonance. This depends on the principle that the nucleii of certain atoms, particularly hydrogen, behave as if they were small precessing magnets in a magnetic field and under such conditions their interaction with the magnetic field makes it possible for them to occupy two neighbouring energy states. The energy difference of magnetic fields of several thousand gauss is such that the switch can be induced by frequencies of about 50–100 Mc/s, again in the microwave region. From the chemical point of view the important thing is that the environment of the nucleus can affect the energy of transition between the two states. Thus, the nature of the bonds between the hydrogen atoms and other atoms can affect this transition. Consequently, in principle, the method could become useful in the finer details of structural chemistry. While these statements can be made confidently, the usefulness of the technique has been wholly dependent on the development of microwave apparatus with suitably added recording devices so that these energy transitions can be determined with a high degree of precision in frequency and magnitude. In combination with other complementary techniques it has formed a most powerful addition to the structural tools of the chemist.

Microwaves of a similar nature have found a most useful but more restricted application in quite a different field though the principles involved are generally similar. An unpaired electron attached to an atom also spins like a little magnet and similarly in a homogeneous magnetic field has two well-defined energy states. Hence, if a substance containing such systems is placed in a wave-guide in such a magnetic field, absorption of microwave radiation can be used as a measure of the number of unpaired electrons in a given volume of material. Such chemical systems are radicals, for example, CH_3, which exist in both the gaseous, liquid and solid phases. In special circumstances their concentration can be as high as 10^{-2} moles per litre and their presence, even their concentration, can be measured by conventional magnetic balances. In the majority of chemical reactions, on the other hand, the concentration may be as low as 10^{-7} moles per litre. Here the new method of electron spin resonance comes into its own, and with suitable recording devices it provides a hitherto unattainable level of sensitivity to enable reactions occurring through the intermediary of free radicals to be studied in a unique way. As in nuclear magnetic resonance, unpaired electrons are also subject to environmental influences and therefore many of these radicals have a complex multi-lined spectrum, characteristic of the radical in question. Thus, one can literally see on a display screen the nature of the radicals changing during the course of a chemical reaction. The resolving power in time is poor, being of the order of many minutes.

Chemical Reactions

To follow the progress of a chemical reaction it is necessary to have analytical methods which will give an accurate record of what is happening chemically at all times. If the reaction is slow and can be stopped at suitable points in its course, the matter is comparatively simple. But analytical difficulties arise in determining the presence of very small proportions of certain compounds the presence of which can modify drastically the course of the reaction. However, it is far more convenient to be able to monitor the reaction continuously, in which case instrumental methods have to be used. Practically all chemical reactions take place through the intermediary of energetic molecules or fragments of molecules which are specially reactive. In fact, the concentration of these molecules determines the rate of reaction as well as its course, and the understanding of the method of creation and disappearance is indispensable in elucidating the mechanism of the reaction. Moreover, the concentration is usually so small and the life-time so short that normal analytical methods are useless in this respect. These problems have now been completely solved by the development of instrumental techniques of great sensitivity. If a reaction is exothermic in its course and, chemically, fairly well defined the rate of evolution of heat may be used. What is needed then is an extremely sensitive means of measuring rises in temperature, and this has to be done in a matter of milliseconds. In liquid systems this can be done by a variety of means. If the volume in c.c. used is small and the system is thermally insulated, then the whole reaction system is virtually adiabatic up to periods of 100 sec. As rise in temperature is then a direct measure of the extent of the reaction, the necessity of measuring small rises in temperature, for example, 10^{-4} °C, results from the following considerations. In, for example, an oxidation reaction initiated externally by ultra-violet radiation when the oxygen is already dissolved in the liquid it takes a short time, from 10^{-3} to about 1 sec, to build up the concentration of the reactive intermediates to a steady value. This lapse of time, together with the known rate of the creation of these intermediates, can be used to calculate their concentration. By the use of established methods the mechanism of the reaction can be completely worked out. Since increase in temperature affects almost every physical property of a liquid, then in principle any physical method is applicable. The direct measurement of temperature by a thermojunction or thermistor is sufficient, but the required sensitivity can only be obtained by the use of very sensitive d.-c. amplifying devices suited especially to these sensing elements for, unfortunately, it is not possible to generate an a.-c. output from the elements as happens in the case of similar devices used in the case of infra-red spectrometers. Fortunately, the response of such elements, which is up to 10^{-3} sec, is normally sufficiently short to cope with most kinds of reaction, although shorter times, for example, 10^{-4} sec, would be desirable. If a liquid is transparent, optical interferometric methods can be used, which, surprisingly, are not especially sensitive even though the precise measurement of the movement of interference fringes by photomultipliers is used as the detecting elements.

Measurement of dielectric constant at moderate frequencies, for example, 50 Mc/s, can be made extremely sensitive, and this, coupled with high-speed oscilloscope recordings, is probably the most sensitive method of all. For example, 10^{-4} per cent of chemical change in as little as 10^{-6} sec can be detected, although naturally it is only applicable to low-loss insulating liquids.

With gas-phase reactions the situation is rather more complicated because heat escapes much more quickly from such systems. Fortunately, the lifetime of the active species is shorter too. The latter factor makes it impossible to use any kind of measuring probes since the response of, for example, a wire, of even 10μ in diameter is something of the order of 10^{-2} sec, but worse still the wires distort the concentration of atomic and radical concentrations in their immediate neighbourhood. So what is measured by this method is not necessarily representative of what goes on in the bulk of the system.

The slight pressure increase which results from the heating of the gas can be measured by what is virtually a condenser microphone of great sensitivity with a diaphragm so designed that it will respond to the rapid rise in pressure. Recording presents no difficulties, but the problem of using such a detecting element in the reaction system other than at room temperature is not easy. Other methods, too, using similar information can be used, but they are not specially dependent on instrumental development. The aforementioned methods, although soundly based and accurate, depend on indirect methods of approach. This has led to various attempts to solve the problem by direct means. In those reactions which can be induced photochemically the reaction can be induced by one means or another and the technique of flash photolysis has proved invaluable. In this case, in gaseous systems, an intense flash of radiation up to an energy of 1,000–10,000 J is delivered to the system, in a time much shorter, for example, one millionth of a second, than the lifetime of the reacting species concerned. This flash generates such a high concentration of active molecules, including electronically excited species, that their concentration can be determined directly by absorption spectrophotometry. Further, the variation of concentration with time can be determined by using a momentary flash for the absorption experiments. Such an unequivocal method of identifying active species has established beyond doubt the mechanism of many gas reactions.

A somewhat similar technique in principle can be applied by using a shock wave to initiate a gas-phase reaction. The transmission of a shock wave through a gas activates the molecules in a time which is short compared with that of most chemical reactions. Thus, when the shock wave is passed there is time to see what happens chemically, not quite at leisure, but at least in the absence of any further activating process. Another advantage of the shock wave is that it is possible to raise gases to temperatures unattainable even by flames and certainly far beyond the melting-point of any impermeable container for the gases. Temperatures up to 10^4 °C can be achieved. This opens the possibility of studying chemical events under quite exceptional conditions. These phenomena occur in times of the order of a millisecond, so once again spectroscopic methods coupled with sensitive photoelectric recording methods are absolutely essential if the full potentialities of the method are to be exploited. The shock-tube technique itself in its present form is borrowed from hypersonic aerodynamics.

Analytical Chemistry

It has already been mentioned that chemistry was mainly concerned with the structure of molecules and the way in which chemical reactions take place. To enable these branches to advance, analytical methods of high sensitivity and precision are, of course, a prerequisite. But analytical methods are needed for much else besides in metallurgy and biochemistry, and many other borderline sciences. Instrumental methods have completely revolutionized this side of chemistry and made it more precise, speedier and more sensitive as well.

On the spectroscopic side infra-red, visible and ultra-violet absorption spectroscopy are used as a matter of course, not simply for structural work but for identification of substances in complex mixtures. This, of course, depends on a firm foundation of structural knowledge in the first place. Similarly, emission spectra are especially useful in identifying elements, particularly metals. The precise method of excitation depends on circumstances, and internal standards provide the means of making the method quantitative. Once this work was done by photographic methods involving measuring the density of spectral lines on a photographic plate. This method is now supplanted by using a battery of photomultipliers each fixed on a chosen spectral line and coupled to digital counting devices. By this method one can now get, within a minute or two, a complete analysis of a complex alloy with as many metals identified as are needed. Speed is essential, for example, in steel works where analysis of melts is needed quickly; without it production would be seriously impeded. The analysis of substances containing alkali and similar metals may be carried out by atomic absorption spectrophotometry. This is a matter of measuring the intensity of absorption of the metal when volatilized in a flame. The measurement of sodium is easily done, but other metals are more difficult. However, photoelectric detectors with suitable amplifiers coupled to a monochromator make it possible to extend the method readily.

Even classical X-ray diffraction analysis has been bent to serve analytical purposes. The method has the great merit of non-destructive testing. The principles are not new, but the apparatus very much depends on several recent developments. A related development is X-ray fluorescence. Here the specimen is irradiated with a beam of X-rays from a high atomic number element such as tungsten and each of the elements present emits by fluorescence its characteristic radiation. This is reflected from a suitable crystal and the intensity of the diffracted beams measured and recorded using Geiger counters. The only limitation is with elements of low atomic number when the radiations are absorbed by air. There is sometimes a positive advantage if the elements the concentration of which is sought are present only in small traces in a matrix of light elements.

An even more striking application of this kind of method is the X-ray microanalyser. Here an accelerated beam of electrons (50 kV) is focused on the specimen itself. By using the techniques learned from electron microscopy the spot can be reduced to 1μ in diameter. Characteristic X-rays are analysed by crystal diffraction and Geiger counters. Elements of low atomic number cannot be so easily estimated except by using a vacuum apparatus. The great merits of this kind of analytical equipment are that minute quantities of material can be analysed in a large bulk of foreign material and that the specimen, for example, an alloy, can be scanned over a considerable area. What is more, by means of the optical microscope used simultaneously, the precise position of the specimen so irradiated can be examined.

It is really outside the scope of this lecture to deal with radioactive methods of analysis. Suffice it to say that apart from the production of radioactive elements, the instrumental development in counters and associated equipment has made the method of extraordinary sensitivity and of direct application. As an example of extreme sensitivity it is possible in the case of strontium to count down to 8×10^{-15} g atoms per litre. Chemical methods are simply out of the question for measuring quantities of material as small as this.

The analysis of gases and vapours has always posed awkward problems for the chemist. The methods are laborious in the extreme and usually needed very large amounts of material of the order of many c.c.s at normal temperature and pressure. In fact, the lack of methods was a vital factor in holding up progress in reaction kinetics and in many analytical problems of a similar kind. Here the situation has changed more than in any other branch of chemistry. First there is the mass spectrometer. After a long period of use as an instrument for the accurate measurement of atomic weights, the development of recording devices instead of photography and means of stabilizing electric and magnetic fields made it possible to carry out chemical gas analysis. With polyatomic molecules the number of ions produced from one molecule is, of course, very high, but here again simple computers with the ability to solve large numbers of simultaneous equations made this kind of analysis a practical proposition. Before the mass spectrometer came into widespread use gas chromatography was invented. Here was really a master invention capable not only of analysing gas mixtures but almost any kind of mixture provided it can be volatilized into a stream of inert gas at a reasonable temperature. The principle of the method is simple in that the substance concerned is partitioned between the stationary liquid phase (supported on a solid) or solid phase and the vapour phase. This partitioning occurs many times during the passage of the vapour mixture through the apparatus so that there is ample opportunity for very exact separation to be achieved—better, for example, than can be achieved by distillation of liquids. The problem is then to devise an apparatus that will detect the most minute concentrations of substances mixed with a carrier gas, and, secondly, to measure the total amount of material emerging from such a column in a fixed time. Again any conventional method of physical analysis is out of the question though sufficiently sensitive thermal conductivity systems are suitable. Even gas density balances of quite exceptional sensitivity are applicable. However, gas concentration detectors of a variety of types coupled to the recording and integrating machines gives the method tremendous sensitivity. In spite of all this the instrument has to be calibrated with known substances. If, however, records of a known substance are, in fact, obtained then the mass spectrometer may have to be brought in for the purposes of identification. One of the by-products of these developments has been the scaling up of the technique as a tool for the preparation of samples of compounds of quite exceptional purity. Any help in improving the effectiveness of separation procedures is a continuing requirement in chemistry.

The instruments briefly described here are of a complex character and even for laboratory use may range up to £25,000 each. Perhaps I should therefore direct attention to true chromatography, which makes use of the simpler materials, is of great elegance, and can be quantitative when necessary.

Here again it is a matter of partitioning a substance between the stationary solid phase—a piece of filter paper—and a moving liquid phase in which the substance is dissolved. By virtue of the movement of the liquid across the paper, substances distribute themselves on the paper depending on the magnitude of the partition coefficient between the two phases. Even greater separation can be achieved by afterwards running another liquid at right angles to the motion of the first. If the substances are coloured the process can be followed visually, hence the name chromatography. If not, they can be made to react with reagents to develop colour, and thus can be observed easily. What is more, it is possible to label some of these substances with radioactive atoms and to scan a chromatogram by suitable radioactive counters to identify the positions of the materials used. Thus, we almost come back to sealing wax and string, for paper chromatography is not much removed from this in its basic simplicity. However, the signs are that although these extraordinary methods are basically simple it is not long before detecting tools are devised to make them more sensitive and more reliable.

In this short space I have tried very briefly to scan a vast field of chemistry and applied physics. There has been no opportunity to indicate the quantitative aspects of the situation and therefore to demonstrate just how much is now possible with these numerous advances. There is much talk about interdisciplinary research these days. There could not be a better demonstration of it than in this field. The advances made are due to real collaboration. The physicists have certainly invented the basic tools—it has been the groups of far-sighted chemists who have seized them with enthusiasm and explored them to the full.

FOSSIL HAND BONES FROM OLDUVAI GORGE

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IN *Nature* of December 17, 1960, Dr. L. S. B. Leakey reported on the discovery of a number of fossil bones of the hand and the foot on a living floor some 20 ft. below the uppermost limit of Bed I, Olduvai. Later (*Nature* of February 25, 1961), Dr. Leakey reported the discovery of a mandible and two parietal fragments of a juvenile from the same site and associated with a well-defined living floor of an Oldowan culture.

Fifteen of the hand bones pertaining to at least two individuals, an adult and a juvenile, have been identified and examined, and are described here. Their allocation is given in Table 1.

Table 1

Juvenile		Uncertain age		Adult	
4 Middle phalanges	*I.U.*	1 Trapezium	*I.U.*	2 Proximal phalanges	*C.D.*
		1 Scaphoid	*I.U.*		
2 Terminal phalanges (fingers)	*C.U.*	1 Capitate	*C.D.*		
		1 Base of 2nd metacarpal	*I.D.*		
1 Terminal phalanx (thumb)	*C.U.*	2 Fragments of middle phalanges	*I.D.*		

C, complete; *I*, incomplete, *D*, damaged; *U*, undamaged.

The middle phalanges (Fig. 1, second row) from the juvenile hand, lacking only their epiphyses, constitute a series II–V from the right hand. They are robust bones, rather more so than phalanges of comparable length of juvenile *Gorilla* and adult *Homo sapiens*. They are strongly curved and, palmad, bear well-defined grooves which are situated in the distal half of the bone for the insertion of flexor digitorum superficialis.

The adult proximal phalanges (Fig. 1, third row) are also more robust than bones of comparable length in modern man; they are strongly curved both longitudinally and transversely, fusiform in shape and deeply hollowed out on the palmar aspect; sharply defined fibrous flexor sheath ridges extend from the base of the bones to their necks.

The terminal phalanges (Fig. 1, top row) which are juvenile, having incompletely fused epiphyses, are characteristically *sapiens* in form. The terminal phalanx of the thumb is of particular interest; it is stout and broad and bears a deep impression for the insertion of flexor pollicis longus.

The carpal bones (Fig. 1, 4th row) are all damaged, but sufficient of their original form remains to determine their structural and functional affinities. The lunate surface of the scaphoid has a rectangular outline; and the tubercle, which is broken off at its root, was probably somewhat elongated, though not as long as in the anthropoid apes. The trapezium has a well-defined saddle-surface but the evidence provided by the other articular surfaces indicates that its 'set' in the carpus was unlike that found in modern man and similar to the condition in *Gorilla*. The capitate, though badly eroded, is generally more *sapiens* than ape-like.

Morphologically, the Olduvai hand bones cannot be closely matched with any known hominoid species living to-day. They bear, however, a greater similarity to juvenile *Gorilla* and adult *H.s. sapiens* than to adult *Gorilla*, *Pan* or *Pongo*. This is due largely to the absence in the fossil bones of any features peculiarly characteristic of brachiators. The adult

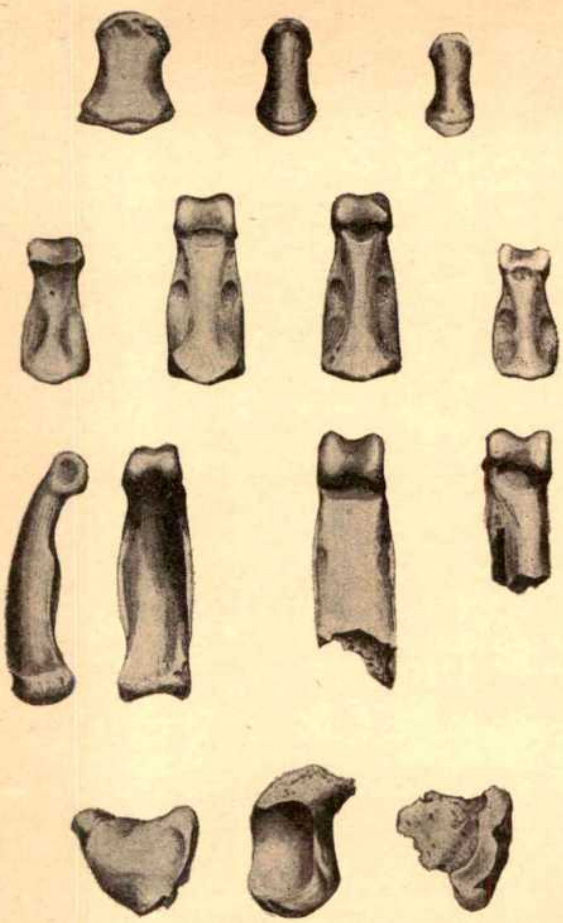

Fig. 1. Hand bone assemblage (drawings by Audrey Besterman). Top row, Juvenile. L. to R., terminal phalanx (thumb); 2 terminal phalanges (fingers). Second row. Juvenile. Middle phalanges, I–V. Third row. L. to R., proximal phalanx (adult), lateral and A.-P. views. 2 proximal phalanges (juvenile). Fourth row. Indeterminate age. L. to R. L. trapezium, L. scaphoid, L. capitate

gorilla hand has a number of specializations that are presumably related to its secondarily terrestrial mode of life and its great body-weight; these features, again, are absent from the fossil bone. The juvenile gorilla hand lacks the secondary specializations of the adult, and it is possibly for this reason alone that its bones have affinities with those of the fossil. The fossil bones differ from those of modern man in a number of features: (1) robustness; (2) dorsal curvature of the shafts of the phalanges; (3) distal insertion of the flexor digitorum superficialis; (4) strength of fibro-tendinous markings; (5) 'set' of trapezium in the carpus; (6) the form of the scaphoid; (7) the depth of the carpal tunnel. The fossil bones resemble modern man in the following features: (1) presence of broad, stout terminal phalanges on fingers and thumb; (2) form of the distal articular surface of the capitate; (3) ellipsoidal form of metacarpo-phalangeal joint surfaces.

There seems little doubt that this assemblage of fossil hand bones all belong to the same species. The difference between the juvenile and adult hand bones are no greater than the differences between the bones of an adult and a juvenile gorilla. In view of this conclusion that the two individuals on the *F.L.K. NN* 1 site are co-specific, all the hand bones are taken into account in the discussion of the functional and systematic implications of the hand as a whole.

While morphologically the precise affinities of the Olduvai hand are indistinct, functionally there seems little reason to doubt that the hand is that of a hominid.

The hand of modern man is capable of two basic prehensile movements that have been termed precision grip and power grip[1]. The power grip is used by man when a secure and strong grip is required for performing an activity in which the elements of delicacy and precision are of secondary importance. In the power grip the object is held as in a clamp between the flexed fingers and the palm, reinforcement and counter-pressure being supported by the adducted thumb. The precision grip, nevertheless, is used by man where a delicate touch and a precise control of movement is required and is achieved by means of a grip between the palmar aspect of the terminal phalanx of the fully opposed thumb and the terminal phalanges of the fingers. The essential osteological correlates of the precision grip are: (1) a fully opposable thumb with a broad spatulate terminal phalanx; (2) broad terminal phalanges on the other digits; (3) a proportion in length between thumb and digits that would permit a full pulp-to-pulp contact between them when they are approximated. While there appears to be little or no doubt that the Olduvai hand fulfils the first two requirements of the precision

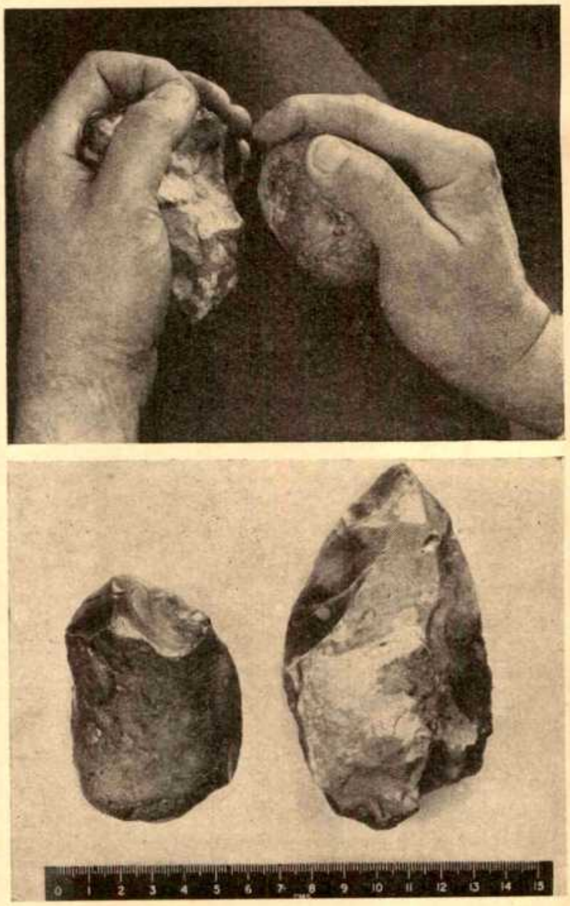

Fig. 2. Top, stone-on-stone technique of hand-axe construction using a power grip only. The flint core is being supported on the knee; bottom left, 'Oldowan' pebble-tool; right, 'Chellean' hand-axe made by me using the above technique

grip, there is no way to be certain about the last in the absence of the thumb metacarpal. While the saddle-surface of the trapezium leaves no doubt that the thumb could be rotated medially about its own axis to face towards the other digits, there is no reason for supposing that the proportions between the thumb and index finger of the fossil form are exactly as in modern man; indeed, the 'set' of the trapezium in the carpus suggests that this is not so. The question therefore is whether the thumb, having undergone rotation, is capable of pulp-to-pulp contact with the remaining digits. In the anthropoid apes while the thumb is opposable *per se*, pulp-to-pulp contact cannot be made owing to the marked disproportion of the length of fingers and thumb. It would seem, therefore, that the Olduvai hand was capable of power grip equal in performance, but, in view of the evidence of the attachment of the flexor tendons, comparatively greater in strength than in modern man. There is less certainty with regard to precision grip, which, while undoubtedly possible, may not have been as effective as in modern man. The overall picture presented by this assemblage is of a short powerful hand with strong, curved digits, surmounted by broad, flat nails and held in marked flexion. The thumb is strong and opposable, though possibly rather short.

At a recent conference at Burg Wartenstein, Austria, organized by the Wenner-Gren Foundation for Anthropological Research, an attempt was made to produce a diagnosis for the genus *Homo*. It was agreed that such a diagnosis could not be made unless certain characters and character complexes were present in combination. Included in these characters, which are referable to the skull, the brain and the post-cranial skeleton, was the criterion that 'the hand is capable of making tools of a recognizable culture'. If one assumes that the artefacts of an early Oldowan culture found on the living floor were the work of the species the remains of which are found there, then this criterion is fulfilled and, in addition, an interesting conclusion is possible: that toolmaking was established in the human lineage long before the hand had assumed its modern human form.

If, on the other hand, one bears the possibility in mind that some other, more advanced form was the toolmaker and the known incumbents of the floor were its victims, then it is in the functional morphology of the hand itself that one must look for evidence of toolmaking. On anatomical grounds there is no doubt that the Olduvai hand was sufficiently advanced in terms of the basic power and precision grips to have used naturally occurring objects as tools to good advantage. There is less certainty about toolmaking, which involves not only a peripheral but also a central intellectual factor as Oakley[2] has long insisted. The report on the Bed I juvenile skull fragments, soon to be published, may throw some light on this question by indicating the approximate cranial capacity of the juvenile skull.

Given the intellectual ability, the construction of the crude, rather small pebble-tools of the type found on the living floor, is well within the physical capacity of the Olduvai hand. Precision grip, which is imperfectly evolved in the fossil hand, is not an essential requisite at this level of craftsmanship as personal experiments in the construction of 'Oldowan' pebble-tools and 'Chellean' hand-axes have shown (Fig. 2).

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[1] Napier, J. R., *J. Bone and Joint Surg.*, **38**, B, 902 (1956).

[2] Oakley, K. P., *Man the Toolmaker*, Fourth ed. (Brit. Mus. (N.H.) 1949).

THE BRITISH MUSEUM (NATURAL HISTORY)

NEW BOTANICAL EXHIBITION GALLERY

By J. F. M. CANNON

Department of Botany, British Museum (Natural History)

THE title of Prof. L. Hogben's familiar book, *Science for the Citizen*, acts as a challenge to anyone concerned with the planning and execution of a museum gallery concerned with some branch of science. The purpose of this article is to place on record the basic considerations and intentions that have guided us in the preparation of the new Botanical Exhibition Gallery which is at least the third in line of succession in the British Museum. Very little seems to be known of the exhibition at Bloomsbury, where all the departments of the British Museum were originally concentrated. A photograph shows it to have been in keeping with the austere traditions of adult education that the more enlightened and liberal Victorians were encouraging. Learning was not to be taken lightly, or perhaps only by those fortunate enough to enjoy a university education. Its successor, which was established after the move of the scientific departments to South Kensington in 1881, came to a violent end on the night of September 9–10, 1940, when, during the course of an air raid, an oil bomb was dropped on the east wing of the Museum. Although disastrous damage occurred and many valuable scientific specimens were destroyed some good resulted as it initiated a replanning of the accommodation so that additional storage space for the scientific collections, together with a fine new exhibition gallery, were made available within the existing shell of the building. The old gallery was stacked with valuable information, but made few concessions to the lay visitor drifting rather aimlessly around the Museum on a wet afternoon. In addition, the passage of time left some exhibits misleading and incomplete, while some major aspects of modern botany remained unmentioned.

It is now our duty to present the credentials of our new venture. First, for whom is it intended ? The obvious answer is that for a national museum the whole population must be the target. This, however, is a drastic over-simplification; to attempt to make a uniform appeal to all grades of intelligence and education over the population would in our opinion be disastrous. Some selection must be made to direct the main impact of the Gallery on to a particular target. Rightly or wrongly we have chosen those taking Ordinary or Advanced Level Certificate of

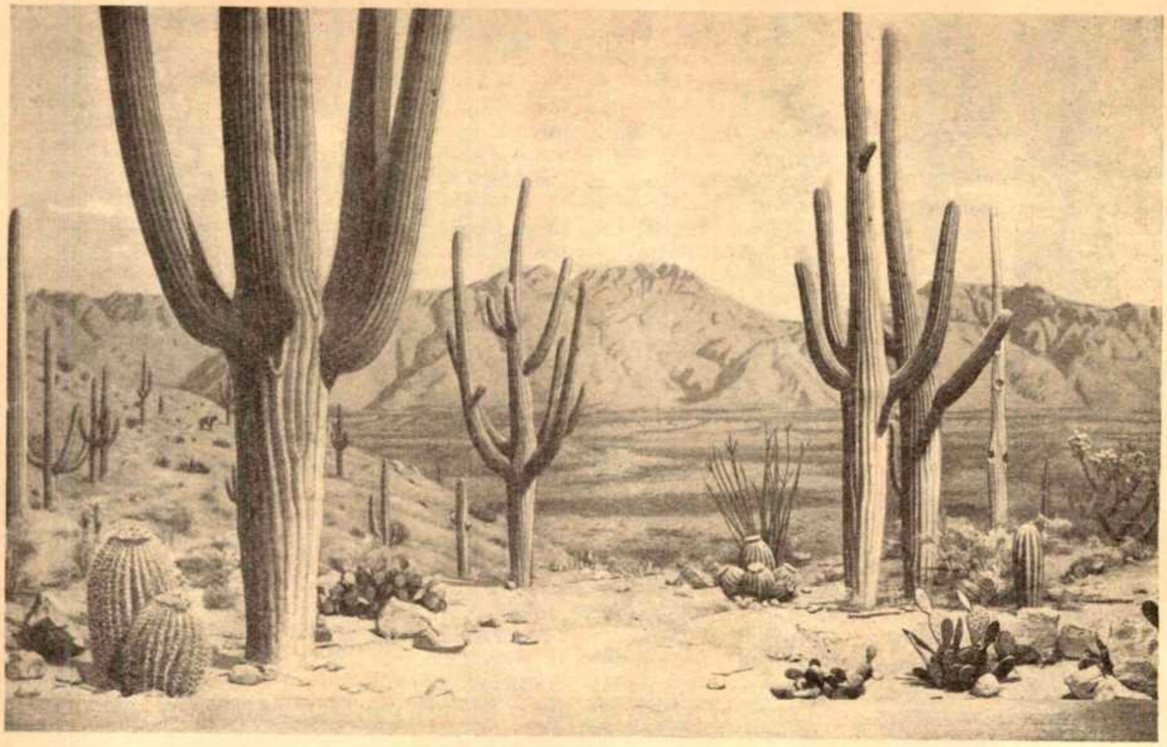

Fig. 1. A diorama of the Arizona Desert showing a group of the giant cactus (*Carnegiea gigantea*) with other desert plants. This is part of an exhibit illustrating plant life under arid conditions

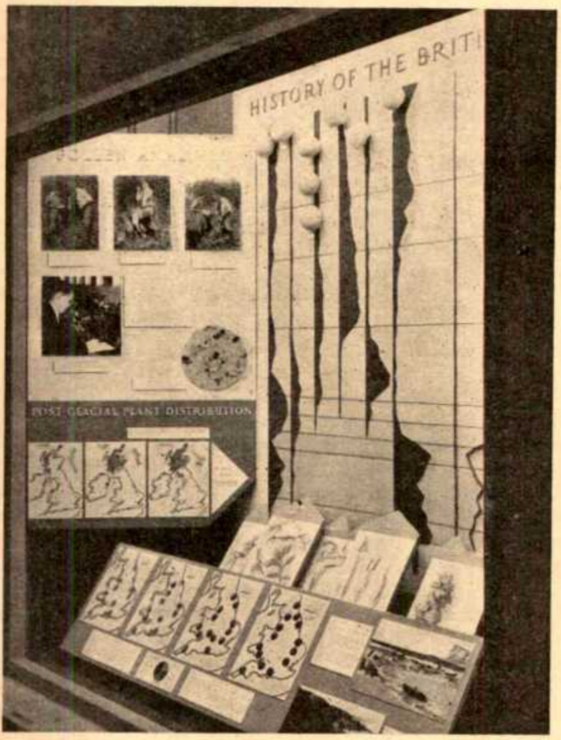

Fig. 2. Part of an exhibit which illustrates the history of the British flora since the last glaciation. The left-hand panel describes the technique of pollen analysis; large models of the most important pollen grains are placed on the diagram at the back

Education courses at school as our central group. Some preliminary research with pupils and teachers suggests that there are many ways in which a museum gallery can supplement and complement the teacher's work in classroom and laboratory. In some instances we can provide models and explanatory diagrams which are beyond the resources of the ordinary school, and which should help to explain some of the more difficult parts of the syllabus; the opportunities for supplementing the syllabus and widening the pupil's botanical horizons are, of course, limited only by the space available. While we are catering especially for this group, we try to ensure that each exhibit contains something of interest for all levels of intelligence. If we achieve nothing more than a new awareness of the extraordinary beauty of plants, then we shall not have failed in our intentions.

What then are our aims? Our chief aim is to present a broad and catholic picture of modern botany, but with the limitations of space and time, special emphasis has been given to certain topics like ecology, because they are of wide general interest and to some extent are the responsibility of everyone. In considering the chief plant groups we have mainly stressed functional morphology, rather than systematics. We have devoted quite a lot of space to plant physiology, so often neglected in museums, but which, as one of the most progressive and advanced aspects of botany, demands attention. We have tried to steer a course between the old 'carbon dioxide + water = sugar + oxygen' (a statement so simplified as to be quite misleading in the absence of further information) and the detailed results of recent scholarship which are gradually revealing the amazingly complex workings of the living cell. We

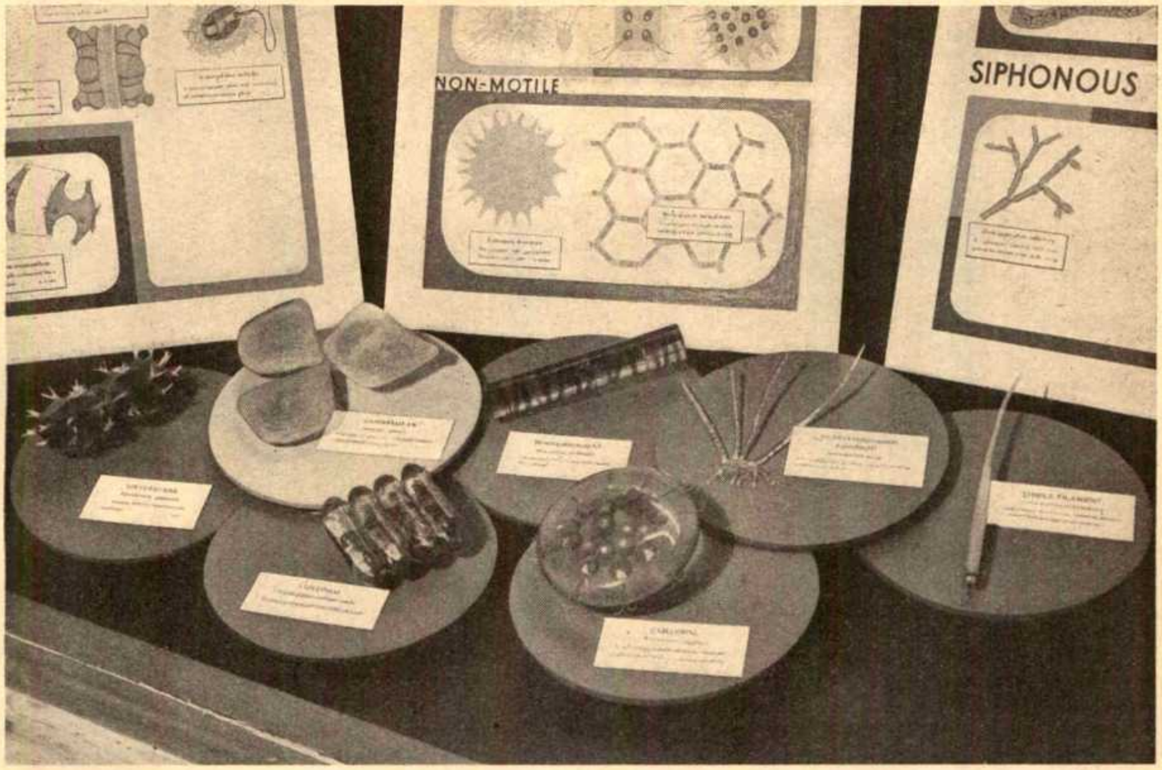

Fig. 3. A series of plastic models to illustrate the increasing complexity of structure in the algae, from unicellular species to those with a complex heterotrichous system of filaments

feel that if we present even a brief glimpse of the network of reactions controlled by enzymes we shall have achieved something.

The dioramas, which are supported by ample teaching material, form our main ecological exhibits. Each exhibit is designed to emphasize one or more basic ecological principles. Thus, the sand dune/salt marsh exhibit deals with succession in space and time, while in the British mountain case the importance of underlying rock in the determination of the land-forms, soil and flora developed is stressed. The Himalayan diorama illustrates the importance of altitude in determining the vertical zonation of vegetation, and the British mountain exhibit demonstrates the effect of altitude on individual plants. The exhibits produced jointly with the Nature Conservancy are concerned with the establishment and scientific management of nature reserves, especially the need for fundamental ecological research before sound management plans can be formulated. What more appropriate end could there be to a biological gallery ? Unless everyone becomes aware of the need for the right use of natural resources, the efforts of disarmament commissions alone will not be sufficient to preserve a world fit to live in.

We should like to commend to our fellow scientists the need for continuing improvement in our relations with the general public. This is no new idea; but it is obvious that many opportunities remain to be taken. There can be no doubt that in problems like conservation the eleventh hour is already with us, while an informed and sympathetic public opinion must form the soundest basis for expanding research. In the last analysis the public holds the purse strings for most scientific research and development and is entitled to full information, both on recent progress and the broad picture of each major branch. A public which feels that scientists have little patience with their ignorance and no time to make their work comprehensible to the layman, is less likely to support scientific research than one whose interest has been stimulated by sound information made freely available. If our new gallery has made some contribution to explaining not only botany but also the kind of work on which modern botanists are engaged, we shall not feel that we have failed.

OBITUARIES

Dr. U. S. Haslam-Jones

Dr. Ughtred Shuttleworth Haslam-Jones, who died on August 7, was the son of the Rev. M. A. Haslam-Jones, then Baptist minister of Hackney, London, but later a clergyman of the Church of England. He was born on July 12, 1903, and educated at various schools as his father moved from one church to another. From Ealing County School he obtained a mathematical scholarship to Queen's College, Oxford, in 1921. At Oxford he was awarded Firsts and the junior and senior mathematical scholarships, and then took a D.Phil. degree under the supervision of G. H. Hardy.

His thesis on the theory of functions of a complex variable contained a more memorable result than such efforts usually do. There is a well-known theorem of Hadamard that if the function $f(z) = \Sigma a_n z^n$ has singularities at points $\alpha_1, \alpha_2, \ldots$ and $g(z) = \Sigma b_n z^n$ has singularities at points $\beta_1, \beta_2, \ldots$, then the singularities of the function $F(z) = \Sigma a_n b_n z^n$ are among the points $\alpha_m \beta_n$. It is far from obvious how to extend this theorem to functions of more than one variable, but an extension to functions of two variables was found by Haslam-Jones. The result, which is not so easy to state as Hadamard's, is in the *Proceedings of the London Mathematical Society* (Ser. 2, 27, 223; 1928).

From 1927 until 1936 he was a lecturer at the University of Liverpool. The rest of his life was spent as Fellow and tutor of Queen's College, Oxford, where he succeeded his old tutor, C. H. Thompson. He was an excellent tutor and was also highly regarded as a lecturer, his usual topic being the theory of functions of a real variable. He served on the Council of the London Mathematical Society from 1937 until 1944, and was a vice-president of the Society from 1940 until 1942. From 1940 until his death he was an editor of the *Quarterly Journal of Mathematics*.

His main scientific interest was in the theory of functions of a real variable, and he published a series of papers on this subject, some of them in collaboration with Dr. J. C. Burkill. The main object of these papers is to show that even very irregular functions, which are not necessarily differentiable in the ordinary sense, still have derivatives in some generalized or approximate sense, except possibly in sets of points of measure zero.

He was a friendly and loyal colleague and will be much missed by all in Oxford.

E. C. TITCHMARSH

Mr. Simeon M. Aisenstein

THE death occurred on September 3 at Chelmsford of Mr. Simeon M. Aisenstein, a pioneer of radio communication in Europe, particularly in the development of thermionic valves.

Mr. Aisenstein was born at Kiev in 1884, and directed his attention to wireless telegraphy as a boy of 16. After studying at the University of Kiev and Charlottenburg Polytechnic, he established a private experimental laboratory prior to the formation of a wireless company. Following a visit to England, this Russian firm was merged into Marconi's Wireless Telegraph Co., Ltd., with Aisenstein as director and principal technical adviser on developments in Russia.

During the First World War, Aisenstein was responsible for all the high-power stations in Russia, and for the maintenance of communications with the Allies. He conducted experimental work in collaboration with the Army and Navy, including pioneer trials in communication with submerged submarines which attracted the attention of the British Admiralty. In 1917, during the Bolshevik revolution, Mr. Aisenstein was imprisoned, but managed to escape to England in 1921, and joined the Marconi Co. A year later he was appointed to a company established by Marconi's in Poland: he was instrumental in the development of this company, particularly in the manufacture of radio valves. In 1935 he went to Czechoslovakia to set up another valve factory, but had to return to Chelmsford in 1939. Here he was at first engaged in the design of radio equipment, and later took over the valve laboratories of the Company. On the formation of the English Electric Valve Co., Ltd., in 1947, he became the first general manager, a position he held until his retirement in 1955, after which he continued to act as a consultant to the Company.

Mr. Aisenstein had been professor at the Moscow Higher Technical School and a vice-president of the Russian Society of Radio Engineers. In recognition of his service, he was appointed a Councillor of State for the Russian Empire in 1915, and was made an Officer of the Legion of Honour by the French Government in the following year. He was the author of many patents and a number of scientific and technical papers.

R. L. SMITH-ROSE

NEWS and VIEWS

Bacteriology at St. Andrews: Prof. W. J. Tulloch

PROF. W. J. TULLOCH has just retired after occupying the chair of bacteriology at Queen's College, Dundee (University of St. Andrews), for 41 years. Throughout this long period, Tulloch brilliantly fulfilled the double role of teacher and research worker besides being dean of the Medical Faculty for many years and supervising the ever-growing diagnostic bacteriological services for both hospital and local authorities. He was an indefatigable and meticulously careful bench worker, and his publications consist largely of a series of major studies—on the mechanism of agglutination, the anaerobic infections of war wounds including the serological types of the tetanus bacillus, the laboratory diagnosis of smallpox, the occurrence of tubercle bacilli and brucellæ in milk, diphtheritic infections in a partially immunized community, and the laboratory diagnosis of gonococcal infections. In most of these fields of study, he was a pioneer who blazed the trail for others to follow. He gave the Harben Lectures in 1934, and for the Lister Lecture of the Royal College of Physicians, Edinburgh, in 1956, he took as his theme "Medicine as an Aspect of General Biology". Tulloch loved to teach and to the end undertook practically all the formal lectures to the undergraduate classes. He was a dramatic and very effective teacher and even in the vivas at the professional examinations, he answered most of his own questions. Undoubtedly he inspired many young men both in and outside his department, and these 'old boys' will long cherish their association with the *maestro*.

Prof. J. P. Duguid

DR. J. P. DUGUID, who succeeds Prof. W. J. Tulloch, is an Edinburgh man, born and bred. Educated at Edinburgh Academy, he went up to the University at the age of 17 and graduated with honours in both science and medicine. After some hospital experience, he was appointed lecturer in bacteriology in 1944, became senior lecturer in 1954 and was given the status of reader in 1960. He gained his M.D. with honours and a gold medal in 1949. Like his predecessor, Duguid is outstanding both as teacher and research worker. In a department with a heavy teaching load, he has taught systematic and practical courses of bacteriology for medical, dental, veterinary and science undergraduate students and in the diploma courses for public health and tropical medicine and other postgraduate classes. He has been largely responsible for the development of the Honours School in Microbiology in Edinburgh. His early research work during and after the Second World War was on the use and mode of action of penicillin, and he made the acute observation in 1946 that penicillin probably acted directly on the cell wall. Later, he did excellent work on the modes of spread of respiratory infections and their control by air disinfection, etc. More recently, he has worked in the field of bacterial cytology and physiology, using electron microscopy and other methods to investigate the structure and function of bacterial capsules, inclusions, fimbriæ and non-fimbrial adhesive factors. He is an assistant editor of the *Journal of Pathology and Bacteriology*.

Pharmacy at London: Prof. W. H. Linnell

PROF. WILFRED H. LINNELL retired from the position of dean of the School of Pharmacy, University of London, on November 1. A native of Cheshire and educated at Stockport Grammar School, Prof. Linnell has very strong ties with the north-eastern part of Britain, where, in Newcastle upon Tyne, he served his four-year apprenticeship in a pharmaceutical business. He joined the School of Pharmacy, in London, as a student in 1914 and obtained his fellowship of the Pharmaceutical Society (together with the Society's Silver Medal) in 1916. Within five weeks he was in France with the Special Brigade, Royal Engineers. After demobilization he returned to Newcastle, as a student of chemistry at King's College, where he obtained an honours degree and was Johnson Fellow and Prizeman in 1921. The award, in 1922, of the Earl Grey Memorial Fellowship took him to Lincoln College, Oxford, where he studied with W. H. Perkin. But local patriotism guided him back to Durham, where he gained his Ph.D. in 1924. After a short period at the Fuel Research Station, East Greenwich, he joined the School of Pharmacy in 1926, where the University of London had just instituted a degree in pharmacy. Prof. Linnell took charge of the Chemistry Department, in which he was appointed reader in 1927, and professor of pharmaceutical chemistry in 1944. On the retirement of Prof. H. Berry in 1956, Linnell became part-time dean of the School and supervised its transference from the old premises in Bloomsbury Square to the modern building in Brunswick Square. He remained part-time dean until his retirement from the chair in 1961, when he became full-time dean of the School. Since 1926 Prof. Linnell has been prominent in all pharmaceutical affairs. He has been examiner to the Pharmaceutical Society for Statutory Examinations since 1927, a member of the Revision Committee of the *British Pharmaceutical Codex* and a member of the British Pharmacopoeia Commission Committee.

Dr. F. Hartley

DR. FRANK HARTLEY, who succeeds Prof. W. H. Linnell, entered pharmacy in 1929, under the old 'oral and practical conditions', and after Part 1 continued his studies for the pharmaceutical chemist examination with an intercollegiate course at University College and the School of Pharmacy, University of London. He was awarded the School's silver medals in pharmaceutics, pharmacognosy and pharmaceutical chemistry and immediately commenced the study of chemistry at Birkbeck College. In 1933 he obtained an honours degree in chemistry with distinction and first prize for the course. From 1933–40 he was successively demonstrator and lecturer at the School of Pharmacy, from which Institution he took his Ph.D. in 1941 under the supervision of Prof. Linnell. Hartley helped supervise the evacuation of the School to Cardiff and then in 1940 joined Organon Laboratories, Ltd., as chief chemist and factory manager. From 1943–46 he was secretary to the Therapeutic Research Corporation and the General Penicillin Committee of the Ministry of Supply. He assumed his present position as scientific services director of the British Drug Houses in 1946. Dr. Hartley is well known in pharmaceutical circles. He has been chairman of the British Pharmaceutical Conference (1957) and is a member of the British Pharmacopoeia Commission Committee. He has also been examiner in pharmaceutical chemistry to the Pharmaceutical Society. His outstanding contributions to the subject were recognized in 1961 by his election to the Sir William Jackson Pope Memorial Lectureship of the Royal Society of Arts. Dr. Hartley has been actively associated for many years with the Royal Institute of Chemistry. He has served many periods as a member of Council and was a vice-president from 1958–60. His numerous friends and associates wish him many years of success in his return to the scene of his earlier triumphs.

Research Committee for Chemical Engineering

FOLLOWING publication of the first report of the Exploratory Committee in the April 1962 issue of *The Chemical Engineer* on the subject of chemical engineering research (see *Nature*, 195, 209; 1962), the Council of the Institution has now set up a Research Committee under the chairmanship of Dr. J. W. Barrett, director, Monsanto Chemicals, Ltd. The Research Committee will be responsible under the Council for the Institution's activities in encouraging, promoting and guiding research in chemical engineering. Broadly, the objects will be: (*a*) to help formulate and implement explicit research programmes in fields of chemical engineering in which fundamental research is thought to be required; (*b*) to help to maintain lively and realistic academic research programmes; (*c*) to suggest speculative and exploratory fields of research detached from the present-day preoccupations of industry; (*d*) to make recommendations as to the part which research institutes might play in chemical engineering research. The specific responsibilities of the Committee will include: (1) recommendation to the Council of individuals and topics for industrial research fellowships, guidance of Fellows after their appointment, receipt of their reports and recommendations for action thereon; (2) establishment of working parties and review of the working parties' activities.

U.S. Centre for Advanced Research in Astrophysics

THE Joint Institute for Laboratory Astrophysics (JILA) has announced a visiting membership programme directed at bringing scientists from throughout the United States and abroad to study at the Institute, which is situated at the University of Colorado in Boulder. The visitors will have complete freedom to work on problems of their own choice for as long as a year. Stipends are available for up to ten scientists during 1963. Visiting memberships are also available for those having other sources of funds. In providing a centre for advanced research in astrophysics, the Institute hopes to stimulate the continuous interchange of ideas from many areas. Much of the present work of the Institute concerns particular stellar atmospheres, atomic and molecular collisions and resonance phenomena. The Institute was set up last spring under the chairmanship of Dr. Lewis M. Branscomb. The original professional staff consists of eight atomic physicists from the National Bureau of Standards in Washington, two astrophysicists from the Bureau's Boulder laboratories, and a group of scientists headed by Prof. Wesley Brittin, chairman of the Physics Department, University of Colorado. The closing date for applications is January 15, and requests for application forms should be addressed to the Secretary for Visiting Members, JILA, 1511 University Avenue, Boulder, Colorado.

Radioactive Fall-out from U.S.S.R. Tests

The total amount of fall-out scattered globally from all the Soviet nuclear tests of 1961 exceeds that produced by the combined testing of the United States and Great Britain for the same time span, according to a study conducted by the U.S. Atomic Energy Commission (*Fallout from U.S.S.R. 1961 Tests*. By G. M. Dunning. Office of Technical Services, U.S. Department of Commerce, Washington 25, D.C., January 1962. 50 cents). The fall-out of fission products, as at the end of 1961, is based on 50 megatons of Soviet nuclear tests against 35 megatons of combined United States and British testing. The details show that total amount of fission products scattered globally from the test sites of nuclear explosions conducted by the U.S.S.R. from all their past tests, up to and including 1961, exceeds that for the United States and the United Kingdom during the same period. The study also shows that, from the 1961 U.S.S.R. testing alone (strontium-90 and cæsium-137), the amount of long-lived radionuclides that may be deposited in the United States (45 mc. per square mile for strontium-90 and 80 mc. for cæsium-137) would be equal to or a little more than that from the United States and United Kingdom tests in the past (40 mc. per square mile for strontium-90 and 70 mc. per square mile for cæsium-137). The amount of long-lived radionuclides from all past Soviet tests (90 mc. per square mile for strontium-90 and 160 mc. per square mile for cæsium-137) would be twice that from all past United States and United Kingdom tests.

The International Council of Scientific Unions

The Year Book of the International Council of Scientific Unions for 1962 comprises a Calendar of Arrangements for 1962 with lists of the officers of the Bureau, members of the Executive Board, national members of the Council and of the scientific members of the Council (Pp. 124. Rome: International Council of Scientific Unions, 1962). These are the international scientific unions, and the Year Book also includes details of the aims of the several unions, the officers of their various commissions, federated associations, committees or sections, and the members of the Committees of the International Council of Scientific Unions, and the joint Commissions and Executive Bodies of the International Council's permanent services. The statutes and by-laws of the Council are also given, together with the rules and charters for the scientific and special committees. There is an alphabetical list of the members of the Bureau, Executive Board, Scientific and Special Committees, Joint Commissions and other organs of the Council. The Treasurer's report and financial statement for 1961 are issued separately as a supplement. Apart from the inclusion of the calendar of arrangements, the items of which have now practically all occurred, the wealth of reference material makes it highly desirable that the Year Book should be published early in the year to which it relates: publication at least in the first quarter could be reasonably expected.

First World Conference on National Parks

The first world conference on "National Parks" was held in Seattle, Washington, during June 30–July 7. The conference was sponsored by the International Union for Conservation of Nature and Natural Resources and co-sponsored by Unesco and the Food and Agriculture Organization, in association with the United States National Park Service and the National Research Council of America. The general chairman of the conference and of the United States Steering Committee was Dr. Harold J. Coolidge, executive director of the Pacific Science Board of the United States National Academy of Sciences, who is also chairman of the I.U.C.N. Commission on National Parks. The object of the conference was to achieve a more effective international understanding and encouragement of the national parks movement on a world-wide basis. Although national parks and reserves have been established by most member countries of the United Nations, some have not yet done so. The theme of the conference was "National Parks of International Significance". The conference directed attention to the necessity of maintaining existing national parks as well as establishing new ones, and enabled Government representatives and other qualified experts to meet together to expand their efforts and co-ordinate their activities. In reviewing past international action in the national park sphere, mention should be made of the London Conventions of 1900 and 1933 for the protection of African fauna and flora. These treaties stimulated conservation programmes in many parts of Africa as well as in other continents, as did the 1942 Convention on Nature Protection and Wildlife Preservation in the Western Hemisphere which has been ratified by twelve American Republics. Many Governments in Africa and Latin America have introduced legislation for setting up and administering national parks and reserves based on the definitions and objectives contained in these important international agreements. A special supplement containing resolutions adopted by the conference is included in the *I.U.C.N. Bulletin* (4; July/September 1962).

Radio Chimica Acta

The temptation to establish new scientific journals appears to be irresistible. The first number of *Radio Chimica Acta* has a foreword by a great German radiochemist, Otto Hahn, in which he poses the rhetorical question as to whether a journal, specially devoted to radiochemistry, can discharge an important function differing from that already performed by existing chemical and physical journals (*Radio Chimica Acta*, Vol. 1, No. 1; September 1962. Pp. 1–48. Price per volume (4 issues), 72 D.M.; 18 dollars. Frankfurt a.M.: Akademische Verlagsgesellschaft; New York and London: Academic Press, 1962). After outlining briefly the history of radiochemistry, he concludes that a place is needed where the results now published in a variety of journals can be brought together. One could give wholehearted support to this view if every paper published fitted neatly into only one of the subdivisions of science and if the boundaries of the subdivisions were unambiguous. However, it is of the nature of science that these two conditions are rarely, if ever, satisfied.

This is well exemplified by the contents of the first number of this journal, which comprises ten papers and two short notes. One paper is straight radiation chemistry, two are concerned with hot atom chemistry, one with complexes of neptunium IV and one is a somewhat technological approach to fractional solvent extraction separation procedures. One cannot help feeling that most of these papers might easily have found a much wider range of readers by publication in the established chemical journals. To have an additional journal for papers of this kind recalls the words of the sonnet "So all ... is dressing old words new, spending again what is already spent". In short, interested workers must pay again to read those papers which they should reasonably be able to expect to find in the journals to which they already subscribe. This is not a situation in which one can welcome another new journal, unless it promises to be of unusually high quality and to meet a really important need which, in this case, seems unlikely.

The National Museums of Ceylon

The National Museums of Ceylon clearly recognize research as one of their functions, and the current issue of *Spolia Zeylanica*, bulletin of the National Museums of Ceylon (29, Part II; 1962), gives renewed proof of the value of their work. The part includes papers on geology, zoology and anthropology, many written by the director, Dr. P. E. P. Derniyagala. The papers are all well produced and illustrated.

Glossary of Atomic Terms

The fourth edition of the booklet *Glossary of Atomic Terms* has been published by the U.K. Atomic Energy Authority. It provides a convenient collection of easily understood definitions of more than 400 terms and abbreviations used in atomic energy. This glossary is intended to be of help to those who are studying atomic energy for the first time. It may therefore be regarded more as a layman's manual of instruction than a source book of highly scientific definitions. For those already familiar with underlying principles, more technically precise definitions have been published by the British Standards Institution in their glossary *BS* 3455: 1962. The glossary is available from H.M.S.O. at 3*s*. 6*d*. net.

Italian Rock Carvings

In the foothills of the north Italian mountains there are several areas where prehistoric figures of animals, signs and weapons occur, carved on rock surfaces. The most famous series has long been known, thanks to the research work of the late C. Bicknell. They occur in the Ligurian Alps around Monte Bego, inland from Mentone. Another group, to be found in the Val Camonica where the valley debouches on to the north Italian plain, was described some years ago by Prof. Marro. While differing in several respects from the Monte Bego series, the Val Camonica art is undoubtedly, culturally speaking, connected with it, and both can be dated to the Early Metal Age. Superpositions make it possible to determine several slightly differing 'layers' at the Val Camonica. More recently, Dr. E. Anati has studied this region, and in the August issue of *Man* he has published a note on some figures of halberds, occurring at a site called locally Roccia dei Corni Freschi which lies near the River Oglio, just north of where it enters the Iseo Lake. Figures of halberds also occur at Monte Bego, but the types are not identical. An important book on his campaigns in the Val Camonica has recently been published by Dr. Anati in the United States, but for some copyright reason the work cannot be obtained in Great Britain.

Anæsthesia and Analgesia

Advances in anæsthesia are among the most dramatic in the whole field of medicine. Inevitably, these advances have meant an increasing degree of specialization, with the result that, outside the sphere of domiciliary obstetrics, relatively few general practitioners have now much experience in anæsthetics. Anæsthesia, however, as Prof. Beecher points out in an article in a symposium on "Anæsthesia and Analgesia" in the August issue of *The Practitioner* (189, No. 1130; 1962), is merely a branch of one of the most fundamental problems in medicine—the relief of pain. It is incumbent on all clinicians to keep in touch with what is being achieved in this field. To help them do so is the purpose of this symposium. In spite of increasing specialization—or perhaps because of it—society is gradually re-learning the old lesson that the essence of the practice of medicine is holism. The patient, not his disease, is the primary concern of the doctor. Nowhere is this truer than in the alleviation of pain—whether by anæsthetic, analgesic or human sympathy.

University News: Belfast

The following appointments to lectureships have been announced: J. Graham (civil engineering); Dr. J. H. Jones (dental pathology); A. Brown (mechanical engineering); Dr. A. M. Rosie (light electrical engineering).

Cambridge

C. Strachey, of King's College, has been elected to a senior research fellowship (Title *B*) at Churchill College.

Columbia

Dr. Tsung-Dao Lee is rejoining the Columbia University Department of Physics as a full-time member of the teaching staff. For the past two years, Dr. Lee has been adjunct professor of physics at Columbia, while serving as a member of the Institute for Advanced Study at Princeton. His new title at the University for the 1962–63 academic year will be visiting professor of physics. It was while he was a member of the Columbia Department of Physics that Dr. Lee was awarded the 1957 Nobel Prize in Physics jointly with Dr. Chen Ning Yang, of the Institute for Advanced Study, for their work in the disproof of the 'principle of conservation of parity'.

London

The following titles have been conferred: *Professor*, on Dr. R. L. F. Boyd (physics), in respect of his post at University College; on Dr. Florence N. David (probability and statistics), in respect of her post at University College; on Dr. Leonard Saunders (pharmaceutical chemistry), in respect of his post at the School of Pharmacy. *Reader*, on Mr. H. H. Bentall (thoracic surgery), in respect of his post at the Postgraduate Medical School of London; on Dr. D. Mendel (physiology), in respect of his post at King's College.

Sheffield

THE following appointments to lectureships have been announced: Dr. J. C. Bowman (honorary lecturer in genetics); Dr. N. M. Atherton (chemistry); Dr. D. Bellamy (zoology); Dr. P. J. Foster (fuel technology and chemical engineering); H. Hartnagel (electrical engineering); Dr. H. K. Kohler (applied mechanics); Dr. J. N. Murrell (honorary lecturer in chemistry); P. Polak (mechanical engineering); J. Swithenbank (fuel technology and chemical engineering); Dr. G. H. Tattersall (building science); Dr. A. K. Walton (physics). D. S. Buist has been appointed to the W. J. Rees research fellowship in refractories technology.

Sussex

THE following appointments to lectureships have been announced: Dr. G. G. C. Routh (economics); I. Hunt (philosophy); Dr. J. M. Radcliffe (theoretical physics); A. D. C. Grassie (experimental physics); Dr. B. L. Smith (experimental physics); Dr. J. D. Smith (chemistry).

Graduate Plastics Programme at Princeton

A GRADUATE programme has been instituted at Princeton University incorporating engineering study and fundamental research in plastics which can lead to the degree of M.Sc.(Eng.) or in certain cases to candidateship for a Ph.D. in the Departments of Mechanical or Chemical Engineering with support of the plastics programme. Instruction covers basic theory and properties, evaluation, production, fabrication, design and application of materials, as well as chemistry of plastics. The programme includes lecture and laboratory classes, and contact with industrial plants representing various interests of the plastics industry. Fellowships covering stipend, tuition and fees are available as well as opportunities for employment as part-time research assistants. Applicants for admission must hold a first degree in engineering or physical science from a recognized institution and must meet general admission requirements of the Graduate School of Princeton University. Further information can be obtained from Prof. Louis F. Rahm, director, Plastics Laboratory, Princeton University, Princeton, N.J.

The 1963 Stanley Clifford Milk Industry Award

THE Central Milk Distributive Committee, in recognition of the valuable services rendered in the dairy industry by Stanley Clifford, who was for many years chairman of the Committee, has established a Fund for the purpose of providing awards to enable a suitable applicant to make a short study tour of the dairying industry in the United Kingdom or abroad. Employees in the dairy industry or in dairy engineering in the United Kingdom who are below the age of thirty are eligible. With the consent of the Central Milk Distributive Committee it has been decided that the 1963 Award shall take the form of a study tour of approximately ten days duration on the Continent and efforts will be made to arrange this to give the greatest benefit to the winning candidate. Candidates should make their application in the form of a short statement covering the following points: (1) length of service in the industry; (2) nature of past and present employment; (3) particulars of any training courses attended; (4) the type of tour the candidate would like to make and the benefit it is hoped to derive from it. Applications for this Award should be made to the Secretary, Society of Dairy Technology, 17 Devonshire Street, London, W.1, not later than January 31, 1963.

Fulbright Travel Grants

UNDER the provisions of the Fulbright programme, travel grants are available to citizens of the United Kingdom and dependent territories to go to the United States of America for academic or educational purposes, provided that they have adequate financial support in dollars for the proposed period of the visit to the United States, and can show proof of admission to an institution of higher learning in the United States for a minimum period of nine months. All grants cover the cost of direct round-trip travel between a candidate's home and the American university or institution. They are available between June 1 and August 15, 1963, for which application must be received by March 16, 1963, and between August 16, 1963, and April 1, 1964, for which applications must be received by June 1, 1963. Application forms and further information can be obtained from the United States Educational Commission in the United Kingdom, 71 South Audley Street, London, W.1.

Announcements

MR. W. A. C. MATHIESON (Dependent Territories) has been appointed chairman of the Executive Council of the Commonwealth Agricultural Bureaux in succession to Dr. V. Armstrong (New Zealand). Mr. H. E. Seneviratne (Ceylon) has succeeded Mr. W. G. Alexander (United Kingdom) as vice-chairman.

THE autumn general meeting of the Iron and Steel Institute and the forty-fifth meeting of the Iron and Steel Engineers Group will be held in London during November 28–29. Further information can be obtained from the secretary, K. Headlam-Morley, Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1.

A SYMPOSIUM on "Citrate Metabolism" will be held by the Bone and Tooth Society at the Institute of Orthopædics, 234 Great Portland Street, London, W.1, on November 9. Further information can be obtained from the honorary secretary of the Society, Prof. B. Cohen, Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2.

THE autumn meeting of the Clay Minerals Group of the Mineralogical Society will be held in the apartments of the Geological Society of London, Burlington House, Piccadilly, London, W.1, on November 9. Further information can be obtained from the honorary secretary, R. M. S. Perrin, School of Agriculture, University of Cambridge, Cambridge.

THE winter meeting of the Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals will be held at Church House, Great Smith Street, London, S.W.1, during December 10–11. Included in the programme is a symposium on "Non-metallic Dispersions in Powder Metallurgy". Further information can be obtained from the Secretary, Powder Metallurgy Joint Group, 17 Belgrave Square, London, S.W.1.

THE INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY

THE report of the Council of the Institute of Physics and the Physical Society for the year 1961 was adopted at the annual general meeting of the Institute and Society held on July 10, 1962. During the year the Council met five times. It has been decided to mark annually the anniversary of the amalgamation of the Institute and Society in May 1960 by a series of meetings and functions, and the first of these was held on May 2 and 3, 1961. Sir John Cockcroft delivered his presidential address entitled "The Development of Physics" (*Bull. Inst. Phys.*, 12, 109; May 1961) in the Royal Commonwealth Society's hall; the first annual dinner of the amalgamated body was held at the Park Lane Hotel, London; and the first annual representative meeting, consisting of the officers of the various branches and groups and past-presidents of the Institute and Society, was held at the headquarters of the Institute and Society, 47 Belgrave Square, London.

During the year under review 1,161 applications for election or transfer to the various grades of membership were received. The total membership on December 31, 1961, numbered 9,120, consisting of 1,268 Fellows, 3,054 associates and 1,997 graduates, in addition to Fellows of the Physical Society, students and subscribers. This represents an increase of 400 over the year. The two technical colleges which applied for recognition for the purpose of the membership regulations relating to approved courses were visited by members of the Membership and Education Committee and both were accepted. Fifty-four technical colleges presented 987 candidates for the Ordinary National and thirty-five colleges 455 candidates for the Higher National Certificate in applied physics. Of the larger number, 239, of candidates for the graduateship examination, 64 were successful; fifteen question papers were set. An examination for Part 1 under the new regulations was also held; only three of the twenty-three candidates who entered were successful.

The Institute and Society was one of the sponsoring societies of the international conference held in the University of Manchester during September 4–8 to mark the jubilee of the discovery of the nucleus by Lord Rutherford. Other conferences and symposia were arranged during 1961.

The report of the committee under the chairmanship of Prof. R. V. Jones on problems facing university physics departments was submitted to the Robbins Committee, and has since received wide publication. All three of the Institute and Society's monthly periodicals, the *Proceedings of the Physical Society*, the *British Journal of Applied Physics* and the *Journal of Scientific Instruments*, increased in size compared with the previous year. A table in the annual report gives details of the material published in the periodicals during 1960 and 1961. Other publications sponsored by the Institute and Society were *Noise in Electronic Devices* and *Selected Papers on Stress Analysis* consisting of papers presented at Group conferences. Two new books, *Automatic Control* by A. E. De Barr and *Viscosity and its Measurement* by A. Dinsdale and F. Moore, are to be published in the "Monographs for Students" series, together with a new edition of J. Topping's *Errors of Observation and their Measurement*. A sixth volume in the series of "Laboratory and Workshop Notes", consisting of selections from the *Journal of Scientific Instruments* and compiled and edited by Dr. Ruth Lang, is shortly to appear.

The Institute and Society maintains ten specialist groups and nine branches in Great Britain, and two branches overseas, in Australia and Malaya respectively. A summary of the activities of these sections is given in the annual report, together with a list of the Council's representatives and nominees on joint and other committees and organizations.

The Australian branch consists of six divisions, and divisional activities have included a conference in Brisbane entitled "Sun–Earth Environment" organized by the Queensland and New South Wales Divisions at which more than forty papers were discussed by 120 delegates, in addition to about fifty scientific meetings and social events.

The activities of the Malayan branch were confined to its members attending colloquia in the Physics Department of the University of Malaya. The largest of the branches, the London and Home Counties Branch, with 4,113 members, held a symposium on "The Soil" during March in conjunction with the Royal Institute of Chemistry and the Institute of Biology. The subject of the Easter conference of the Education Group was "Communication: Physics for the Non-Specialist", and the summer meeting consisted of a visit to the research laboratories and new factory of Hilger and Watts, Ltd. During February the Electronics Group joined with the British Institution of Radio Engineers in promoting a meeting on "Tunnel Diodes". In the autumn a conference was held in Leamington on "The Physics of Gas Discharge Devices". The Low Temperature Group held a number of whole-day and half-day meetings. Topics discussed included: ultrasonic absorption and attenuation in metals at low temperatures; problems in thermal insulation; the generation of high d.-c. magnetic fields; superconductivity; ferromagnetism and antiferromagnetism at low temperatures; and the thermodynamic properties of fluid helium. The Simon Memorial Prize was awarded to Dr. I. M. Lifschitz of the Academy of Sciences of the Ukrainian S.S.R., Kharkov.

On the recommendation of its Awards Committee the Council approved some changes in the arrangements concerning the various awards (lectureships, medals and prizes). It authorized a new award, to be called the Maxwell Medal and Prize, for outstanding contributions to theoretical physics carried out within ten years of the award. The recipients of the Duddell Medal, C. V. Boys Prize, and Charles Chree Medal and Prize, were Dr. J. B. Adams, Prof. A. W. Merrison and Dr. Scott E. Forbush, respectively. The text of the C.V. Boys Lecture, entitled "Counter Techniques in High Energy Nuclear Physics: Rare Decays of pi and mu-mesons", has been published (*Proc. Phys. Soc.*, 78, 1116; Dec. 1961). The Guthrie Lecture was delivered by Dr. D. Shoenberg on the subject of the de Haas–van Alphen effect and the electronic structure of metals (*Proc. Phys. Soc.*, 79, 1; Jan. 1962), and the Thomas Young Oration on the application of frequency response techniques in optics by Dr. H. H. Hopkins. The Holweck Medal and Prize was presented by the French Physical Society to Prof. A. B. Pippard in Paris on May 12.

The total response to the Physics Trust Fund Appeal at the end of the year under review was £117,146. The cost of the educational and scientific work of the Institute and Society now runs at about £25,000 a year, reflecting the substantial planned increase to meet which the Trust Fund Appeal was mainly established. The accounts included in the annual report cover the first full year's working of the amalgamated Institute and Society. S. WEINTROUB

NUCLEAR RESEARCH IN FRANCE

THE Nuclear Research Centre of Fontenay-Aux-Roses is affiliated to the Commisariat a l'Energie Atomique and employs a staff of 1,000 (the total staff of the Commissariat is 14,000 persons) (*French Science News*, No. 2, April–June 1962). The Centre comprises the following departments:

(1) *Atomic Reactors*

(*a*) *Reactor protection studies* made with reactors *Zoë* and *Triton*. *Zoë* is a moderated, heavy-water cooled, natural uranium reactor which produces a maximum thermal neutron flux of 10^{12} neutrons/cm^2/sec. It was the first reactor built in France. *Triton* is a double-core, 20 per cent enriched uranium, water-moderated swimming-pool reactor, cooled by forced water-circulation. It develops a power of 150 kW and has a flux of 10^{13} neutrons/cm^2/sec.

A 600-keV *Sames* accelerator is also available in the Department and is used for the measurement of monoenergetic neutron propagation and diffusion in matter.

(*b*) *Critical experiments*. Sections of various materials are measured in a reactor by the oscillation method: *Zoë* and *Minerve* are used for this purpose. The latter is a low-power (100 W) experimental reactor, which produces maximum thermal flux of 5×10^9 neutrons/cm^2/sec. It is also used for measuring neutron absorption in samples of the materials used for the construction of reactors, and for neutron studies on lattices moderated with light water.

(*c*) *Liquid metals*. This section carries out research on the use of certain metals in the molten state as coolants in atomic reactors. The main application is the cooling of fast neutron reactors by means of sodium, sodium–potassium alloy, lead, lead–bismuth eutectic or mercury. The results of this research are to be applied to the cooling of *Rapsodie*, which is to be built at the Nuclear Centre at Cadarache.

(2) *Controlled Fusion*

(*a*) *Research on controlled fusion*, carried out by a joint Commissariat-Euratom research group. Research covers three main subjects: the 'striction' programme, where induced discharges, carrying 150,000 amp, are studied; the 'continuous mirror' programme, which uses accelerated ion injections; and the 'pulsed mirror' programme, which uses an apparatus for studying adiabatic compression.

(*b*) *Theory of ionized gases*. This section carries out the theoretical work required for controlled fusion research.

(3) *Chemistry*

(*a*) *Investigations, research and chemical applications*. The laboratories are equipped for the study and development of: the chemical processing of uranium, thorium and beryllium ore; the purification of 0·8 90 per cent enriched uranium; the purification of elements such as beryllium and zirconium; the processing of chemical plant waste.

(*b*) *Analytical studies*. This section carries out analytical studies which are new or which are better adapted to atomic energy problems, and the initial development of special equipment such as automatic analysers.

(*c*) *Contracts*. Work here consists mainly of the technical checking of contracts between the Commissariat and other organizations.

(4) *Plutonium*

This department has a series of 'hot' laboratories for the handling and study of radioactive materials.

(*a*) *Plutonium chemistry*. Here processes whereby the constituents of an irradiated fuel are separated and recuperated in pure forms are studied. Work follows three main lines: improvement of the solvent process; influence of progress made in fuels and cladding; dry processes.

Plutonium base ceramic and transuranium elements are also investigated.

(*b*) *Plutonium metallurgy*. The initial research on plutonium alloys was to examine aluminium base alloys. A plutonium technology workshop was built and metal transformation techniques were adapted to radioactive materials.

(5) *Mineralogy*

Analytical work is carried out as well as applied research work on radiogeology.

(6) *Mines and Mining Research*

The main department is at Fontenay; but its activities cover the whole of France, former French territories in Africa, and Madagascar.

(7) *Biology Department*

(*a*) *Atomic hygiene and radiopathology*. The fundamental problems of radiological protection are studied. In atomic hygiene, the aim is to set the security-level of irradiation and radioactive contamination.

Work on radioactive contamination deals with problems of industrial hygiene concerning the possible formation of liquid or solid radioactive aerosols in laboratories or workshops, and with problems of contamination via the digestive tract or via the skin.

Two laboratories carry out analyses which show any slight contamination, whether normal or accidental, of persons or of the biological environment.

(*b*) *Radiation check and radioactive engineering*. Activities are as follows: operation of the photometry and micrometry laboratories for checking the degree of irradiation by photographic or nuclear emulsions; supervision of the radioactivity of nuclear installation surroundings; the regular radiochemical analysis of rain and run-off; the study of natural radioactivity mainly by radon and thoron assay.

RHEOLOGY IN AUSTRALIA AND NEW ZEALAND

AT the Jubilee Conference of the Australian and New Zealand Association for the Advancement of Science, held at the University of Sydney during August 20–24, a Sub-Section on Rheology was created by members of the New South Wales Branch of the British Society of Rheology, under the secretaryship of Dr. L. Dintenfass (Department of Medicine, University of Sydney) and under the presidency of Prof. R. C. L. Bosworth (Department of Physical Chemistry, the University of New South Wales). This was the first time that rheologists had been able to form a definite group within a general conference in Australasia. Some twenty-seven papers were presented to actively interested audiences.

At the opening session the president pointed out that rheology had had a particularly long pre-scientific incubation period prior to the development of scientific principles, thought and instrumentation. In Australia and New Zealand rheological principles are involved in many primary and secondary industrial activities in medicine, agriculture and engineering, but, at the moment, there is in these countries no tertiary education course devoted to the study, the teaching or the research in rheological principles as such. The time is surely coming when this omission must be made good. The address at this session was given by Mr. R. S. T. Kingston, officer-in-charge of the Timber Physics Section, Division of Forest Products, C.S.I.R.O., South Melbourne, Victoria. Mr. Kingston reviewed the historical development in rheological theory with particular emphasis on non-linear phenomenological theory and non-colinear stress arising from non-linear strain velocities. Mr. Kingston was equally concerned with thermodynamical and statistical interpretations.

A session was devoted to rheology in biological systems. Dr. M. C. M. Dodgson (Prince of Wales Hospital, Randwick, N.S.W.) gave a paper on the rheology of brain, in which the mechanical properties of brain cell material were considered. Dr. Dintenfass gave a paper on viscosity and thixotropy of blood, and Dr. A. A. Palmer (Sydney Hospital) on the flow of blood in capillaries. The viscosity of blood decreases as the rate of shear increases; the viscosity of blood samples remains nearly constant at relatively higher rates of shear. Abnormal blood may show thixotropy even at these relatively higher rates of shear. Acceleration and deceleration in branching capillaries can lead to segregation of blood cells from plasma. Dr. B. Bloch (Sydney Hospital) and Dr. G. W. Hastings (Department of Polymer Science, the University of New South Wales) gave two papers on the use of plastics and adhesives in bone fractures.

A number of papers was concerned with the rheological properties of natural products. The rheology of protein solutions was considered by Dr. A. G. Parts (Department of Physical Chemistry, University of Sydney), who based his measurements on the dielectric loss and the viscosity of amino-acid solutions. Mr. M. Feughelman (Division of Textile Physics, C.S.I.R.O., North Ryde, N.S.W.) gave a paper on the structure and the mechanical properties of wool and related the properties with the nature of a number of bonds holding polypeptide chains together. Dr. B. J. Rigby (Division of Textile Physics, C.S.I.R.O., North Ryde, N.S.W.) gave a paper on thermal transitions in collagen. The transition temperature of wet unconstrained material apparently correlates with the amount of hydroxyproline present in the collagen. Mr. F. C. H. Ottaway (Bread Research Institute, North Ryde, N.S.W.) presented a paper on the rheology of bread dough. Initial fermentation changes the properties of the gluten content and no entirely satisfactory methods are available in dough-testing machines.

A number of papers was presented on the subject of the rheology of wood and wood products. Dr. M. C. Probine (Dominion Physical Laboratory, D.S.I.R., Lower Hutt, New Zealand) presented a paper on the rheological and morphological properties of plant cells. Mr. L. D. Armstrong (Division of Forest Products, C.S.I.R.O., South Melbourne, Victoria) considered the effects of moisture change on the creep of wood. Both the strength and the modulus of elasticity of wood decrease as the moisture content is decreased. Mr. H. G. Higgins (Division of Forest Products, C.S.I.R.O., South Melbourne, Victoria) gave a paper on the structural rheology of pulp and paper, and Prof. M. Chaikin (School of Textile Technology, the University of New South Wales) gave one on the rheology of non-uniform fibres, with special reference to textile fibres subjected to longitudinal extension.

The extension of rheological principles to chemical engineering problems and the more customary uses of these principles and the assessment of properties of the products of chemical industry were considered in a number of papers. Dr. T. Hagyard (Department of Chemical Engineering, University of Canterbury, Christchurch, New Zealand) gave a paper on the viscosity of fluidized bed systems, which he illustrated by showing high-speed photographs and high-speed cinematographic records of very nearly uniform spheres. The structures shown were those of hexagonal structures over limited regions with structureless intergranular materials and with occasional 'holes'. Mr. A. Sherwood (Department of Mechanical Engineering, University of Sydney) gave a paper on the determination of stresses in gelatine models. The newer silicone plastics are also being investigated for their suitability for this purpose. Mr. R. E. Price (Central Research Laboratory, Imperial Chemical Industries, Australia and New Zealand, Ltd., Ascot Vale, Victoria) gave a paper on post-extrusion swelling in thermoplastic melts, and Dr. F. Connors (Department of Polymer Science, University of New South Wales) gave one on the rheological characteristics of polyvinyl chloride. Mr. K. R. Hall (Chemical Research Laboratories, C.S.I.R.O., Fishermen's Bend, Victoria) gave a paper on the mixing operations of non-Newtonian fluids.

A number of papers was concerned with the basic properties of certain classes of materials, and others outlined new techniques of instrumentation. Mr. D. R. Axelrad (Department of Mechanical Engineering, University of Sydney), for example, gave a paper on the flow of metals, in which he outlined present-day theories of high-temperature creep. Dr. R.

Tanner (Department of Mechanical Engineering, University of Sydney) gave a paper on non-Newtonian lubricants. Greases and those systems which approximate in their behaviour to Bingham solids give higher bearing friction than Newtonian lubricants. Mr. A. W. Findlay (Monash University, Clayton, Victoria) gave a paper on the rheology of pigment suspensions and outlined a modified Bingham approach to the properties of these systems. Mr. R. Bell (Taubmans Industries, Ltd., St. Peters, N.S.W.) gave a paper on normal stresses in fluids, and stressed the need for emphasis on truly accurately level surfaces. Changing strain velocities can give rise to large normal stresses in many systems, including glycerine and golden syrup.

Dr. H. A. J. Battaerd (Central Research Laboratory, Imperial Chemical Industries, Australia and New Zealand, Ltd., Ascot Vale, Victoria) gave an account of a high-speed cinematic record of the performance of an impact tester utilizing a falling sphere which was applied to polythene films. Dr. P. U. A. Grossman (Division of Forest Products, C.S.I.R.O., South Melbourne, Victoria) outlined some unconventional testing methods in the study of creep and vibrational properties of solids.

The Sub-Section held one conjoint meeting with the Pharmaceutical Sciences Section. Two papers were presented, one by Dr. L. Dintenfass on micro-rheological methods and techniques, and one by Prof. R. C. L. Bosworth on the rheological properties of emulsions. The papers were followed by a panel discussion in which the hosts expressed many ways in which the rheologists could be of help to them.

R. C. L. BOSWORTH

THE BRITISH GELATINE AND GLUE RESEARCH ASSOCIATION

THE twenty-third research panel meeting of the British Gelatine and Glue Research Association was held in London on May 23. The chairman of the Association, Mr. S. G. Hudson (Richard Hodgson and Sons, Beverley), who presided, directed attention to the Association's wide range of interests as reflected in the programme for the day.

The first paper, "The Chemical Nature of Elastin", was contributed by Dr. S. M. Partridge (Low Temperature Research Station, Cambridge). The identification, occurrence, physical properties and isolation of elastin were reviewed. The ease of removal of other proteins from elastin varies with the tissue concerned, but even with the ligamentum nuchae of cattle, autoclaving is necessary. It is recognized that some structural changes are possible during isolation; nevertheless, severe damage is unlikely since there is only 1 g mole of *N*-terminal residue per 350,000 g of the isolated protein. The isolated protein is readily broken down into fragments by treatment with acids and the fragments are clearly multichain, indicating the existence of cross-links in the original protein. Enzymatically degraded elastin was chromatographed to give a coloured peptide believed to be the 'core' of the elastin structure. The 'core' gives, on acid hydrolysis, at least four substances of some complexity and these are being examined. The discussion centred round the nature and significance of the 'core'.

In the second paper, Mr. J. A. Mackenzie (R. and P. Scherer, Slough) described "The Manufacture of Gelatine Capsules by the Rotary Die Process". Sheets of plasticized gelatine gel are fed on to a pair of revolving dies and the capsules are automatically filled and sealed on the die. The process calls for a high standard of precision engineering and also for considerable ingenuity in using the unique physical properties of gelatine to the best advantage. Given these factors, capsules of a great variety of shapes and sizes can be made at very high throughput-rates and with a considerable degree of automation.

Dr. R. N. Coleman (Geo. Nelson, Dale, Warwick) gave the third paper, entitled "The Dispersion of Concentrated Gelatine Gels in Hot Syrups". Sometimes high-grade gelatines are encountered which prove to be difficult to disperse in hot, concentrated sugar solutions when using the usual method of soaking the gelatine in water and then adding it as a swollen gel. The difficulty can be minimized by completely dissolving the gelatine in hot water before addition to the syrup: the grist size of the gelatine is important in assisting easy solution in this method. Even so, a few gelatines remain which cannot be dispersed unless the amount of water in the system is increased.

The final paper, "The Cross-linking of Glues with Epoxy Compounds and Formaldehyde Donors", by J. A. Gibbs, I. R. Whiteside and J. Wootton (British Gelatine and Glue Research Association), was presented by Mr. I. R. Whiteside. There has been a general desire to get away from formaldehyde as a cross-linking agent for animal glues because it is toxic and unpleasant to handle. There is a need also for cross-linking agents which can be mixed with glue to give compositions of reasonable pot-life and which can be cured to insolubility after application. It was shown that these requirements could be met by using either dimethylol urea as a donor of formaldehyde in solution or an epoxy resin.

D. A. SUTTON

EDUCATION OF TECHNICIANS AND TECHNOLOGISTS

AN international conference on this topic was held in Cracow, Poland, during September 6–10, 1961, to discuss the education and further training of technical personnel.

The conference was initiated by the Association of Engineers of the General Technical Federation of Poland (*Scientific World*, 6, No. 1; 1962). The Associations of Engineers and Ministries of Higher Education of Czechoslovakia, the German Democratic Republic, Yugoslavia, Poland, Hungary and the Soviet Union also took part in the organization of the conference. Representatives of the Prague Regional Centre of the World Federation of Scientific Workers and of the Higher Schools and Scientific Institutes

Section of the Union of Polish Teachers, as well as an observer from the International Labour Office, attended the Conference.

The reports delivered and discussed were: (1) the influence of national economic needs on higher education (Prof. J. Tymowski, Poland); (2) education in intermediate-grade technical schools (H. Haas and G. Straumer, German Democratic Republic); (3) the training of managerial personnel in Soviet secondary technical schools (Prof. B. A. Kuzmin, U.S.S.R.); (4) problems of higher technical education (Prof. I. Gerendas, Hungary); (5) the training of managerial personnel and the reform of higher technical schools in the Soviet Union (Prof. B. T. Zhadan, U.S.S.R.); (6) systems of practical training and methods of improving qualifications by institutions other than schools in Czechoslovakia (Prof. J. Koritta, J. Jirasek and Dr. I. Auerhan, Czechoslovakia); (7) further education of engineers and technicians employed in various branches of industry (Prof. S. Koncar-Djurdjevic, Yugoslavia); (8) planning for the demand for engineers and technicians (T. Wodzynski, Poland); (9) training of teachers at secondary and higher technical schools (Prof. H. Lohlmann, German Democratic Republic).

All participants emphasized the need to improve the contact between theory and production, as much by increasing the number of students wishing to become technicians or engineers as by multiplying and varying the type of practical work in production during the course of study.

Jointly with the University of Moscow and the Moscow Bauman Technical School, the World Federation of Scientific Workers held an international symposium on the training of scientists, engineers and teachers in Moscow in September 1962. Taking part in the work of the symposium were more than 200 individuals, excluding Soviet scientists, from 50 countries.

The following topics were discussed in the reports delivered at the plenary session of the symposium: (1) science and technology in the world of the future; (2) aims, tasks and conditions of education of specialists in higher educational institutions; (3) higher scientific and technological education in newly developing countries; (4) higher education and international relations. The symposium was divided into three sections: scientific and technological education, education in social and historical sciences and the training of teachers.

The reports submitted on scientific and technological education were classified under the following headings: (1) specialization in higher scientific and technological establishments; (2) mutual relations between theoretical and practical education; (3) the role of scientific research in the educational system; (4) methods of education without interrupting work (part-time and correspondence courses); (5) further improvement of the qualification of scientists, technologists and of the teaching staff; (6) the role of the humanists and social sciences, including the study of languages, in the education of students in science and technology.

At the section which dealt with education in social and historical sciences the following subjects were raised: (1) specialization in higher education in social and historical sciences; (2) mutual relations between theoretical and practical education; (3) the role of scientific research in the educational system; (4) methods of education without interrupting work (part-time and correspondence courses); (5) study of natural sciences by students in social and historical sciences.

The reports delivered in the section on teaching were under the following headings: (1) the training of teachers in universities and in special training colleges; (2) the problems of training teachers in sufficient number in newly developing countries; (3) further improvement of the qualification of teachers; (4) extra-mural education for teachers.

Further information concerning the symposium can be obtained from Dr. P. Biquard, 10, Rue Vauquelin, Paris 5e, France, or Central Committee, Educational and Scientific Workers' Union, 42 Leninsky Prospect, Moscow, U.S.S.R.

THE BRITISH COAL UTILISATION RESEARCH ASSOCIATION

THE annual report of the British Coal Utilisation Research Association for 1961, which includes abstracts of reports and published papers, besides lists of members of Council, committees and senior staff, notes that about 40 per cent of the research income was obtained from research contracts and work sponsored on a repayment basis, and that this figure seems likely to increase. Contract research during the year included work on the behaviour of coal-ash slags, total gasification of coal, smoke emission from domestic solid fuels, and research on the characteristics of graphite moderators for nuclear reactors. Much of the work of the Association is now concentrated on a comparatively few clear-cut projects, including automatic boiler equipment, especially inexpensive coal-handling equipment for small plant. Evaluation of earlier field trials with the small pipe central heating system has confirmed that the running as well as the installation costs are low for solid-fuel-fired installations. The feasibility of automatic measurement of the rate of condensation of sulphuric acid from flue gases has been established and a tentative method formulated for assessing the suitability of a coal for cyclone firing from the viscosity-temperature characteristics of the slag and the theoretical gas temperature in the plant. Viscosities of slags from a wide range of coals are being determined.

Long-term researches are concerned mainly with the mineral matter in coal, and further work has been done with the slagging fixed-bed gasifier to produce low-cost gas from the cheaper grades of coal. A new basic research programme has been initiated on the combustion of coal, particularly pulverized coal, and some initial experimental problems in the combustion and gasification of coal in which coal is heated rapidly under strictly defined conditions have been overcome. The reactivity of graphite to oxidizing gases at 450°–900° C depends on the extent of the surface area associated with both the micron-sized capillaries and those capillaries of molecular sizes that are accessible to the gases, while interpretation of a series of nuclear magnetic resonance spectra suggests that the proportion of aliphatic hydrogen in tertiary CH groups may be higher than was hitherto thought. The property of coking appears to be related to the rate of decomposition of chloroform-soluble material at near 400° C and the differences between low-rank and coking coals are now largely understood in general terms. Radioactive labelling using tritium has been used to determine the origin of fine material produced when a range of sizes is broken in a centrifuged mill; photoelasticity has been applied in a preliminary study of the stress distribution in particles and mill elements; and the energy required for fracture has been determined by controlling the propagation of a crack in a coal specimen.

NUTRITION OF THE EXPECTANT AND NURSING MOTHER

PREGNANCY imposes considerable nutritional stress on the mother. The growth of the fœtus, and enlargement and hypertrophy of the organs of reproduction and lactation, and the increase in the volume of circulating blood are important physiological changes occurring during pregnancy and lactation which increase the nutritional requirements during this period. To give precise information on the physiological changes that occur in Indian women, and the magnitude of the nutritional strain to which they are exposed, in recent years the Indian Council of Medical Research has embarked on a series of investigations. Studies have been made on the body composition in terms of cell mass, extra-cellular water, fat and active formation of lean body mass during the second trimester of pregnancy*.

It has been observed that the basic metabolism is increased in nursing women to the extent of nearly 26 per cent over the average basic metabolic rate of normal adult women; this is, presumably, due to the energy required in the production of milk. These, and other studies, are providing clear-cut knowledge about the nutritional requirements during pregnancy and lactation of Indian women. Such knowledge will lead to a more realistic planning of diets during these periods of physiological stress.

Attention was also directed to the composition and nutritive value of breast milk in Indian women. Special investigations have been undertaken by the Council to determine the concentration of nutrients in breast milk of poor Indian women and to explore the possibility of improving the nutritive quality of this milk by supplementing the mother's diet, thus benefiting in one operation both the mother and the suckling infant. Studies on the chemical composition revealed that, while the concentration of proximate principles compared well with those observed in well-nourished populations, the concentration of several vitamins in the samples from poor Indian women was much lower. It was also observed that supplementation of the maternal diet with thiamine and ascorbic acid led to significant increase in the concentration of those vitamins in breast milk, although a similar effect was not observed with vitamin A and iron.

Studies on the output of breast milk in different stages of lactation revealed that poor Indian women are in a state of almost continuous lactation throughout the child-bearing period of their lives. The implications of this observation from the point of view of health and nutritional status of the mother require careful consideration. One study, using various levels of dietary protein and measuring nitrogen balance, revealed that 0·14 g of nitrogen per kg per day would be the minimum nitrogen intake needed for maintaining equilibrium. On this basis, the minimum amount of dietary protein requirements, between 22 and 28 weeks of pregnancy, would work out to 0·9 g per kg per day, or 40·5 g per day for a pregnant woman of average weight of 50 kg.

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* Indian Council of Medical Research. Report for the years 1958–59 and 1959–60. Pp. iv + 274. (New Delhi: Indian Council of Medical Research, 1960.)

SCOTTISH PALÆOBOTANY

TWO papers, recently published by the Royal Society of Edinburgh, add to, and at the same time simplify, knowledge of previously known plants*.

K. M. Lele and J. Walton have investigated *Zosterophyllum*, which, after repeated investigation, is appearing as a tufted rhizomatous Pteridophyte widespread on Lower Devonian marshes. The most useful of the new material consists of axes freed when rock was dissolved in the preparations of a balsam transfer; some of these axes had rotted before preservation and consist merely of the vascular core inside the cutinized epidermis. The well-developed stomata are numerous on the upper branches but rare below and confirm Lang's conclusion that this is a land plant. The course of the vascular strand in the H-shaped branches of the base of the plant is now clear and this branching, though remaining characteristic, becomes understandable and *Zosterophyllum* takes its place as a normal rather than a problematical early plant.

A. G. Long's paper is the latest of a series dealing with petrifactions from the Lower Carboniferous of Berwickshire. *Tristichia* is a new stem; it is slender (only 2–3 mm wide) and bears petioles as wide as itself, but much less vascular. The petioles (also known as compressions) fork repeatedly and their thread-like ends bear tiny terminal pinnules. Similar fertile petioles considered to be of the same plant bear terminal cupules from each of which numbers of seeds were macerated out, and the seeds are held to be the same as *Stamnostoma* previously described as petrifactions. Thus the microsporophyll alone remains unknown, but pollen grains are described from the tops of the seeds. *Tristichia* can now take its place along with *Tetrastichia* and other Lower Carboniferous Pteridosperms.

The Royal Society of Edinburgh is to be congratulated on maintaining the quality of its plates.

TOM M. HARRIS

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* *Transactions of the Royal Society of Edinburgh.*

Vol. 64, No. 17 (1960–61): Contributions to the Knowledge of *Zosterophyllum myretonianum* Penhallow from the Lower Old Red Sandstone of Angus. By K. M. Lele and J. Walton. Pp. 469–475 + 2 plates. 6*s*.

Vol. 64, No. 18 (1960–61): *Tristichia ovensi* gen. et sp. nov., a Protostelic Lower Carboniferous Pteridosperm from Berwickshire and East Lothian, with an Account of some Associated Seeds and Cupules. By Albert G. Long. Pp. 477–489 + 4 plates. 8*s*. 6*d*.

(Edinburgh: Royal Society of Edinburgh, 1961.)

CHEMOTHERAPY IN MALARIA CONTROL

MASS chemotherapy has been used in malaria control programmes on a number of occasions. Where it is possible to issue pyrimethamine or chloroquin at weekly or even fortnightly intervals to all members of a community, rapid reduction of the parasite-rate will be achieved: under such conditions it is immaterial whether or not spraying is carried out. In experiments carried out in many parts of the world there has been a rise in parasite-rate after cessation of treatment because most of the experiments have been on small communities living in otherwise uncontrolled areas. In rural Africa, however, malaria is not found in small communities, but in a high proportion of the inhabitants of vast territories. There is a large, seldom static, often unco-operative and usually very low density population. The cost of spraying and regular treatment, particularly in using trained reliable staff and finding transport, tends to be high.

With these factors in mind an experiment was carried out to ascertain the reduction of parasite-rate which could be achieved under field conditions with a minimum number of treatments in a high proportion of a reasonably large population as opposed to regular treatment of a small community. The study was undertaken by Dr. E. T. Reid of the Research Laboratory, Salisbury (*Central African Journal of Medicine*, 8, No. 5; May 1962).

Two similar areas, Fungwe and Chimanda in Southern Rhodesia, were each sprayed with BHC (benzene hexachloride) annually for seven years. After five years parasite-rates remained in the region of 20 per cent and mass chemotherapy was undertaken in one of the areas.

As many people as possible were treated on each of three occasions in the Fungwe area, using a dose of 600 mg chloroquin base and 50 mg pyrimethamine. Treatment was at the beginning of the transmission season in 1959, in the middle of the next non-transmission season and at the end of the second transmission season. Parasite-rates in the area fell rapidly after these experimental treatments from 23·5 per cent to 2·58 per cent during the period of the experiment (18 months). In the control area the parasite-level remained at approximately 20 per cent. It is considered that malaria control projects in rural Africa do not sufficiently take into account people who, for one reason or another, avoid treatment or having slides taken, and who form foci of infection of unknown importance.

PALÆOLATITUDE OF EVAPORITE DEPOSITS

By E. IRVING and J. C. BRIDEN

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IN many problems connected with Earth science it is useful to make numerical estimates of past latitudes and the directions of crustal features relative to the ancient meridian. Two broad lines of inquiry may be recognized: (1) studies of palæogeography which require estimates of the palæolatitude spectra of (say) a fossil genus or a type of sediment; (2) investigations of structural patterns in the crust relative to the Earth's rotational axis with the view of studying the stress history of the Earth. Palæomagnetic methods afford the means of making these estimates since the measured palæomagnetic inclinations give information on the past latitude, and the declinations information on the azimuth relative to the ancient meridian. As an example of this method results relating to the palæolatitudes of evaporite deposits are discussed in this article.

Evaporite deposits (that is, beds of chlorides and sulphates such as halite, gypsum and anhydrite, etc.) are often taken as indicators of past climate since they may be supposed to have been laid down in regions of excess evaporation; for example, their most probable place of occurrence is where temperature is high, at least at some season of the year, and rainfall fairly low. Yet the present geographical distribution of Palæozoic and Mesozoic evaporites[1,2] is asymmetrical about the Earth's equator (Fig. 4*a*), the bulk of them being in the northern hemisphere in intermediate or high latitudes often in regions which nowadays experience both heavy rainfall and low temperatures. This could be due to changes in the climatic zones, or changes in land distribution (in particular changes in latitude of the places where evaporites now occur) or to these factors working together. Some workers have suggested that the distribution can be explained in terms of a steady northward shift of evaporite occurrences going back through geological time. The shift has been estimated previously for northern continents at 0·05°/m.y.[1], and as 0·07°/m.y. for America and 0·11°/m.y. for Europe and Africa[3].

The first numerical estimates of the palæolatitudes of evaporite deposits from the palæomagnetic data were made for beds of Silurian to Triassic age from the United States; the palæolatitude range was calculated to be 0–30°[4]. Afterwards, maps showing the parallels of palæolatitude for Europe and North America were published[5–8]; these confirm, with added details, the range given here. Blackett[3] has shown that the rate of northward shift of the evaporite occurrences is similar to, but generally less than, the palæolatitude changes calculated from the palæomagnetic data (0·08°/m.y. for the Americas and 0·14°/m.y. for Europe). A further result obtained by Blackett[3] is that estimates of 'salt latitude' of reference localities made on the basis of an assumed model of the past position of the ancient evaporite zones (the ancient evaporites are assumed to be centred at a latitude of 30°, as appears to be the case nowadays (ref. 2, Fig. 32), yield changes comparable with, but systematically less than, the changes in palæomagnetic latitude; the 'salt latitudes' for the Mesozoic and Palæozoic are less than the palæomagnetic latitudes by about 10°.

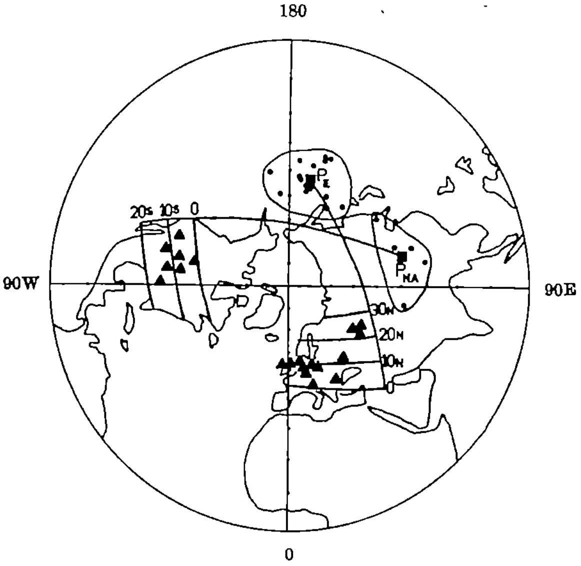

Fig. 1. Map of the northern hemisphere showing the distribution of evaporites during the Permian in Europe and North America. Evaporites are denoted by solid triangles. Individual palæomagnetic pole determinations are denoted by dots; the sets of poles from Europe (see, for example, refs. 13 and 14) and North America (ref. 15) are encircled, the mean positions P_E and P_{NA} being denoted by solid squares. The parallels of palæolatitudes are drawn across that continent to which they apply and give the values for individual deposits. It may be noted that P_E is some 63° of longitude east of P_{NA}, the arc angle between them being 47°

The purpose of work reported here is to investigate more precisely than hitherto the range of palæolatitudes in which evaporites were laid down at different times. For example, one of the possible explanations for the discrepancies just noted is that at some periods evaporite deposition was centred about the equator and this may reflect a climatic zonation different from that at present. This study is based exclusively on palæomagnetic results and methods and has the advantage that it is independent of arguments about the nature of past climates.

The directions of fossil magnetism measured in a rock unit may be used for estimating the position of the palæomagnetic pole relative to the sampling area at the time the rock was deposited. If a sequence of results in time is available from a particular continent it is possible to work back and calculate the palæolatitude of any place in that continent at any time, provided no great relative movement of the sampling area within the continental framework has occurred. The justification for this method is that there is now much evidence that polar shifts within geological periods are small in many cases: for example, in the Permian and Triassic of Europe and North Asia, and in the Triassic of North America (see for example, ref. 9). Thus, continent by continent and period by period (for those periods and continents for which palæomagnetic evidence is available), it is possible to estimate the palæolatitude of evaporite deposits.

The palæomagnetic data available at present have been used, and has been summarized in various compilations[10–13]. The estimates may be obtained by solving spherical triangles or graphically using stereograms. The latter method is sufficiently accurate for present purposes and has been used here. The procedure is illustrated in Fig. 1 in which the estimated palæolatitudes of the Permian evaporites of Europe and North America are given.

The problem of weighting arises, since a local gypsum bed should not be given equal weight with (say) the Zechstein evaporites. The method used here should suffice to a first approximation. The primary reference used here is Green[1], who has drawn a series of maps of the northern hemisphere for separate geological periods, and it is presumed that his method of notation gives a weighting approximately proportional to areal extent. He used polar projections from which geographical co-ordinates can easily be read off. These results have been supplemented from other sources (detailed in the figure legends); but this weighting procedure has, so far as possible, been preserved throughout. Detailed distribution maps have previously been given by Lotze[2] but using

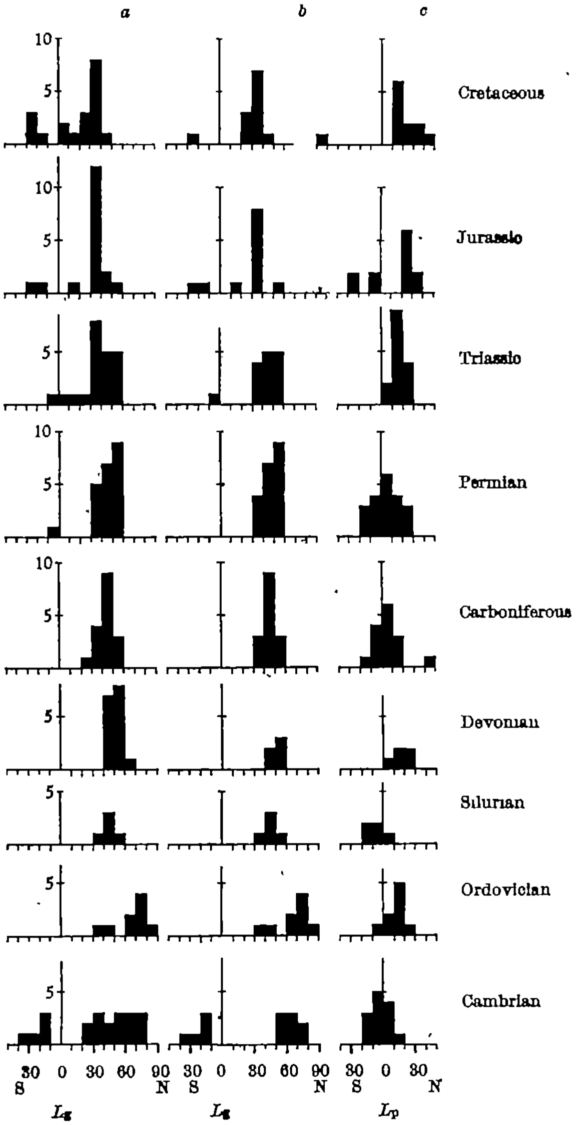

Fig. 2. Frequency histograms for different periods. Localities for all continents except Antarctica and Australia have been compiled from refs. 1, 16 and 17. Information from Australia has only recently come to light and has been obtained from the Bureau of Mineral Resources, Canberra. There are no data from Antarctica. Frequency is plotted vertically, and latitude horizontally at 10° intervals. (In some circumstances there may be advantages in plotting the frequency per unit area but this has not been done.) In this and in Figs. 3 and 4, column (*a*) shows present geographical latitude (L_π) of all evaporite occurrences; column (*b*) shows (L_π) of those occurrences for which palæomagnetic data is available; column (*c*) shows the palæolatitudes (L_P)

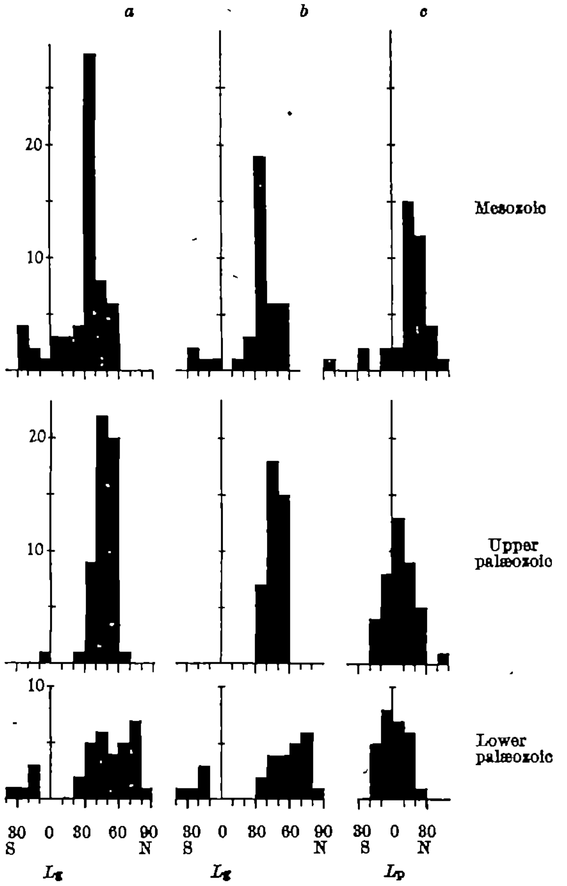

Fig. 3. Frequency histograms for broad time groups. Conventions as for Fig 2. Latitude at 10° intervals

oblique projections with a 90° network so that accurate geographical co-ordinates cannot readily be obtained. These distributions given by Lotze and Green are essentially similar and the use of one in preference to the other would not, in the present context, yield different results.

The estimates of palæolatitude are made on the basis of the assumption that the directions of magnetization observed in rocks reflect the ancient field direction, and that the Earth's magnetic field has been that of a geocentric axial dipole. The results are subject to statistical errors, the average accuracy (P = 0·05) being about 10°. This means that the results given in Figs. 2, 3 and 4 will be subject to a substantial dispersion not attributable to changes in palæolatitudes.

Relative movement between the evaporite occurrence and the palæomagnetic sampling region is assumed to be unimportant. Relative translation would only become important if it were of comparable magnitude to the experimental error, that is, relative movement within the same continental framework of the order of 1,000 km. Relative rotations cannot produce an error greater than twice the distance between the two areas which rarely exceeds the statistical error. It must also be assumed that the directions of the Earth's field did not change between the time the evaporite was deposited and the time of magnetization of the formations studied palæomagnetically. Polar shifts within geological periods are often small, and in such cases as the Permian and Triassic of Europe, where evaporite deposits and the levels studied palæomagnetically are interbedded, such errors will be negligible. Errors arising from either of these sources will only increase the scatter of values; they do not affect the mean result.

Tertiary evaporites were not studied because the changes in magnetic field appear to be rapid at this time, and the dating of many Tertiary formations studied palæomagnetically is poor, and more geological and palæomagnetic information is required before a quantitative treatment can be attempted.

Values were obtained to the nearest degree and are grouped in intervals of 5° or 10° in the frequency histograms of Figs. 2, 3 and 4. In Fig. 2 the results for each geological system are shown. In Fig. 3 they are combined into the three broad age-groups, Lower and Upper Palæozoic and Mesozoic. Fig. 4 gives the cumulative results. Because of the incompleteness of the palæomagnetic record, estimates of palæolatitudes cannot be made for all evaporite deposits. Each figure shows the present latitude distribution of evaporite occurrence and also the distribution of the values used. These are similar, suggesting that those deposits for which estimates were obtained are a representative sample of the whole.

The present latitudes of evaporites are mostly intermediate or high and predominantly northerly. The full range is 31° S.–83° N. but 73 per cent lie between 30° and 60° N. In contrast, the estimates of palæolatitudes are generally low, ranging from 59° S.–44° N., with 76 per cent of the values lying within 20° of the palæoequator. They are consistent with the view that evaporites were laid down preferentially in tropical latitudes and hence that areas of excess evaporation were in low latitude.

There is also the possibility that the effect is due to coincidences in the disposition of land and shallow seas where evaporites might be laid down; there is the possibility, through an accident of palæogeography, that in the Palæozoic and Mesozoic these did not occur in high latitudes. To investigate this the

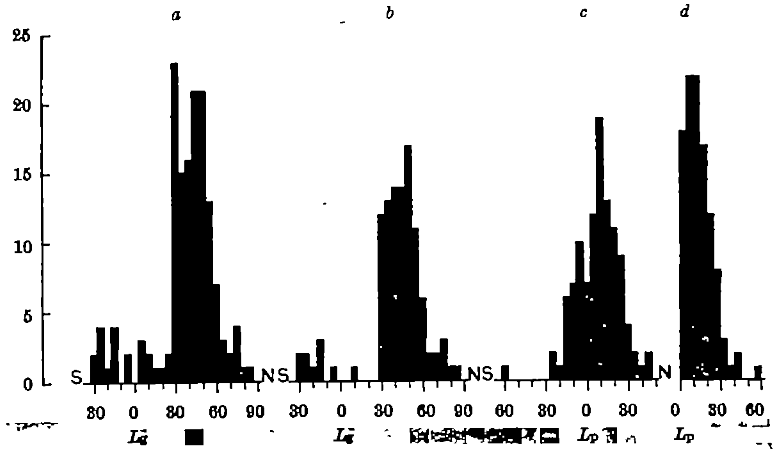

Fig. 4. Cumulative frequency histograms. Conventions as in Fig. 2, except that a 5° plotting interval is used, and in an additional diagram (d) L_p is plotted irrespective of whether the values are north or south of the palæoequator

palæolatitudes of lands and shallow seas have been estimated from magnetic results obtained from a large number of shallow water and terrestrial lavas and sediments, by the same method as has been used for evaporites. It is found that in the Lower Palæozoic the values are mainly low (less than 30°) so this may be the cause of the observed evaporite distribution. However, there is the possibility that palæomagnetic data covering this time are inadequate. In the Upper Palæozoic and Mesozoic it is found that although the most abundant occurrences of land and shallow seas are in low latitudes, their spread is much greater (80° N. to 80° S.) than that of the evaporites. For example, the palæolatitude of Australia throughout the Mesozoic determined palæomagnetically is high: there are no substantial evaporites although there is abundant terrestrial and shallow marine deposition. The possibility of palæogeographic coincidences as a general explanation of the distribution of Upper Palæozoic and Mesozoic evaporites is therefore rejected.

Comparison between the results of these latter ages suggests the possibility of detailed changes within the tropics. The Mesozoic values are predominantly in the range 10–30° N., which is broadly consistent with, although somewhat lower than, what would be expected from the distribution of present-day evaporite accumulations. The predominance of northern hemisphere values suggests the paucity of land and shallow seas in low southerly latitudes at this time, and this is supported by the analysis of palæolatitudes referred to above. In the Upper Palæozoic, however, the distribution is symmetrical about the palæoequator and the values are mostly within 20° of it. The zone of excess evaporation would appear therefore to have been centred on the palæoequator at this time. This could account for the discrepancy between the 'salt' and palæomagnetic latitudes already referred to[3], and indicate that the use of palæoclimatic models based on present climate zonations may in detail be misleading. The experiments carried out by Opdyke[7] using other models of climate zonation are of much interest in this context.

These results are consistent with the view that evaporites were laid down preferentially in low latitudes and that the contrast between the past and present latitude distribution is due mainly to changes in latitude, although a subsidiary effect due to the past distribution of land and shallow seas may be present. The results are to be considered as an early attempt to apply a comparatively new method to distribution problems in geology. Although future palæomagnetic observations may change the picture given here, the power of the method as a tool in studying such problems is apparent.

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BORON CONTENT IN RELATION TO ORGANIC CARBON IN CERTAIN SEDIMENTS OF THE BRITISH COAL MEASURES

By Dr. R. M. C. EAGAR

The Manchester Museum

HARDER[1,2] has shown that much of the boron present in sediments is located in the muscovite structure of fine-grained illite, notably in the fraction smaller than 2μ, and that appreciable but smaller quantities of boron may occur in the coarser fractions of grain size 20–60μ, where it is found in tourmaline. Where tourmaline makes a very small and relatively constant contribution to a sedimentary series, as has been demonstrated, for example, in the more psammitic rocks of the German Upper Carboniferous Coal Measures[3], variation in the total boron content of the associated pelitic rocks is likely to approximate to variation in boron associated with the illite. Part of the boron in illite is a measure of palæosalinity, as well-known experiments have demonstrated. However, the process by which it appears that boron cations are installed in the lattice of dioctahedral mica is dependent on both time and temperature[2]; and, furthermore, the total boron associated with the illite can in varying measure be attributed to boron already installed in detrital illite of the source material, which may have been released by the weathering of pre-existent marine or hypersaline sediments. It is thus possible for varve-clays to have very high boron contents[1]. Nevertheless, where contributions from detrital tourmaline and other source materials remain relatively constant and conditions of sedimentation are little altered, any changes in salinity of the water in the basin of deposition might be expected to leave corresponding changes in the total boron content of the sediments concerned. Ernst and others[4,5] have in fact shown that there exists a broad correlation between total boron (microchemically determined) and palæosalinity, in so far as it is revealed by fossils, in Upper Carboniferous (Westphalian *B*) Coal Measures, both in Germany and also on a few horizons in Belgium and northern France. Comparable results have been quoted for German Devonian rocks where average boron contents are considerably less[4]. As Ernst, Harder and others have emphasized, relative changes in the boron content of rocks within a single profile, for example, in a borehole, are likely to be of more significance, both for purposes of correlation and as evidence for the palæoecologist, than absolute boron contents, whether or not these are related to illite contents.

Working on sediments of various ages, Walker[6] has successfully separated very fine-grained fractions of illites and his adjusted boron contents of illites in certain marine and hypersaline rocks show general agreement with figures for corresponding American sediments[7]. Walker, however, found the supposedly freshwater British Purbeck Beds, with illite contents of 509 and 554 p.p.m., were indistinguishable from marine Jurassic beds (520 p.p.m.). These figures contrast with those of 110 and 135 p.p.m. for illite in certain American freshwater rocks[7] and may indicate merely that the Purbeck Beds acquired their illite from the weathering of previously formed marine Jurassic sediments, which were uplifted at this time.

Recent palæoecological work by me on 37 ft. of pelitic sediments in the British Coal Measures (Upper Carboniferous, Westphalian *A*) of Worsborough district, near Barnsley, has been supplemented by chemical work on 21 samples carried out by Mr. R. Y. Gillespie and Mr. A. Parr-Burman of United Analysts, Ltd., East Boldon, and microchemical determinations of boron on the same samples by Dr. W. Ernst. Advice on chemical procedure and on the interpretation of the results has been given by Dr. G. D. Nicholls. Free silica was determined by the method of Trostel and Wynne[8]. Total carbon was determined by combustion in oxygen in a conventional combustion train, the evolved carbon dioxide being absorbed in Midvale design soda lime tubes. The carbon combined in mineral carbonates was determined by treating a portion of the sample with phosphoric acid and absorbing the carbon dioxide, again in soda lime and in asbestos. Organic carbon is defined as total carbon minus carbon from mineral carbonates. When proportions of carbon dioxide were small (less than 1 per cent by weight) carbonate was calculated as siderite ($FeCO_3$). When proportions of carbon dioxide were greater than 1 per cent approximate determinations were made of the iron, calcium and magnesium constituents and the proportionate weights of the carbonates calculated accordingly[9]. Sulphur determinations were calculated as iron sulphide. Clay minerals were then calculated as 100 per cent minus the weights of silica, organic carbon, carbonates and iron sulphide. In ten of the samples very rough checks on the content of the clay minerals were made by X-ray diffraction with the help of Dr. J. Zussman, and in three cases clay mineral content was checked by ignition loss/moisture absorptions tests[10] carried out by Mr. P. S. Keeling of the British Ceramic Research Association.

Both organic carbon and total boron vary widely (Fig. 1), the latter from 12 p.p.m. (in band *A*) to 141 p.p.m. (below band *B*). These figures may be compared with those selected for northern European Coal Measures (of almost identical age), about 109 p.p.m. (B_2O_3 = 0·035 per cent) to delimit marine from brackish water, and about 62 p.p.m. (B_2O_3 = 0·025 per cent) to separate brackish and fresh water[5]. Furthermore, it will be seen (Fig. 1) that where organic carbon is appreciable (over 1·5 per cent) total boron and organic carbon show an antipathetic relationship. Boron tends to vary with clay mineral content, as might be expected, but the inverse correlation between boron and organic carbon is slightly better than the correlation between boron and clay minerals. Nevertheless, in the case of three peaks of organic carbon out of six (bands *B*, *C* and *E*), the fall in total boron slightly precedes the rise in organic carbon. Moreover, if the total boron were assumed to reside in or to be associated with the clay minerals, then the curve boron/clay minerals tends to follow that of total boron and is thus again largely antipathetic to organic carbon. Comparing the richly carbonaceous bands *A*–*F* with the grey shales and shaly mudstones between them, the former, averaging 16·59 per cent organic carbon, have a mean boron content of 48 p.p.m., whereas the latter, averaging 1·05 per cent organic carbon, have a mean boron content of 98 p.p.m., a result confirming preliminary published figures[11].

Re-examination of the results of Nicholls and Loring[12] suggests a similar relationship between boron and carbon in a Coal Measures cyclothem at Bersham Colliery, near Wrexham (Fig. 2). These measures differ as a whole from those at Worsborough in having lower contents of organic carbon (0·19–2·64 per cent) and higher contents of free silica, that is, detrital and authigenic quartz (29·2 per cent at horizons *B.C.*3 and 9, to 43·2 per cent at *B.C.*4). At Worsborough the corresponding figures for free silica are 1·0 per cent (in band *A*) to 24·1 per cent (at 19 ft. above the coal). Total boron contents are as a whole lower at Bersham (see below) and much less variable (note the increased scale in Fig. 2). Nevertheless, the curve of total boron dips appreciably at the horizon of *B.C.*3 where organic carbon exceeds

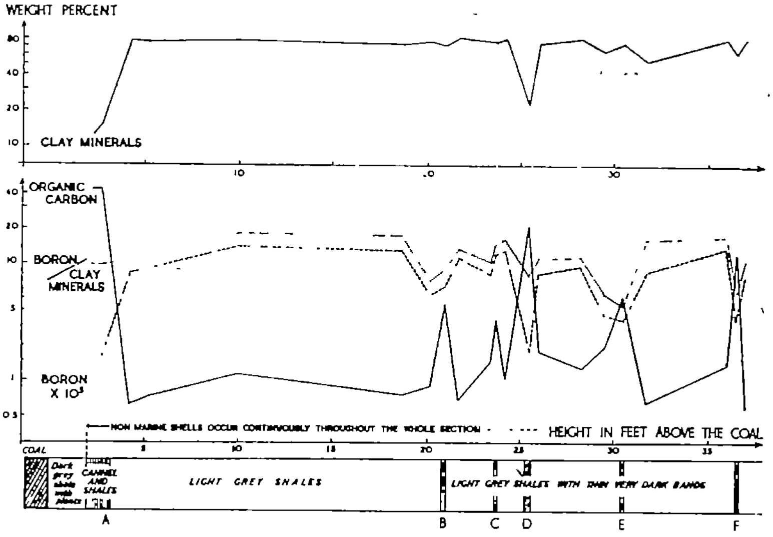

Fig. 1. Section above the Flockton Thin Coal of the Lower Coal Measures at Cold Bath Opencast, Worsborough, near Barnsley, Yorkshire. The horizon is in the lower half of the *modiolaris* Zone, near the top of Westphalian *A* and about 200 ft. below the Clay Cross (mid-*modiolaris* Zone) Marine Band. Geochemical data from analyses by United Analysts, Ltd., East Boldon. Boron determined microchemically by Dr. W. Ernst, North Rhein-Westphalian Geological Survey, Krefeld

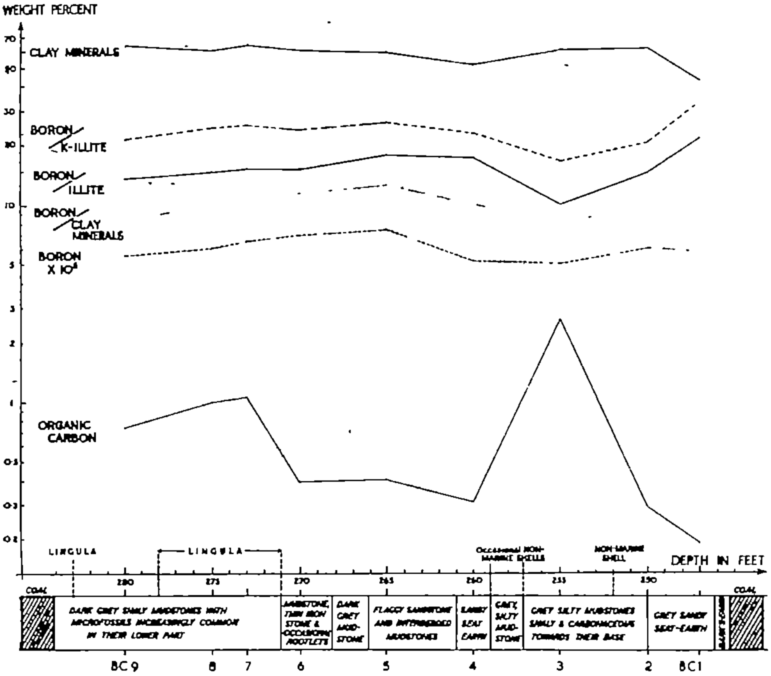

Fig. 2. Section between the Red (base) and Crown (top) Seams of the mid-*modiolaris* Zone of the Coal Measures, from an underground bore-hole at Bersham Colliery, near Wrexham, North Wales, after Nicholls and Loring, 1962, Fig. 1 and Table 9. Boron determinations were made spectrographically. Geological descriptions and palæontology adapted from D. Magraw, late of H.M. Geological Survey, in Nicholls and Loring. Depths refer to depths below the Powell Seam

what appears to be the critical value (1·5 per cent) at Worsborough. At Bersham the curves for boron/clay minerals, boron/illite and boron/potassium/illite are of essentially the same form as that of total boron. They all show more antipathy to organic carbon than sympathy with total clay mineral content (as in Fig. 1). As noted by Nicholls and Loring, increased values of the boron ratios in the seat-earths of horizons *B.C.*1 and 4 may not be real but result from detrital tourmaline in the much-increased proportions of quartz in these samples. On the other hand, I have noted that certain measures yielding high contents of boron at Worsborough, those underlying bands *B*, *E* and *F* of Fig. 1, apparently correlate through intermediate sections with thick beds of seat-earth type, yielding rootlets, at Everbridge Opencast, south of Chesterfield and nearly 30 miles south of Worsborough. Relatively high boron and illite contents, moreover, have been noted recently in several seat-earths in the Ruhr Coal Measures[13].

If the total boron in the Worsborough samples is located in the illite, then from very rough estimates by X-ray diffraction boron in illite ranges from the order of 77 p.p.m. (band *E*) to 144 p.p.m. (band *B*); whereas in the grey shales the illite contains much higher proportions of boron, computed values of 202 p.p.m. and 238 p.p.m. (at 5 and 22 ft. respectively above the coal) being probably too low.

Nicholls and Loring[12] have already pointed out that in the *Lingula*-bearing beds of Bersham, which represent the horizon of the widespread mid-*modiolaris* Zone Marine Band, boron values are no higher than in the remaining part of the section. For nine samples in the cyclothem total boron averages 60·3 p.p.m., comparing with an average of 82·9 p.p.m. for 21 samples from the Worsborough section which, it is stressed, contains a non-marine fauna of anthracosiid and myalinid lamellibranchs, ostracods, fish debris and *Spirorbis*. Furthermore, the boron content at Worsborough is relatively low in the dark, richly carbonaceous bands which palæoecological evidence suggests were laid down under tranquil conditions with relatively slow deposition of sediment[14]. These bands also contain the ostracod *Geisina arcuata* (Bean), which is generally considered to have been of brackish water habitat[15] and was probably benthonic[16]. On the other hand, boron values are high in the grey shales or shaly mudstones which the same evidence suggests were laid down under relatively turbulent conditions[14], probably with faster depositional rate, and in water which from general stratigraphical considerations one might reasonably infer to have been fresher. Furthermore, such grey shales contain non-marine lamellibranchs comparable with supposedly 'up-river' assemblages described from other horizons[17]. Moreover, decreases in the size of shell faunas such as take place at Worsborough as one passes from dark carbonaceous shales to light grey shales, by comparison with similar changes in modern brackish water faunas, are more likely to indicate decreases than increases in the salinity of the habitat[18]. Thus the evidence from total boron contents at Worsborough appears at variance with all other evidence regarding palæosalinity. The same disagreement is apparent when the Worsborough section as a whole is compared with that of Bersham, where fossil evidence suggests marine or pene-marine conditions but boron values are lower. On the other hand, at Worsborough boron values vary widely and inversely with organic carbon when the latter is appreciable, crossing values which in north Europe have been regarded as marking boundaries between marine and brackish and between brackish and fresh water. Data from Bersham follow the same pattern as at Worsborough both with regard to total boron and, with certain assumptions, to its roughly estimated content in clay minerals, but with smaller proportions of both organic carbon and clay minerals boron shows less variation.

That boron is concentrated by plants and in coals is well known. Relatively low concentrations (about 4–25 p.p.m.) of boron found in 'sapropels' of several salt water lakes in the U.S.S.R.[19] may be of relevance to the results given here. These results will be described in more detail and more fully discussed elsewhere. It may be pointed out here, however, that no matter how the total boron at Worsborough proves to be distributed among the constituents of the samples, factors other than the salinity of the waters in which these sediments accumulated have influenced their boron contents. Even when one takes into consideration contributions from detrital and authigenic illite, detrital and perhaps authigenic tourmaline and the possibility of excess borates being available from volcanic activity in the neighbourhood[20], probably very shortly before the deposition of the Worsborough sediments[21], variation in total boron content is inexplicable without consideration of the content of organic matter. It appears that the presence of organic matter and relatively low contents of total boron in these sediments are related, directly or indirectly.

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POSSIBLE SIGNIFICANCE OF LINEAR PLOTS OF MOISTURE EXPANSION AGAINST LOG OF A TIME FUNCTION

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IN a recent article[1] on some moisture expansion relationships for a fired kaolinite–hydrous mica–quartz clay it was established that natural moisture expansion was linear against log of time for all firing temperatures from 800° to 1,200° C. and exposure times of 4 hr.–90 days. Plots of the autoclave expansions against the log of time were also linear for specimens fired from 1,000° to 1,150° C. for autoclave times from 2 to 200 hr. However, the significance of these results was not apparent. It should be noted that the samples, after firing, were cooled to 300° C. and then removed from the kiln and cooled to room temperature in a dry desiccator, so that the measurements of length and of weight increase recorded the effect of the first exposure of the samples to large amounts of water vapour in the air and in the autoclave.

A search of the literature has revealed that kinetic processes that proceed linearly with a log function of time are by no means unusual in ceramics and chemistry. It has been established[2], for example, that the Roginsky–Zeldovich (R–Z) equation:

$$dq/dt = ae^{-bq}$$

where q is the amount of gas adsorbed at time t, and a and b are constants, holds over all the known types of chemisorption. It has also been applied to the kinetics of the following: luminescence, photoconductivity of solids, creep of metals, magnetic viscosity, magnetic permittivity of $BaTiO_3$, oxygen–uranium oxide system, oxygen–praseodymium sesquioxide system, and the dehydroxylation of muscovite mica. The integrated form of this equation shows that:

$$q = \frac{1}{b} \ln (abt + 1)$$

The R–Z equation is valid[2] if it is possible to linearize a plot of q against log $(t + t_0)$ by a suitable choice of $t_0 = \frac{1}{ab}$. It is consistent with an activated adsorption in which the activation energy gradually increases as q increases. A theoretical justification for the formula has been developed by Halsey[3] and a physical interpretation has been given to the term t_0 which, in chemisorption, takes into account desorption that accompanies sorption. The greater the amount of desorption the higher will be t_0.

In so far as moisture expansion is concerned, it has been verified that the results for natural moisture expansion of the fired clay previously described[1] can be represented by an equation of similar form to the R–Z equation. Calculation for a linear regression of expansion on log $(t + t_0)$ (using values of $t_0 = 0{\cdot}01$, $0{\cdot}1$ and $1{\cdot}10$ hr.) indicates highly significant relationships in which the sum of the squares of the deviations from the regression equation are at a minimum when $t_0 = 0$, for the majority of firing temperatures between 800–1,200° C. For $t_0 = 0{\cdot}01$ and $0{\cdot}1$ hr. the difference in the sums of squares from those for $t_0 = 0$ are negligible, whereas for $t_0 = 1$ hr. they are not. It is thus concluded that t_0 is too small to be distinguishable from zero, using the results so far obtained for the natural moisture expansion of this fired clay. Plots of natural moisture expansion against log $(t + t_0)$ for samples fired at 900°, 1,000° and 1,075° C. are given in Fig. 1. The variance ratio[4] for the sample fired at 900° C. is 486 for $t_0 = 0$, 470 for $t_0 = 0{\cdot}1$ hr., and 351 for $t_0 = 1$ hr.; for the

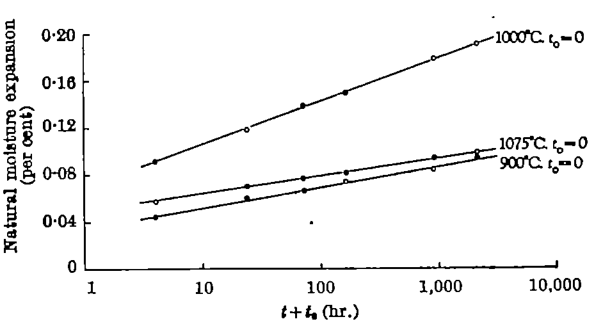

Fig. 1. Plots of natural moisture expansion against log $(t + t_0)$ for a kaolinite–hydrous mica–quartz clay fired to temperatures of 900°, 1,000° and 1,075° C.

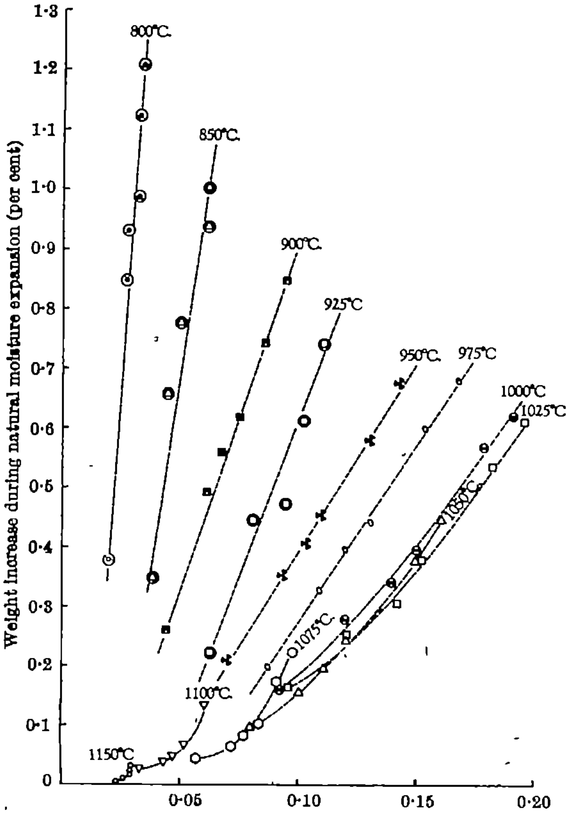

Fig. 2. Relationship between weight increase and natural moisture expansion at exposure times of 1/6, 1, 3, 7, 39 and 90 days for undried samples of a kaolinite–hydrous mica–quartz clay fired from 800° to 1,150° C.

sample fired at 1,000° C. it is 4,440 for $t_0 = 0$, 4,434 for $t_0 = 0{\cdot}1$ hr., and 3,079 for $t_0 = 1$ hr.; for the sample fired at 1,075° C. it is 1,268 for $t_0 = 0$, 1,171 for $t_0 = 0{\cdot}1$ hr. and 662 for $t_0 = 1$ hr., and for all the data n_1 and n_2 (the number of degrees of freedom) are equal to 1 and 4, respectively. The constants a and b of the R–Z equation are set out in Table 1.

Table 1. CONSTANTS OF THE ROGINSKY–ZELDOVICH EQUATION FOR THE DATA OF FIGS. 1, 3 AND 4

q	Firing temperature (°C.)	b	a	t_0 (nearest hr.)
Natural moisture expansion (Fig. 1)	900	134·8	Very high	0
	1,000	63·9		0
	1,075	156·2	indeterminate	0
Weight increase during natural moisture expansion (Fig. 3)	900	11·5	Indeterminate	0
	1,000	11·8	0 01689	5
	1,075	18·8	0·00062	86
Autoclave moisture expansion (Fig. 4)	1,000	44·7	0·02237	1
	1,150	40·0	0·01250	2
	1,200	28·1	0·00324	11

The data for natural moisture expansion are related to the weight increase of the samples, as shown in Fig. 2. Linear relationships exist up to a firing temperature of 1,000° C., but at higher temperatures (1,025°–1,150° C.) the relationships are curved. Since the experimental points along any temperature line are for exposure times ranging from 4 hr. to 90 days it might be expected that the weight increase could also be represented by an equation of similar form to the R–Z equation.

Calculations have consequently been made for a linear regression of weight increase on log $(t + t_0)$ using values of t_0 from 0·1 to 100 hr. Highly significant relationships are obtained in which the sums of the squares of the deviations from the regression equation are at a minimum when $t_0 = 0$ for samples fired up to 900° C., but for samples fired to higher temperatures these sums of squares require t_0 to take on increasing values with increasing firing temperature before minima are obtained. Plots of weight increase against log $(t + t_0)$ for samples fired at 900°, 1,000° and 1,075° C. are given in Fig. 3. The variance ratio for the sample fired at 900° C. is 206 for $t_0 = 0$, 200 for $t_0 = 0{\cdot}1$ hr., and 160 for $t_0 = 1$ hr.; for the sample fired at 1,000° C. it is 354 for $t_0 = 0$, 786 for $t_0 = 4$ hr., 800 for $t_0 = 5$ hr., and 773 for $t_0 = 6$ hr.; for the samples fired at 1,075° C. it is 40 for $t_0 = 0$, 1,462 for $t_0 = 85$ hr., 1,464 for $t_0 = 86$ hr., and 1,461 for $t_0 = 87$ hr.; for all samples $n_1 = 1$, $n_2 = 4$. The constants a and b of the R–Z equation are set out in Table 1.

It has also been verified that the results for autoclave moisture expansion of samples of the clay previously described[1], fired from 1,000°–1,200° C. and autoclaved for periods from 2 to 200 hr., can be represented by an equation of similar form to the R–Z equation. Calculation for a linear regression of autoclave expansion on log $(t + t_0)$ using values of $t_0 = 1$–15 hr. indicates highly significant relationships in which the sums of the squares of the deviations from the regression equation are at a minimum when t_0 is of the order of 1 hr. for samples fired to temperatures from 1,000° to 1,100° C. For samples fired to higher temperatures t_0 must increase in value with increase of firing temperature to maintain these sums of squares to a minimum. Plots of expansion against log $(t + t_0)$ for samples fired at 1,000°, 1,150° and 1,200° C. are given in Fig. 4. The variance ratio for the sample fired at 1,000° C. is 516 for $t_0 = 0$, 787 for $t_0 = 1$ hr., and 600 for $t_0 = 2$ hr.; for the sample fired at 1,150° C. it is 403 for $t_0 = 0$, 1,308 for $t_0 = 1$ hr., 3,147 for $t_0 = 2$ hr.; and 2,377 for $t_0 = 3$ hr.; for the samples fired at 1,200° C. it is 83 for $t_0 = 0$, 8,850 for $t_0 = 10$ hr., 9,420 for $t_0 = 11$ hr., and 3,850 for $t_0 = 12$ hr.; for all the data $n_1 = 1$ and $n_2 = 3$. The constants a and b of the R–Z equation are set out in Table 1. Data are not available to study the relationship between the water adsorbed during autoclaving and the time of autoclaving.

The fact that the natural moisture expansion, the accompanying weight increase, and the autoclave moisture expansion for one fired clay follow, within the limits specified, a kinetic equation of similar form to the R–Z equation is interesting and suggestive of

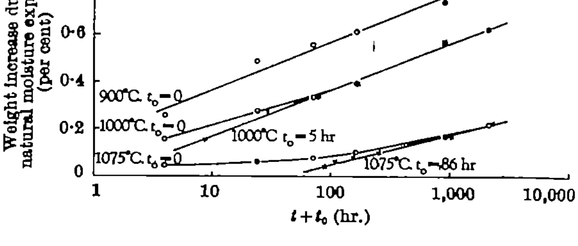

Fig. 3. Plots of weight increase during natural moisture expansion against log $(t + t_0)$ for a kaolinite–hydrous mica–quartz clay fired to temperatures of 900°, 1,000° and 1,075° C.

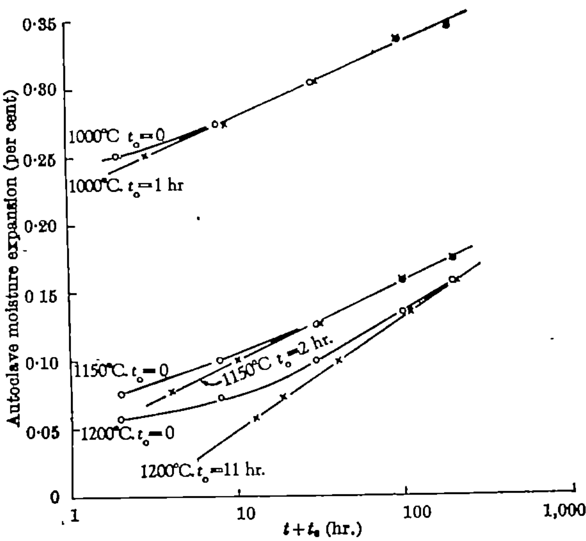

Fig. 4. Plots of autoclave moisture expansion against log $(t+t_0)$ for a kaolinite–hydrous mica–quartz clay fired to temperatures of 1,000°, 1,150° and 1,200° C.

new ways of studying the kinetics of moisture expansion. However, it does not establish that chemisorption or a chemical reaction is taking place, or the nature of the processes (the same is true of any other kinetic law), although it indicates that if chemical processes are involved there is an increase in activation energy as the water is adsorbed and expansion proceeds. Such an increase in activation energy might arise from adsorption sites which are at increasing depths below the surface of the reactable material[5]. In conformity with this there is strong but not conclusive evidence[6] from water adsorption data and surface area measurements of the fired kaolinite–hydrous mica–quartz clay to suggest that the adsorbed water has penetrated deeply beneath the surface, and is chemically combined rather than chemisorbed on the surface.

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USE OF ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY IN INVESTIGATIONS OF FUNCTIONAL METAL COMPONENTS IN MICRO-ORGANISMS

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ELECTRON paramagnetic resonance (EPR) spectroscopy[1] has been successfully used for identifying free radicals and the valency state of transition metal ions that occur in biological materials[1–4]. The technique has also furnished evidence for the functioning of certain transition metals in the catalytic turn-over of oxidative enzymes[2–4]. This communication presents a few examples of the use of EPR in studies on the function of transition metals in microbial metabolism.

Iron. A broad scan of the EPR spectrum of particles prepared from *Azotobacter vinelandii* (Fig. 1) shows sharp absorption lines at $g=1·94$, 2·00 and 4·3, superimposed on a broad underlying signal centred close to $g=2$. These particles, as isolated, are partially reduced, since on oxidation with ferricyanide the broad signal at $g=2$ and that at $g=4·3$ increased, whereas that at $g=1·94$ disappeared. On the other hand, reduction with dithionite resulted in the disappearance of the broad signal ($g=2$) and the signal at $g=4·3$, but the $g=1·94$ signal increased tenfold. There was little change in the sharp signal at $g=2·00$.

By analogy with the spectra obtained with iron complexes in other materials[6,7] it is likely that the absorptions at $g=2$ and 4·3 are associated with Fe^{3+}. The sharp signal at $g=2·00$ is probably due to flavin semiquinones. The $g=1·94$ signal in Fig. 1, which increased markedly on reduction, has been seen in materials of animal origin by Sands, Beinert and Lee[8–10]. These authors attribute this signal to a reduced form of non-hæm iron which functions near flavin in the respiratory chain. The signal appears under reducing conditions, such as deprivation of oxygen in the presence of endogenous substrates, addition of specific substrates or of chemical reductants. Although several variants of this general signal type have been observed, the main characteristics are an absorption peak (maximum slope in first derivative curve) at $g=1·93$ to $g=1·95$, pronounced temperature sensitivity (short relaxation time) (for general reviews of the EPR technique see refs. 11 and 12) and in most cases a minor component at $g=2·00$ near the free radical absorption. This characteristic EPR signal has been found in whole heart and liver tissue, in isolated mitochondria and in particles and enzymes derived from them, for example, succinic and DPNH dehydrogenases[2,10,13]. More recently the signal has also been identified in a series of metal containing enzymes, namely, xanthine oxidase of milk[4,5], aldehyde oxidase of rabbit liver, and dihydro-orotic dehydrogenase from *Zymobacterium oroticum*[5].

In the azotobacter particles the reduced iron species ($g=1·94$), which is obviously closely related to the reduced non-hæm iron complex found in animal

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* Sabbatical leave at the University of Wisconsin, October 1961–July 1962.

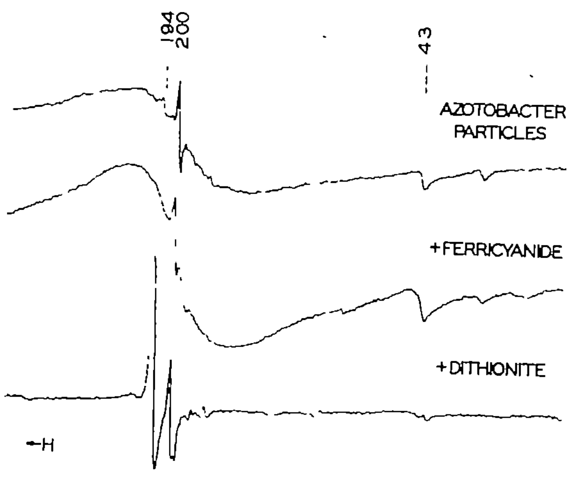

Fig. 1. EPR spectra (first derivative in all figures) of particles from *Azotobacter vinelandii OP* (34 mg protein/ml.) suspended in 0·01 M phosphate buffer, *p*H 7·5) prepared by ultrasonic treatment of the cells for 15 min (ref. 16). Particles collected between 25,000*g* and 144,000*g* for 60 min in a Spinco preparative centrifuge. Spectra recorded at −170°. Upper curve, spectrum of particles as isolated; middle curve, after oxidation with ferricyanide; lower curve, after reduction with dithionite. The *g* values of the various signals are indicated

tissues, was at best only a small fraction of the iron appearing in the oxidized form at $g = 2$ and 4·3 (Fig. 1). The iron signals in the oxidized state may be wholly or partly due to hæm proteins, whereas that at $g = 1·94$ has been observed in materials devoid of hæm iron[3,5,9,10]. The non-hæm iron species is probably associated with a new type of electron carrier[3,10].

The two smaller signals at lower field in the dithionite-reduced spectrum of Fig. 1 are probably components of a Mn^{++} sextet. The other components of this sextet are obscured by the iron signal.

The non-hæm iron complex at $g = 1·94$ occurs in a variety of micro-organisms, as shown in Fig. 2. A comparison with an EPR spectrum of a DPNH dehydrogenase from beef heart[14] confirms that the iron species ($g = 1·94$; $g = 2·00$), in addition to its occurrence in *Azotobacter*, is present in extracts of *Micrococcus lactilyticus* and in *Escherischia coli*.

Manganese. A Mn^{++} signal (six equally spaced components, centred at $g = 2·01$) overlaps the iron signal in a number of bacterial preparations, for example, in *E. coli* and in *Clostridium pasteurianum* (Fig. 2). In crude extracts of *C. pasteurianum* the Mn^{++} signal was so strong that it obscured that of the non-hæm iron complex. The addition of pyruvate to either preparation enhanced the signal at $g = 1·94$–1·95 and decreased the intensity of the Mn^{++} signal, presumably by the formation of Mn^{++} complexes. The iron signal can also be differentiated from the Mn^{++} signal by its different temperature sensitivity. Since Mn^{++} has a relatively long relaxation time, the iron signal at $g = 1·94$ decreased rapidly relative to that of Mn^{++}, when the temperature was raised. The separation of a 'nitrogenase' fraction from the crude extract[15] eliminated practically all the Mn^{++} and a very clear signal of the non-hæm iron appeared when this preparation was treated with hydrogen gas or when pyruvate was added (Fig. 2). The Mn^{++} sextet was recovered in the fraction concerned in the evolution of hydrogen and carbon dioxide from pyruvate, after inactivation of the nitrogenase activity by heat treatment. It is not known at present whether Mn^{++} has a specific function in the metabolism of pyruvate by this fraction. Similarly the significance of relatively large amounts of manganese that we have readily detected by EPR in other bacteria, for example, *Aerobacter aerogenes*, *Bacillus polymyxa*, *Nitrobacter agile*, *Rhodospirillum rubrum* and *Pseudomonas aeruginosa* and in algæ and higher plants is not clear.

Molybdenum. EPR spectroscopy together with low-temperature optical reflectance spectroscopy has been used to study particles from *Azotobacter vinelandii OP*. Particles, prepared from cells grown without combined nitrogen, fix atmospheric nitrogen[16,17] and these were compared with those extracted from the same organism, grown with an ammonium salt as the source of nitrogen (Fig. 3). These are designated N_2 and NH_4^+ particles, respectively. Thus the N_2 particles had a signal at $g = 1·97$ which varied in intensity with batch, but was always more intense in particles that were actively fixing nitrogen. A signal of this *g* value has been observed previously in nitrate reductase from *Pseudomonas aeruginosa*[18] xanthine oxidase of milk[4,5] and aldehyde oxidase of rabbit liver[5] and is probably associated with Mo^{5+} in the three enzymes. The Mo^{5+} signal in these enzymes disappears on oxidation or on further reduction and these effects are also observed with the $g = 1·97$ signal in the *Azotobacter* particles. It is likely, therefore, that this signal in *Azotobacter* is also due to Mo^{5+}, but at its present intensity no fine structure, indicative of Mo^{5+}, could be resolved. The state of molybdenum after extensive reduction, when the Mo^{5+} signal disappears, is not known at the present time.

When the N_2 particles were gassed with hydrogen and a medium factor[16] was added, the iron signal increased markedly indicating reduction of the electron carriers. Combined optical spectroscopy showed that the cytochromes were extensively reduced under these conditions as shown in Fig. 4

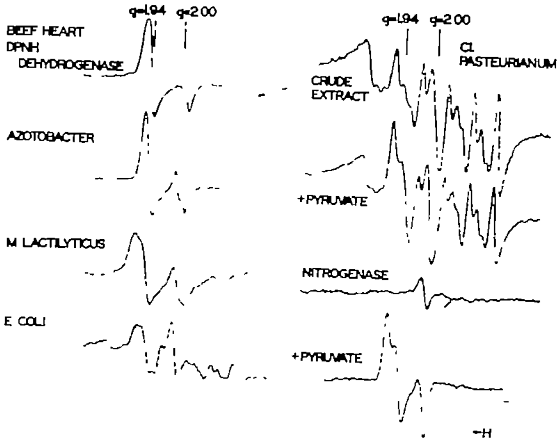

Fig. 2. EPR spectra of various preparations showing the typical signal at $g = 1·94$ recorded at −170°. Left column from top to bottom: DPNH–coenzyme *Q* reductase from beef heart (50 mg protein/ml., ref. 14); particles from *Azotobacter vinelandii* (36·5 mg protein/ml.) prepared as in Fig. 1; crude water extracts of *Micrococcus lactilyticus* prepared with the ultrasonic probe for 15 min, centrifuged at 10,000*g* for 15 min, supernatant fraction adjusted to *p*H 8·0 (95 mg protein/ml., ref. 23); and *Escherischia coli* (*K*12) grown anaerobically, extracts prepared in 0·05 M phosphate *p*H 7·5 as described for *M. lactilyticus* (34·5 mg protein/ml.). All preparations reduced with dithionite

Right column from top to bottom: a crude extract of *Clostridium pasteurianum* in 0·05 M phosphate buffer, *p*H 6·6, centrifuged at 20,000*g*, supernatant fraction used (32 mg protein/ml.); the same extract plus pyruvate (final concentration 0·07 M); a purified nitrogenase preparation of *Cl. pasteurianum* (84 mg protein/ml.) in 0 05 M phosphate, *p*H 6·5; nitrogenase plus pyruvate as above. In *E. coli* and in the crude extract of *Cl. pasteurianum* Mn^{++} signals are visible which overlap with the $g = 1·94$ line. The latter becomes visible when the extracts are completely reduced

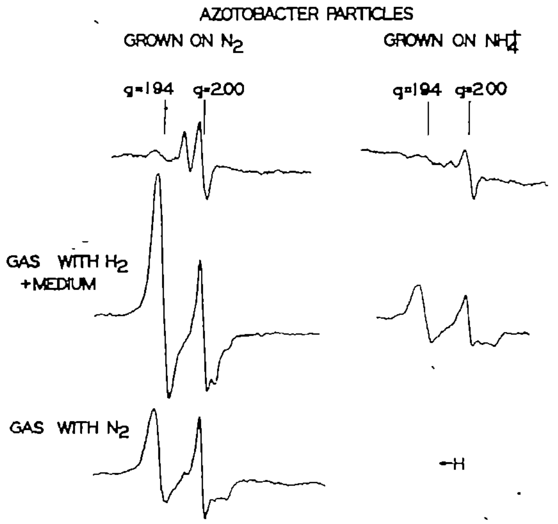

Fig. 3. EPR spectra of particles from *Azotobacter vinelandii OP* (48 mg protein/ml.). Cells disrupted in culture medium, in which they were grown, with an ultrasonic probe (ref. 16). Particles collected between 25,000 and 144,000*g* for 60 min in a Spinco preparative centrifuge. Particles aerated at 0° for 10 min before assay partially to oxidize the respiratory carriers. Spectra recorded at −170°. Left column, particles actively fixing nitrogen (0·12 atom per cent ^{15}N excess); right column, particles that do not fix nitrogen, prepared from cells grown with an ammonium salt as nitrogen source.

Upper row, spectra of particles as isolated. In the N_2-fixing particles there is a signal at $g = 1·97$, probably associated with Mo^{5+}. Middle row, after addition of 10 μl. of culture medium, in which organism had been growing, and gassing with hydrogen. Lower curve, after gassing with nitrogen after hydrogen

(upper curve) and the Mo^{5+} signal disappeared. The same treatment had some effect on the NH_4^+ particles, but the extent of the reduction was less (Fig. 3). Addition of hydrogen or the media factor singly was less effective than in combination (Fig. 4, centre curve). The addition of nitrogen gas to the nitrogen-fixing particles resulted in a partial re-oxidation of the electron transfer components as shown by EPR and optical spectroscopy. There was no effect when helium was used instead of nitrogen and no reversal was achieved with a nitrogen treatment of the NH_4^+ particles.

Copper. Cell-free extracts have been prepared from *Nitrosomonas europaea* that will oxidize hydroxylamine to nitrite, provided a suitable carrier, such as mammalian cytochrome *c*, phenazine methosulphate or methylene blue was added[19]. Particles prepared from this bacterium contain flavin and cytochromes of the *b*, *c* and *a* type. The EPR spectra of this particle clearly show that they contain a copper protein with a signal centred around $g = 2·1$ and the hyperfine structure typical of Cu^{2+} (Fig. 5). The observed signal is strikingly similar to that recently reported for cupric chloride in pyridine-chloroform solution[20]. On addition of substrate the copper signal of the enzyme undergoes complex changes, which indicate that the metal is involved in the oxidase system. (Similar results were observed with whole cells but the spectral changes were more rapid.) These changes are not those expected should copper be fully reduced, otherwise the signal would be similar to the type seen when sodium dithionite was added (Fig. 5). In earlier studies, Beinert *et al.* showed that the interaction of hydroxylamine with the copper of cytochrome oxidase from beef heart resulted in drastic changes in the EPR spectrum. By analogy it might appear that also in the *Nitrosomonas* oxidase hydroxylamine

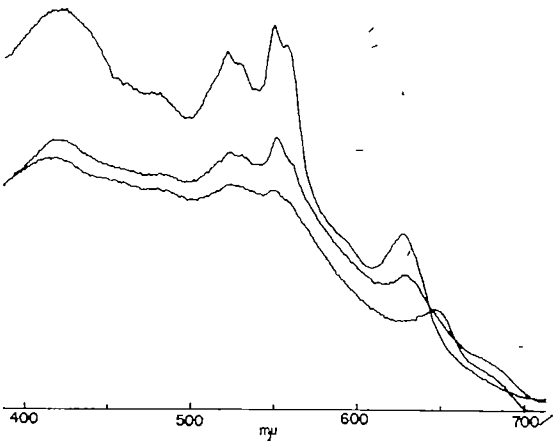

Fig. 4. Optical reflectance spectra of *Azotobacter* N_2 particles as in Fig. 3, recorded at −90° with a Cary spectrophotometer, model 14, and a modified diffuse reflectance attachment (ref. 2). Ordinate: absorbance in arbitrary units as obtained by reflectance. Lower curve, particles as obtained after aeration (see legend to Fig. 3); centre curve, after gassing with hydrogen; upper curve, after gassing with hydrogen and subsequent addition of 10 μl. of culture medium

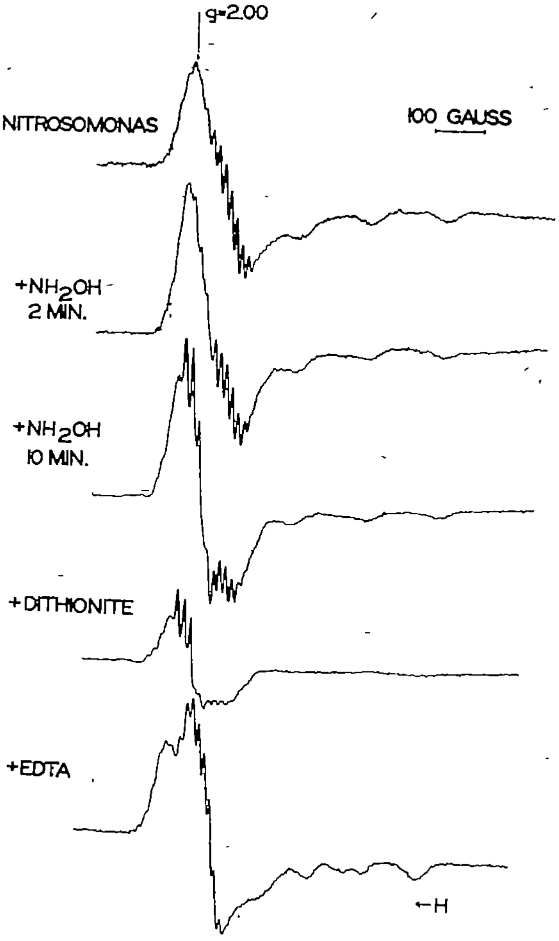

Fig 5. EPR spectra of particles from *Nitrosomonas europaea* (31 mg protein/ml.) at −170°. Cells suspended in 0·05 M *tris* buffer, *p*H 7·5, treated with the ultrasonic probe for 20 min, particles between 15,000 and 144,000*g* for 60 min suspended in the buffer. Top to bottom: untreated particles; 2 min at 0° after addition of hydroxylamine, final concentration 0 02 M; 10 min after addition of hydroxylamine; after reduction with dithionite; 30 min with 3·5 mM EDTA final concentration. The three lower curves were recorded at a lower amplification, 0·8 times for curves 3 and 4, and 0·6 times for the bottom curve. Similar results were obtained with whole cells

attacks the copper directly. This interpretation of the spectral changes is, however, inconsistent with the observation that the EPR spectrum changes much faster when cytochrome *c* is present together with the enzyme and hydroxylamine. The spectral changes therefore seem to arise as a consequence of electron transfer from hydroxylamine via cytochromes to the oxidase rather than by a direct non-specific interaction between the copper protein and hydroxylamine.

Beyond this we are unable to interpret the complex spectra of this copper protein. It would appear, however, that spectra of two different species of copper are superimposed. This interpretation is supported by the effect of ethylenediamine tetraacetic acid (EDTA) on the particles, as shown in the lowermost spectrum. The two copper components respond in a different way; the signal of the copper EDTA complex rises at the lower field, whereas the other component appears to be unaffected. These changes can be reversed by dialysis against 0·05 M *tris* buffer.

This survey shows that EPR spectroscopy, which has already proved its usefulness in investigations with mammalian enzyme systems, may also provide a new parameter in determining the function of transition metals, among which are iron, copper, manganese and molybdenum, in microbial metabolism. Since the application of this technique to biochemical systems is relatively new, many of the observed spectra cannot be interpreted fully at the present time. As more experience is gained, however, a whole range of catalytically active metal complexes might be identified by making critical comparisons between materials of different origin. Thus the field of active iron complexes in particular has gained prominence recently. It is now clear that the iron porphyrin compounds are only one group of a whole array of iron compounds that participate in metabolism. One of the recently discovered groups are the iron proteins of low molecular weight, such as photosynthetic pyridine nucleotide reductase (PPNR)[21] and ferredoxin[22] which participate in electron transport. Another newly discovered type of catalytically active compound is discussed in this communication, which is readily identified by its EPR spectrum in the reduced state. It should be emphasized that the latter group of iron proteins is quite different in all known properties from the ferredoxin and PPNR types.

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INFRA-RED ABSORPTION BANDS OF THE PEPTIDE GROUP

BENDIT[1] has recently suggested that the absorption at 3,190 cm^{-1} in keratin and at 2,400 cm^{-1} in deuterated keratin arises in the amorphous regions only, and he considers that the assignment of these two bands is difficult. He rejects the assignment of the corresponding bands in monosubstituted amides[2] and polyamides[3,4] to overtone and combination modes enhanced by Fermi resonance. Badger and Pullin[5] first proposed this explanation for the 3,080 cm^{-1} band in collagen. We consider that the position and intensity of the bands in the NH and ND stretching regions of all N substituted amides may be likewise explained.

Keratin is an unsuitable substance for examining the assignment of the 3,190 cm^{-1} and 2,400 cm^{-1} bands for three reasons. These are, first, the complication of the spectrum by the broad and ill-defined bands in the NH and ND stretching regions which arise from the NH and OH and ND and OD bonds in the side groups; secondly, the uncertainty of the physical structure of keratin[6], which prevents a conclusive interpretation of the dichroism measurements; thirdly, the lack of a fully N deuterated keratin spectrum.

Keratin[1] shows a strong peak at 3,290 cm^{-1}, a shoulder at 3,190 cm^{-1} and a weak peak at 3,050 cm^{-1}. In addition, a very broad absorption extends from about 2,800 cm^{-1} to about 3,400 cm^{-1} which is almost completely removed on deuteration; this absorption is presumably due to the side-groups. The 3,290 cm^{-1} and 3,050 cm^{-1} bands are reduced in intensity on deuteration. The 3,190 cm^{-1} shoulder does not disappear as claimed by Bendit but is also only reduced in intensity; the asymmetry of the 3,290 cm^{-1} band is quite clear in Bendit's spectrum. The two new ND bands at 2,460 cm^{-1} and 2,400 cm^{-1}

have approximately equal intensity and are assigned by Bendit to the modes corresponding to the 3,290 cm^{-1} and 3,190 cm^{-1} NH absorption in the amorphous regions. (The fate of the 3,050 cm^{-1} band on deuteration is not considered by Bendit.) He suggests that the equal intensity of the ND bands is accounted for by assuming the complete exchange in material exhibiting the 3,190 cm^{-1} band (amorphous) and the partial exchange in material exhibiting the 3,290 cm^{-1} band (amorphous and crystalline). If completely deuterated keratin showed approximately equal ND bands this assumption would be invalidated; complete deuteration has not been achieved with keratin[7], but in other amides where it has been achieved the two ND bands are found to be approximately equal in intensity. Though there are significant and as yet incompletely understood intensity differences between the accessible and inaccessible regions the 3,190 cm^{-1} and 2,400 cm^{-1} absorptions are not restricted to the accessible regions. (In deuterium exchange studies it is usually assumed that the regions accessible to deuterium oxide are amorphous and that the inaccessible regions are crystalline. This over-simplification only confuses our understanding of the structural problems and we shall use the terms accessible and inaccessible.)

The absorption of the side-groups and the uncertainty of the difference in structure between the accessible and inaccessible regions necessitates very cautious interpretation of the dichroism measurements, especially in the light of recent proposals for the structure of keratin[4]. In addition, Bendit's point (c) is not valid as the parallel dichroic ratio shown by the 3,290 cm^{-1} NH band after deuteration is less than 3. Hence the contribution of the deuterated α helices to the 'perpendicular' spectrum must be greater than one-third of their contribution to the 'parallel' spectrum.

We consider that the following examples are in accord with the Fermi resonance assignment and contrary to Bendit's proposals.

Crystalline substances. Two (or more) amide ND bands of approximately equal intensity are present in the spectrum of single crystals of fully N deuterated α diglycine[8] in which the ND_3^+ bands are separated from the COND bands in the polarized spectra. In single crystals of deutero-acetanilide the ND bands are complex and bands on the high-frequency side of the main peak have no equivalents in the NH region[9]. Crystalline deutero NN′dimethyl oxamide[10] and deutero-diformyl hydrazine[10] show multiple bands in the ND region.

NN′diacetylhexamethylenediamine[11] shows a band at 3,185 cm^{-1} and various completely crystalline polyamide oligomers show a 3,200 cm^{-1} band[3].

Liquids. Pure liquid N alkyl amides show both the 3,200 cm^{-1} shoulder and the 3,300 cm^{-1} bands, for example, N ethyl acetamide[12] and N butyl acetamide[13]; the latter, on deuteration, shows two ND peaks of equal intensity[2]. In many cases, spectra recorded with a sodium chloride prism do not show the 3,200 cm^{-1} shoulder after reproduction[2,11,14] but the doubling of the ND band is clearly seen, for example, N methyl acetamide[15].

Polymeric materials. In polyamides of different crystallinity (estimated by X-rays) there is no parallel between the crystallinity and the intensity of the 3,200 cm^{-1} shoulder, for example, in the low crystallinity copolymers[3]. Poly-DL-alanine in the α form was found[15] to have a single NH band at 3,305 cm^{-1} and after almost complete deuteration the two ND bands at 2,465 cm^{-1} and 2,400 cm^{-1} were nearly equal in intensity.

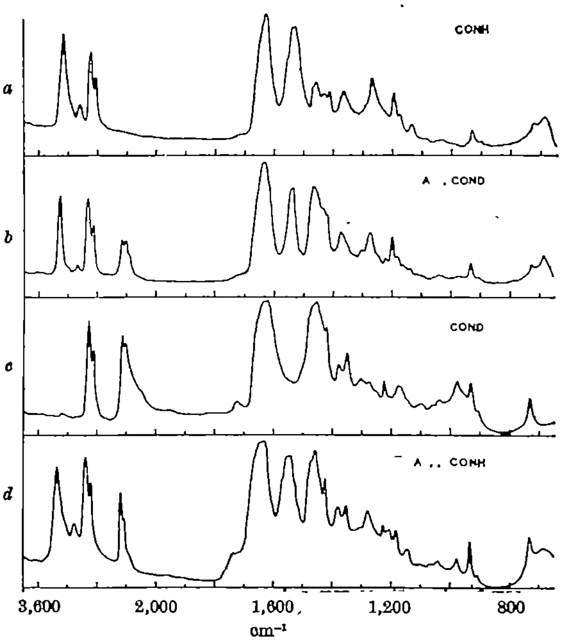

Fig. 1. *a*, Normal nylon 6 6; *b*, nylon 6·6 after exchange with excess deuterium oxide at room temperature until no further exchange occurs (60 h); *c*, fully N deuterated nylon 6·6; *d*, N deuterated nylon 6·6 after exchange with water (conditions as for *b*). *A*, Accessible groups

The absorption bands of accessible and inaccessible CONH and COND groups in nylon 6·6 have been obtained[16] by deuterating the accessible part of the normal polymer with deuterium oxide and by hydrogenating the accessible part of a fully deuterated polymer with water (both at room temperature). The spectra are shown in Fig. 1. Figs. 1*a* and *b* show that on partial deuteration nylon behaves like keratin; a small shoulder remains at 3,200 cm^{-1} and the two strongest ND bands (2,470 cm^{-1} and 2,414 cm^{-1}) are equal in intensity. On complete deuteration (Fig. 1*c*) the ND bands are still of approximately equal intensity and on hydrogenation of the accessible regions (Fig. 1*b*) the residual inaccessible deuterated groups show two ND bands. Furthermore, the relative intensities of the 3,300 cm^{-1} peak and the 3,200 cm^{-1} shoulder is almost the same as in the normal polymer (Fig. 1*a*). According to Bendit's suggestion the 3,200 cm^{-1} shoulder should now be much greater in intensity. It is clear that accessible and inaccessible amide groups in 6·6 nylon show a different relative intensity for the two ND bands, but the 2,414 cm^{-1} band is still quite strong in the inaccessible regions. This problem is under investigation, and an explanation in terms of combination bands, the intensity of which depends on the extent of Fermi resonance, is most probable since significant differences in the amide fundamental frequencies are detectable between accessible and inaccessible groups.

In silk films the main NH peak is at 3,290 cm^{-1} and the 3,200 cm^{-1} shoulder is absent[17-19]. On deuteration of the accessible material, silk shows two ND bands with approximately equal intensity[19,20]. The inaccessible NH band is considerably narrower than the total NH band and on subtracting these two bands it is found that the accessible NH absorption consists of a broad but single band with no sign of a shoulder in the 3,190 cm^{-1} region[20]. This result is confirmed by hydrogenating the accessible material in a fully deuterated film. The two bands in the fully deuterated film are at 2,465 cm^{-1} and 2,415 cm^{-1}, but in this case the lower frequency band is slightly more intense; this intensity difference is maintained during the hydrogenation process.

These results for crystalline substances, liquids and polymeric materials show that the assignment of the 3,190 cm^{-1} and 2,400 cm^{-1} bands to amorphous (accessible) material in keratin as suggested by Bendit[1] is untenable.

The details of the assignments on the basis of overtone and combination bands enhanced by Fermi resonance still require elucidation, but we consider that this is the most probable explanation for all the bands in the NH and ND regions. For polymeric materials, a prerequisite is the complete assignment of the amide fundamentals for both the proto- and deutero-compounds in both the accessible and inaccessible regions.

A long-standing criticism of this type of explanation for the 3,080 cm^{-1} band, assigned to the overtone of amide II, has been based on the presence of a similar band in lactams where amide II is absent; this difficulty has been convincingly overcome[21].

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THE evidence presented by Cannon *et al.* invalidates my suggestion that the 3,190 and 2,400 cm^{-1} bands are associated exclusively with the amorphous phase in keratin. However, I do not think that their assignment in terms solely of Fermi resonance offers a complete explanation; it now appears likely that these bands do not have a unique origin, and that they are a composite of the peptide modes favoured by Cannon *et al.* and other bands characteristic of the material. This opinion is based on the following considerations.

(1) There are limitations when applying the frequency assignments of the amide group to proteins. In the first place, in simple amides or polyamides there are no intra-chain interactions to give rise to frequency perturbations, such as were investigated by Miyazawa and Blout[1] for the amide I and amide II bands. Secondly, side-chain absorption modes may occur, not all of which are broad and ill-defined. For example, the CONH$_2$ absorption of such groups as glutamine has been shown by Elliott[2] to occur at 3,225 cm^{-1}, with a weaker band near 3,370 cm^{-1}, and I thank Dr. R. D. B. Fraser for directing my attention to this. The effect is exhibited in the spectra of silk, keratin and gliadin (Figs. 2*a* and *b*), which show good correlation between CONH$_2$ content and the absorption at 3,200 and 2,400 cm^{-1}. (The CONH$_2$ content of these proteins, expressed as a percentage of the number of residues containing amide groups to the total number of residues is about 1, 10 and 40 per cent respectively.) Another source of side-chain contribution may be basic groups such as arginine, which absorb strongly near 3,200 cm^{-1}.

(2) Important evidence in favour of my original suggestions was the apparent disappearance of the 3,190 cm^{-1} band on deuteration, while Cannon *et al.* claim that the asymmetry of the main NH peak in deuterated keratin indicates that the band is still present. This claim cannot be regarded as established, as the main NH peak has various asymmetrical features in most polypeptides, both on the high- and low-frequency side[3]. Even in the case of poly-DL-alanine, which is given by Cannon *et al.* as an example of a substance exhibiting a single peak, asymmetry is clearly apparent. It is also worth noting that in a number of proteins tested, the disappearance of the shoulder near 3,200 cm^{-1} is more rapid than the decrease in intensity of the main NH peak, thus indicating that at least part of the absorption near 3,200 cm^{-1} is caused by accessible material.

(3) The variations of relative intensity of the ND doublet on re-hydrogenation, particularly as they are not accompanied by any significant frequency shifts of the peaks, are consistent with the presence of two components, although this type of behaviour does not rule out a Fermi resonance assignment. The effect, which is shown for gelatin in Fig. 2*c*, has also been observed for a number of other proteins, including keratin. Furthermore, whatever the correct assignment of the ND doublet, it is clear from the

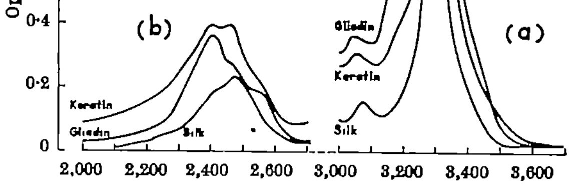

Fig. 2. *a*, Spectra of undeuterated films of gliadin and silk, and of keratin in the form of a section of horse tail hair; *b*, spectra of gliadin, silk and keratin, partially deuterated from the vapour phase; *c*, spectra of partially deuterated gelatin during the re-hydrogenation process. Times of exposure of specimen to water vapour are stated

re-hydrogenation results that the accessible and inaccessible phases exhibit different absorption intensities at 2,400 cm^{-1}, which must reflect, at least in part, structural differences between these phases.

(4) As most materials containing amide groups have an absorption mode near 3,200 cm^{-1}, which is assigned by Cannon[4] to the combination band amide I + amide II, enhanced by Fermi resonance, its absence in silk, which exhibits these fundamental modes as prominently as any other protein, calls for comment.

(5) In view of the above considerations, as well as the evidence presented in my earlier report, it would appear that in keratin the assignment of the controversial NH and ND modes exclusively to overtone and combination bands enhanced by Fermi resonance is not justified, and that other factors may well play an important part.

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ISOTOPIC OXYGEN INCORPORATION IN XANTHOPHYLLS OF *SPINACEA OLERACEA* QUANTASOMES

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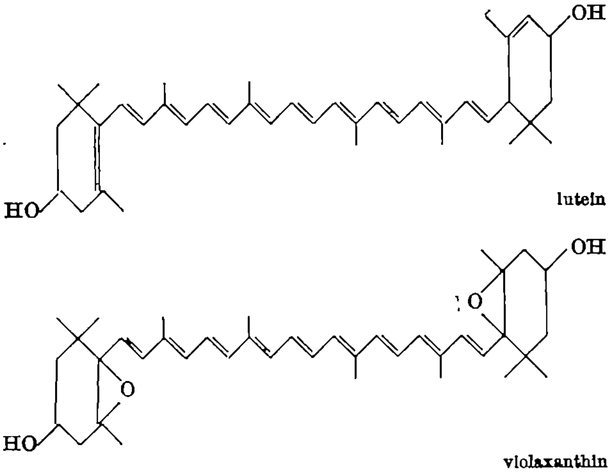
CAROTENOIDS in general, and xanthophylls in particular, are universally associated with the chlorophylls of photosynthetic organisms[1]. Among the possible functions for the xanthophylls, the role of oxygen transport resulting from the photolysis of water[2] was first suggested by Dorough and Calvin[3]. Sapozhnikov *et al.* have shown that the two major leaf xanthophylls, lutein and violaxanthin (Fig. 1) are interconvertible: dark aerobiosis resulted in an increase in violaxanthin at the expense of lutein, while strong light anaerobiosis led to the reversal of this reaction[4,5]. The existence of this relationship was confirmed by Blass, Anderson and Calvin using $^{14}CO_2$ labelling of these xanthophylls and showing them to have very nearly equal specific activities[6].



lutein

violaxanthin

Fig. 1

Yamamoto, Chichester and Nakayama have reported recently that no oxygen-18 enrichment was detectable in violaxanthin from *Spinacea oleracea* chloroplasts exposed to $H_2{}^{18}O$ and Hill reaction with ferricyanide, but that a small incorporation was observed in lutein[7].

Hill and Scarisbrick demonstrated that illuminated chloroplasts evolve oxygen in the presence of an artificial electron acceptor[8]. More recently, Park and Pon isolated chlorophylls and carotenoids-containing lamellæ from *Spinacea oleracea* chloroplasts which possessed enhanced Hill activity[9]. These authors provided evidence to suggest that the ultimate sub-units which have this activity are oblately spherical particles 100 × 200 Å, to which the designation 'quantasome' has now been given.

In this article, we report on an investigation similar to that of Yamamoto, Chichester and Nakayama, but with quantasome aggregate fractions of *Spinacea oleracea* which can evolve oxygen in the presence of a suitable electron acceptor, but which lack the ability to fix carbon dioxide.

Locally grown fresh spinach stalks were used throughout. Selected leaves were ripped from the stalks by hand, and the quantasome fraction was prepared according to the procedures described by Park and Pon[9]. Hill activity, chlorophylls and xanthophylls concentrations were determined by optical methods against appropriate controls[10]. The electron acceptor used for the Hill reaction was recrystallized 2,3′,6-trichloroindophenol, sodium salt (Eastman Kodak *P*3467) (TCI). Oxygen-18 enriched water and 99·4 per cent gaseous molecular oxygen-18 were obtained from the Isotope Division of the Weizmann Institute of Science (Rehovoth, Israel). Pure samples of lutein and violaxanthin used in co-chromatography were provided by Dr. Harold H. Strain, Argonne National Laboratory, Chicago, Illinois, and Dr. James H. C. Smith, Carnegie Institution of Washington, Stanford. California.

In all experiments to be described the quantasome fractions were diluted with 0·01 N phosphate buffer *p*H 7·8 to yield chlorophyll concentrations of 4 ± 0·2 mg/ml. and initial oxidized TCI concentrations of 2·7 ± 0·3 μM/mg chlorophyll. For experiments with $H_2{}^{18}O$, 3·0-ml. aliquots of diluted quantasome preparations containing 10 ± 0·5 atom per cent oxygen-18 were placed in 20-ml. sample flasks with an argon atmosphere. For experiments with molecular oxygen, 3·0-ml. aliquots containing no excess $H_2{}^{18}O$ were placed in 20-ml. sample flasks connected

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Table 1

	Exp. 1	Exp. 2
	$H_2{}^{18}O$	${}^{18}O_2$
Incubation mixture	10 00 ± 0·10	99·6 (20 per cent)
Atmosphere	Argon, 100 per cent	Argon, 80 per cent
Tank CO_2	0·202 ± 0·008	
Qo_2 (μM TCI reduced/mg chlorophyll h *)	(262)	(237)
Lutein: Dark	0·203	0·206
Light	0·206	0·258
Violaxanthin: Dark	0·225	0·213
Light	0·212	0·218
Boiled control (Lutein + violaxanthin)	0·203	0 201

* Assayed on diluted aliquots.
All data given in atom percentage oxygen-18.

to a vacuum glass-manifold arrangement which permitted the introduction into the sample flasks of a gaseous atmosphere consisting of 20 per cent oxygen-18 and 80 per cent argon by volume at atmospheric pressure. Each experiment consisted of two 3·0-ml. aliquots, one of which was kept in total darkness for 30 min at 20° ± 2° C, while the other was kept in darkness during the initial 25 min and then exposed to 10,000 ft.-candles heat-filtered incandescent illumination for the following 5 min. After 30 min, both the dark control and the illuminated samples were diluted with 15 ml. of a boiling mixture of acetone–methanol (7 : 2). Each sample was then repeatedly extracted until colourless, under reduced illumination, with 5-ml. fractions of peroxide-free diethyl ether, after the addition of 50 ml. aqueous saturated sodium chloride solution to the sample. The diethyl ether fractions from each sample were then combined, dried with sodium sulphate, brought down *in vacuo* to a small volume and stored under argon in a deep freeze for further treatment.

Lutein and violaxanthin were separated from chlorophylls without recourse to saponification of the diethyl ether extracts by adsorption chromatography on linear polyethylene powder. Linear polyethylene powder, melt index 0·9, was obtained through the courtesy of the Dow Chemical Co., Midland, Michigan, and was prepared for adsorption chromatography according to the method described earlier[6].

Each extract was developed with 85 per cent aqueous methanol on a 32 × 150 mm linear polyethylene column under positive argon pressure. By this procedure, it was also possible to recover quantitatively the TCI used as the Hill reagent. TCI, violaxanthin and lutein were obtained by elution. Each xanthophyll eluate was diluted with 10 ml. thiophene-free benzene and 100 ml. saturated sodium chloride aqueous solution. The benzene phase was quantitatively recovered, dried over sodium sulphate, filtered and stored in the deep freeze for further purification. Each benzene extract, evaporated *in vacuo* to a small volume, was chromatographed on a pretreated 32 × 150 mm 'Micro-Cel *C*' (Johns-Manville Co., synthetic calcium silicate, 95–200 m^2/g active surface), and developed under argon-positive pressure with 10 per cent acetone in light petroleum ether. The desired band was obtained by extrusion of the column. Pretreatment of the 'Micro-Cel *C*' adsorbant consisted of pyrolysis at 400° C for 1 h followed by washing with dry light petroleum ether. The separation and purification obtained by this method could not be distinguished from a procedure which involved a crystallization step.

Two-dimensional co-chromatography with authentic samples of lutein and violaxanthin on Kieselguhr-treated paper[11] developed in darkness under an argon atmosphere with isopropanol in petroleum ether, in one direction, followed by 20 per cent chloroform in petroleum ether in the other, did not yield more than one detectable component[12].

Sufficient quantities of lutein and violaxanthin were thus obtained without the necessity of using dilution with carrier and its attendant uncertainties. Neoxanthin, however, was not available in the required amount to obtain meaningful data.

Each of the two xanthophylls from these experiments were then converted to carbon dioxide according to the procedures of Rittenberg and Pontecorvo[13]. All glassware was preheated at 500° C for 12 h, and both samples and oxidant were exhaustively dried in high vacuum to remove residual oxygen and water from the system. Pyrolysis with mercuric chloride was carried out at 500° C for 1 h, and the oxygen-16/oxygen-18 ratio was determined by one of us on the Consolidated Electrodynamics Co mass spectrometer model 21–130, a cycloid tube type which permits accurate measurements with relatively small samples. Results are given in Table 1.

It can be seen from Table 2 that results in Table 1 confirm all previously reported data on oxygen incorporation in xanthophylls[3,6]. In all cases and conditions examined, very little isotope appears in these compounds. The technique used for obtaining the results reported here was also applied to the carotenoid spheroidenone of the photosynthetic bacterium *Rhodopseudomonas spheroides* in an oxygen-18 atmosphere. These experiments yielded very substantial incorporations of the label into this compound, thus confirming the validity of the method used[14]. It is possible to estimate the minimum expected incorporation if only one xanthophyll oxygen per quantasome was involved in oxygen transport during photosynthesis. There are approximately 180 chlorophyll molecules per *Spinacea oleracea* quantasome[15], and about one-third this number of carotenoid molecules, of which 40 are xanthophylls[16]. The relative fraction of lutein, violaxanthin and neoxanthin are 0·57, 0·26 and 0·17, with 2, 4 and 3 oxygen atoms, respectively[7]. Thus, we can calculate the total number of xanthophyll oxygens as follows:

Table 2

Organism	Treatment	Xanthophylls	$H_2{}^{18}O$ atom (per cent)	Atom (per cent) incorporated	Ref.
Chlorella pyrenoidosa (whole cells)	5 h high-intensity illumination	unresolved	3·01	0·245	(3)
Chlorella pyrenoidosa (whole cells)	dark	unresolved	3·06	0 233	(3)
Spinacea oleracea (chloroplasts)	$Fe(CN)_6^{+++}$ 30 min high-intensity illumination	Lutein Violaxanthin	3·95	0·225 0·206	(6)
Spinacea oleracea (chloroplasts)	$Fe(CN)_6^{+++}$ Dark	Lutein Violaxanthin	3 95	0·224 0·206	(6)
Spinacea oleracea (quantasomes)	TCI, 25 min dark, then 5 min light	Lutein Violaxanthin	10·0	0 206 0 212	this article
Spinacea oleracea (quantasomes)	TCI, 30 min dark	Lutein Violaxanthin	10 0	0 203 0·225	this article

Lutein = 40 × 0·57 × 2	45·6
Violaxanthin = 40 × 0·26 × 4	41·6
Neoxanthin = 40 × 0·17 × 3	20 4
Total xanthophylls oxygen atoms per quantasome	107·6

Water in equilibrium with the system contained 10·00–0·20 = 9·80 atom per cent excess oxygen-18.

If only one of these 108 oxygen atoms per quantasome could exchange with the $H_2{}^{18}O$ the resulting overall xanthophyll enrichment would be:

$$\frac{9\cdot80}{108} = 0\cdot09 \text{ atom per cent excess oxygen-18}$$

But the maximum incorporation actually observed was:

$$0\cdot22–0\cdot20 = 0\cdot02 \text{ atom per cent excess}$$

From these considerations it is possible to conclude that the xanthophylls of green plants probably do not function as principal oxygen carrier in photosynthesis.

This work was done during the tenure by one of us (E. A. S.) of an advanced fellowship from the American Heart Association and was supported, in part, by the U.S. Atomic Energy Commission.

We thank Prof. C. C. Delwiche for advice on the theory and use of the mass spectrometer and Prof. R. B. Park for information on the preparation of *Spinacea oleracea* quantasome fractions.

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HISTAMINE METABOLISM *VIA* THE NUCLEOTIDE PATHWAY

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Enzymatic Formation of Histamine Riboside from Histamine Ribotide

A PREVIOUS communication[1] from our laboratory described the formation of histamine ribotide from histamine-adenine dinucleotide using a phosphodiesterase and some properties of this ribotide. The present report, which is a continuation of studies to elucidate the possibility of liberation of free histamine from histamine dinucleotide *in vivo*, describes the formation of riboside from histamine ribotide by a human prostatic acid phosphatase, and some chemical and biological properties of histamine riboside.

The degradation of histamine ribotide was carried out by the use of the partially purified human prostatic acid phosphatase[2], in a medium containing 2 μmoles of ribotide, 0·2 ml. of 0·2 M acetate buffer (*p*H 5·0), 0·1 ml. of 0·01 M magnesium chloride and 0·2 ml. of enzyme, in a total volume of 0·6 ml. The liberation of inorganic phosphate was 73 per cent of the theoretical value after 60 min incubation at 37° C. The rate of the reaction appears to be somewhat slower than that with 5′-adenylic acid. The use of a purified preparation of 5′-nucleotidase from snake venom (*Agkistrodon halys blomhoffi* Boie)[3] or bull semen, in an amount sufficient to decompose 5′-adenylic acid completely in 5 min, failed to give evidence for the presence of inorganic phosphate in 60 min which should be liberated from histamine ribotide. A comparatively large amount of crude snake venom liberated inorganic phosphate from histamine ribotide, but this was probably due to the non-specific monoesterase contained in the venom.

Histamine riboside was isolated from the reaction mixture of histamine ribotide and prostatic enzyme after incubation at 37° C for 3 h at *p*H 5·0 in a nitrogen phase, by McIntire's method[4] for purification of tissue histamine, which involves butanol extraction at *p*H 12·5 and adsorption–desorption by cotton acid succinate. The riboside can be separated from the starting ribotide by the use of 'Dowex-50' (H^+ form), but column chromatography with cotton acid succinate has the advantage of allowing the use of a more dilute acid (0·004 N hydrochloric acid) as the eluting solution. Each fraction of the eluate was submitted to optical density measurement for the histamine moiety at 220 mμ. The fractions containing the riboside were lyophilized, and the riboside was crystallized from a mixture of ethanol and ether (1 : 1). The crystals were so highly hygroscopic at room temperature that accurate measurement of the melting-point was impossible; but the analytical values shown here clearly indicate that this sample is the histamine riboside. One μmole of this sample contained 0·99 μmole of ribose, measured from periodate consumption, by taking the optical density of histamine dihydrochloride at 220 mμ, at *p*H 7·0, as the standard. Hydrolysis of the riboside by heating with 2·5 N hydrochloric acid at 170° C for 5 h in a sealed tube showed that the amount of histamine bound was 0·93 (calc. 1·00) by the assay with guinea pig ileum and 0·96 by the spectrofluorometric method[5], both values agreeing well with the theoretical value. This sample of riboside did not contain free histamine detectable either biologically or fluorometrically, and inorganic phosphorus was not detected in the foregoing acid hydrolysate.

Paper electrophoretic studies indicated that the basicity of histamine riboside is fairly close to that of histamine, that is, on electrophoresis in 0·1 M phosphate buffer (*p*H 7·2) at 300–400 V, 1 m.amp/cm, for 120 min, histamine moved 4·6 cm towards the cathode, while the riboside moved 3·5 cm in the same direction. Under the same conditions, the ribotide and dinucleotide moved 1·0 and 0·4 cm in the reverse direction, respectively. The R_F values on paper chromatography, using a mixture of 0·1 M acetic acid/ethanol (1 : 1) as a solvent, were 0·82 for the riboside, 0·53 for the dinucleotide and 0·68 for the ribotide. The ultra-violet spectrum of the riboside showed an absorption maximum at around 210 mμ, agreeing with that of the ribotide. The diazo reaction of the riboside with sulphanilic acid developed a yellow coloration on the paper chromatogram. The absorption spectrum of this reaction solution lacked the peak at 530 mμ characteristic of free histamine, as in the case of the ribotide and dinucleotide, and one peak at around 380 mμ was noted. Similar to the ribotide which was previously described, the orcinol reaction for ribose was completely negative. Attempted formation of a fluorescent product by condensation with *o*-phthalaldehyde, observed by Shore *et al.*[5] with histamine, failed in these three compounds which have a histamine–ribose bond. This result could be utilized for the determination of free histamine in the presence of these compounds.

As in the case of the ribotide[1], 0·1–0·3 μmole of histamine riboside caused a slow increase of tone of the guinea pig ileum piece sensitive to 0·0005 μmole of histamine, apparently distinguishable from the action of histamine. Arterial pressure of a pentobarbitone-anæsthetized cat, which showed a characteristic fall with 0·001 μmole of histamine, was not affected by intravenous injection of 0·2 μmole of the riboside or 0·5 μmole of the ribotide. Only a higher concentration (0·5–1·0 μmole) of the dinucleotide showed a very slight and transitory fall, somewhat slower than that of histamine. The local blueing[6] of the skin by intracutaneous injection in a guinea pig previously administered pontamine sky blue by cardial puncture was negative in all these three histamine derivatives even with 0·5 μmole, while histamine was positive at 0·001 μmole.

Nucleotide Pathway of Histamine

Previously, we confirmed the work of Alivisatos *et al.*[7] that histamine-adenine dinucleotide is synthesized from histamine and diphosphopyridine nucleotide (DPN)[1]. Following up this investigation, we subjected this dinucleotide to the action of phosphodiesterase from snake venom and were able to isolate a pure preparation of histamine ribotide[1]. Further, we succeeded in the formation and isolation of histamine riboside from the resulting ribotide with the use of human prostatic acid phosphatase (preceding communication). Recently, Abdel-Latif *et al.*[8] reported an alternative pathway of enzymatic formation of histamine ribotide or riboside by the direct interaction of histamine on the ribotide or riboside of nicotinamide. Preliminary evidence[9,10] also indicates that histamine ribotide is formed from histamine and phosphoribosyl pyrophosphate in the presence of adenosine triphosphate. In earlier experiments, imidazoleacetic acid riboside was isolated from urine of rats injected with ^{14}C-labelled histamine or imidazoleacetic acid by Tabor and Hayaishi[11], and Karjala[12]. These findings suggest a possibility that histamine metabolism *via* the nucleotide pathway plays some physiological part *in vivo*.

This report deals with results of *in vitro* experiments conducted to find out whether or not this pathway includes the degradation of histamine riboside and the reverse reaction, the formation of the riboside, by nucleosidases especially by nucleoside phosphorylase. The liberation of free histamine from histamine riboside was investigated taking as its criterion the contraction of the guinea pig ileum suspended in a Tyrode bath kept at 37° C in the presence of the riboside and enzyme preparation. Nucleoside hydrolase[13], prepared from *Lactobacillus delbrueckii*, did not cause a contraction of the ileum, but when 50 mμmoles of histamine riboside were added after, simultaneously, or before the treatment with enzyme, the ileum did contract, and this contraction was relaxed by antihistamine ('Neoantergan') (Fig. 1). However, nucleoside phosphorylase[14] from rat liver, nicotinamide ribosidase[15] from bovine liver, hæmolysed human erythrocyte and snake venom (*Agkistrodon halys blomhoffi* Boie) did not cause the contraction of the ileum under the same conditions.

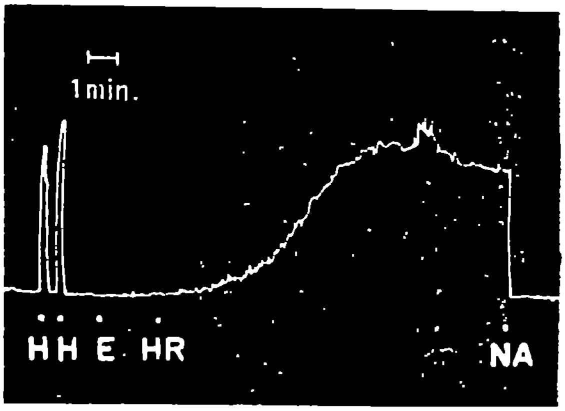

Fig. 1. The liberation of free histamine from histamine riboside by nucleoside hydrolase from *Lactobacillus delbrueckii*. *H*, histamine 2 mμmoles; *HR*, histamine riboside 50 mμmoles; *E*, nucleoside hydrolase (1 mg protein); *NA*, 'Neoantergan' 0·02 μg in 10 ml. Tyrode bath (37° C)

The liberation of ribose-1-phosphate or ribose from the histamine riboside was estimated by the orcinol reaction. It was found that the orcinol reaction of the medium remained negative even after 1–5 h incubation at 37° C; this medium contained 1·5 μmoles of histamine riboside, 30–320 units of nucleoside phosphorylase[14] purified from rat liver, 1·0 ml. 0·1 M phosphate buffer (*p*H 7·4), in a total volume of 3·0 ml. In the presence of diamine oxidase[16] partially purified from hog kidney the oxygen uptake proved to be 38·4 μl. by histamine, 1·8 μl. by histamine riboside, 2·4 μl. by histamine ribotide and 1·9 μl. by histamine dinucleotide in 2 μmoles each for 1 h. Therefore, this enzyme can be said to act almost specifically on free histamine only. In view of the fact that the phosphorolytic cleavage of inosine was coupled with the xanthine oxidase reaction[17], an excess amount of diamine oxidase was added to the reaction system just mentioned, including histamine riboside and nucleoside phosphorylase, but an acceleration of the reaction failed to take place. On the other hand, in the case of nucleoside hydrolase from *Lactobacillus delbrueckii*, the liberation of an equivalent quantity of ribose and histamine was observed by the orcinol reaction and by the bioassay with guinea pig ileum. In an attempt to form histamine riboside from histamine and ribose-1-phosphate in the presence of nucleoside phosphorylase, with a total volume of 3·0 ml. of the medium containing 1·5 μmoles of inosine, 50 units of nucleoside phosphorylase, 400 units xanthine oxidase and 1·0 ml. of 0·1 M phosphate buffer (*p*H 7·4) under the conditions where ribose-1-phosphate can be liberated quantitatively from inosine, an equivalent amount of histamine was added to the medium. However, the formation of histamine riboside was not demonstrable because free histamine determined by the diazo reaction did not decrease in the reaction mixture even after 1–4 h incubation.

From these results, it seems that the nucleotide pathway of histamine does not involve the process of phosphorolytic cleavage of riboside and the reverse reaction. Previously, we reported that histamine-containing nucleotides or nucleoside all show no histamine-like effect as proved by tests on the contraction of guinea pig ileum, the fall in blood pressure of the cat and on the local accumulation of circulating dye in guinea pig skin (preceding communication). These facts, signifying that even *in vivo* the histamine–ribose bond of these compounds is difficult to split, agree well with the results of the present experiment. Consequently, the final product(s) of the nucleotide pathway of histamine must be histamine riboside and/or its derivatives, and the fact that these intermediates are physiologically inactive offers a promising possibility of this pathway being included in a part of the detoxication mechanism of histamine.

In the early period of our studies, from the fact that the anaphylactic release of histamine from guinea pig lung was inhibited by nicotinamide[18], an inhibitor of DPNase and nicotinamide ribosidase, we proposed a tentative hypothesis that part of the released histamine might be liberated from the nucleotide pathway. Thus we assumed that either one of two processes, sensitive to nicotinamide, the formation of histamine dinucleotide by DPNase or the degradation of histamine riboside by nucleosidase might be contained in this pathway. It has been recently confirmed, however, that the DPNase activity of guinea pig lung tissue is not affected by sensitization or antigen–antibody reaction[18]. Furthermore, as it was not possible to catalyse the degradation of histamine riboside with mammalian enzymes in the present study, the nucleotide pathway seems not to be involved in the mechanism of nicotinamide inhibition of histamine release.

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INFLUENCE OF RIBONUCLEIC ACIDS FROM THE LIVER ON IMPLANTATION AND GROWTH OF TRANSPLANTABLE TUMOURS

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SEVERAL investigations have shown that ribonucleic acids (RNA) from normal tissues can inhibit the growth of tumours[1-3]. The results obtained are in agreement with our own[4]. This article summarizes our results and those of some more recent experiments.

The cells of a transplantable strain of the liver mucous cancer (Malyugina's strain) were used as a recipient. The preparations of RNA of donor cells were obtained by the phenol method at $pH = 6{\cdot}0$ and contained neither protein nor DNA. Tumour cells before implantation into outbred rats were incubated with RNA in Hank's solution at room temperature (about 25°). The suspension of tumour cells incubated under similar conditions without RNA served as a control. The results of the experiment were checked 25 days after inoculation. In all more than 900 rats were used for these experiments.

Incubation with liver RNA results in a strong inhibition of tumour growth (Fig. 1). The effect depends on concentration of the RNA and time of incubation. No tumours were observed after 8-h incubation at concentrations of 0·1 mg/ml. and higher. At the site of inoculation there appeared small, glassy nodules the transplantation of which was not successful. The inhibitory effect of the liver RNA on the growth of the liver mucous cancer is notable even at 0·05 mg/ml. The cells incubated with the liver RNA do not display any morphological deviation from the control. The viability of these cells has been confirmed by a successful explanation *in vitro*.

RNA from the cells of the liver mucous cancer produced a weaker effect (Fig. 1). The tumours grown from the cells incubated with RNA from the mucous cancer were easily transplanted. The unavoidable admixture of RNA from normal tissues probably may explain the inhibitory effect of RNA preparations from the mucous cancer.

The treating of RNA preparations with RNase (0·2 mg/ml. of RNA was incubated with 0·004 mg/ml. of RNase at 37° for 1 and 2 h) fully eliminated the effect. The completeness of inactivation has been confirmed by the fact that in a special experiment with mucous cancer cells incubated only with RNase (without RNA) the inhibition of tumour growth is greater than in the experiments with RNA treated with RNase. It is worth mentioning that the inhibitory effect of RNase is well known.

Contrary to RNA, preparations of liver DNA do not inhibit tumour growth.

The difference between the experiment and the control decreases considerably when rats are injected

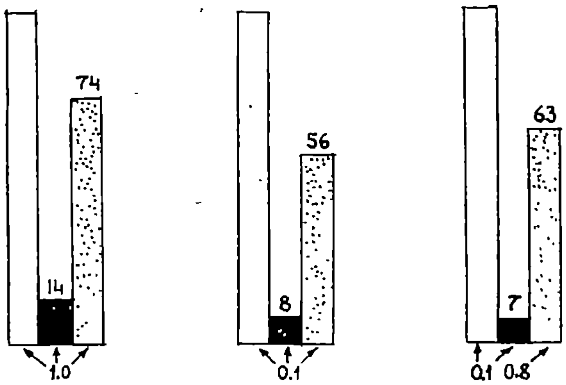

Fig. 1. Influence of preliminary incubation of implanted cells performed with RNA from the rat liver and mucous cancer of the rat liver on the growth of mucous cancer.
White, control; stippled, RNA from mucous cancer; black, RNA from rat liver.
Left, 2·5-h incubation; middle, 8-h incubation; right, 8-h incubation. Figures above columns designate the average weight of tumours (in percentage of the control assumed to be 100 per cent) and figures under the columns are concentration of RNA in incubation medium

with cortisone. This gives some grounds for believing that changes which occur in tumour cells under the influence of liver RNA may reduce their resistance towards protective mechanisms of the animal. Therefore, on weakening that protection, the effect of RNA either becomes insignificant or disappears altogether.

We have compared the effect of liver RNA on the growth of the liver mucous cancer and sarcoma 45 (Fig. 2). It was found that the growth of sarcoma 45, unlike the liver mucous cancer, was not suppressed by liver RNA. (As is seen from Fig. 2, the weight of tumours even increases in comparison with the control. It may be explained, probably, by the improvement of the survival conditions of the cells due to the enriching of the incubation media.)

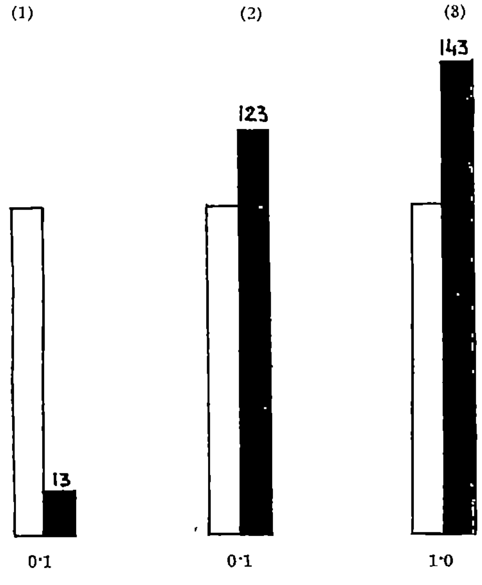

Fig. 2. Comparison of effects from preliminary incubation (8-h) of implanted cells performed with liver RNA on the growth of mucous cancer of the liver and sarcoma 45.
White, control; black, experiment–incubation with RNA from rat liver. Figures above columns designate the average weight of tumours (in percentage of the control assumed to be 100 per cent) and figures under the columns–concentration of RNA in incubation medium 1, mucous cancer of the liver; 2 and 3, sarcoma 45

However, this difference is not to be regarded as an evidence of the organospecific effect of the liver RNA, for different kinds of interpretations of the results obtained are quite possible. Perhaps, not all tumour strains react to RNA from normal tissues in the conditions of our experiments.

The results of the following experiment probably may testify to the organospecific character of the effect of liver RNA from normal tissues (Table 1). As usual, the cells of the liver mucous cancer were used as a recipient. These cells were treated with RNA extracted from a rat kidney. In this case RNA concentration exceeded by ten times the usual concentration of liver RNA (1·0 mg/ml. instead of 0·1 mg/ml.) with the maximal time of incubation.

Table 1. INFLUENCE OF PRELIMINARY INCUBATION OF IMPLANTED CELLS PERFORMED WITH RNA FROM THE RAT KIDNEY AND GUINEA PIG LIVER

Preparation	RNA concentration (mg/ml.)	Time of incubation (h)	No. of animals	Average weight of tumours	Growth inhib. of tumours (%)*
Control (without RNA)	—	8	19	5·1	
RNA from rat kidney	1·0	8	20	2·4	53
RNA from guinea pig liver	1·0	8	40	2·7	47

* The coefficient of inhibition was calculated by means of the formula $\frac{Mc - Mex}{Mc} \times 100$, where *Mc* and *Mex* present the average weight of tumours in the control and experiment respectively.

Despite these conditions, the effect was weaker than in all experiments with liver RNA of lower concentration. Our experiments also show that the action of RNA from the guinea pig liver is characterized by the same weak inhibitory effect (Table 1).

When discussing our results it is necessary to take into consideration the fact that the inhibitory effect of RNA from rat liver on the growth of the tumour, for example, the liver mucous cancer, may not be explained by the presence of admixtures of toxic agents for, on one hand, this explanation is not in agreement with the data showing different effectiveness of preparations obtained from cells of the rat liver, mucous cancer of the rat liver, rat kidney and guinea pig liver; on the other hand, RNase inactivates preparations. Besides, as already mentioned, we have not found any morphological changes in the cells treated with RNA before implantation.

Thus, our experimental results, as well as those obtained by some other authors[1–3], indicate that the capacity of tumour cells for autonomic growth decreases after their incubation with RNA from normal tissues. Possibly, this effect, in a certain degree, is organospecific.

We believe that an investigation of this effect may be helpful for the analysis of the nature of epigenetic changes[5,6] and their role in the carcinogenesis. The results of the effect of RNA from normal tissues on the growth of tumours seem to be important also for the evaluation of the generalized virus theory of tumour origin.

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FROST INJURY IN LIVING CELLS

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BY freezing the β-lipoprotein of human plasma at a definite low temperature with various single salt solutions, Lovelock found that the higher the eutectic temperature of the suspending solution above the freezing temperature the greater the degree of denaturation of the lipoprotein[1]. This may illustrate, as he suggested, a new type of frost injury in extracellularly frozen cells. It seemed, however, very difficult to apply his method in the case of living cells, especially of animal origin, because they are very susceptible to a high concentration of single salt solutions. Fortunately the egg cell of the sea urchin is remarkably resistant to some single-salt solutions. This made possible experiments to examine the effects of salt solutions on a freezing cell at eutectic temperatures of the solutions.

Unfertilized eggs of *Hemicentrotus pulcherrimus* were used as material. The egg cells of this species of sea urchin were found to be very resistant to a rapid rate of freezing. In a frozen mass of egg cells suspended in sea water the cells could tolerate a rapid cooling rate of at least 20° C/min without any formation of intracellular ice. The media for the egg cell suspension were: 0·54 M potassium nitrate; 0·54 M potassium chloride; 0·54 M sodium nitrate; 0·54 M sodium chloride; 0·38 M magnesium chloride and 0·34 M calcium chloride solutions. None of these media seems to exert any harmful effect on the egg cells at least for 1 h at 15° C. When transferred into normal sea-water from these media nearly all the egg cells could cleave normally after insemination.

Test-tubes containing 0·5 ml. of egg suspension were cooled in special cooling baths in which brine stratified well from top to bottom according to the temperature. The temperature of the egg cell suspension was measured with a fine thermocouple inserted in the suspension, and was recorded automatically. The egg cell suspension in each test-tube was first cooled in the coldest bottom layer of brine. When the suspension was cooled to a desired temperature higher than the temperature of bottom layer, the test-tube was pulled up to the upper brine layer which had been kept at the same desired temperature. In this way the cell suspensions were cooled to various planned temperatures from −2·5° C to −35° C within about 3 min and kept at the same temperatures for 1 min. Each test-tube was then rapidly transferred into the water at 20° C. When the egg cell suspension was planned to be cooled to a temperature higher than −10° C, it was seeded in each test-tube with small ice-crystals when it was cooled to about −2° C. Egg cells frozen and thawed in this way were observed under a microscope for counting the intact cells. The results of the experiment are shown in Table 1 in which the percentage survival is based on the number of cells capable of resuming development after insemination.

Table 1 shows clearly that at least in solutions of single potassium salts all the suspended cells suffer fatal injury within only 1 min at a freezing temperature just below the eutectic point of the surrounding solution. In the solutions of sodium salts the suspended cells are rather resistant to freezing. They promptly die, however, at a freezing temperature about 10° C below the eutectic point. Freezing at temperatures below −35° C seems to be fatal to the suspended cells regardless of the kind of medium. When 1 M sucrose solution was used as the medium instead of single-salt solutions, all the suspended egg cells were killed at a freezing temperature of −20° C within only 1 min, while the eutectic point of pure sucrose solution was −13·9° C. In sea-water with added sucrose, on the other hand, about 70 per cent or more egg cells could survive freezing at −20° C at least for 1 min. These observations suggest that some very drastic changes promptly take place in the freezing egg cells when they have been cooled to a dangerous temperature below the eutectic point of the surrounding medium. The freezing process of the egg cells suspended in some of these media was, therefore, observed in a special cold box.

A small amount of egg suspension, covered with silicone oil, was cooled on the stage of a remote-control inverted microscope with special heating devices. The tip of a fine thermocouple was inserted in the egg suspension for measuring the temperature. When the egg suspension was cooled to −4° C it was seeded by means of an ice-tipped pipette. In the egg cells frozen in this way a remarkable change was found, especially in the solutions of potassium salts. When the frozen suspension was cooled to a temperature slightly below the eutectic point of the medium, fine crystals of the potassium salt suddenly appeared as many pine-needle-cluster-like crystals on the surface of homogeneous ice (Fig. 1). In such a case, if the frozen suspension was re-warmed to a temperature above the eutectic point, these salt crystals rapidly disappeared leaving underlying ice crystals covered with the concentrated salt solution. Such salt crystallization at the eutectic point could be repeated many times by fluctuating the temperature of the frozen suspension. When one of such salt crystals grew to the surface of an egg cell, a pronounced darkening rapidly spread from the spot touched by the salt crystal to the whole cell. There is no doubt that in this instant a flashing[2] or an intracellular formation of very fine ice crystals occurred in the cell. In the

Table 1. PERCENTAGE SURVIVAL AFTER A SHORT PERIOD OF FREEZING IN THE EGG CELLS IN VARIOUS SINGLE SALT SOLUTIONS

Freezing temperature (°C)	KNO_3	KCl	$NaNO_3$	NaCl	$MgCl_2$	$CaCl_2$	Sea water
− 2·5	90						
− 5	0	85					
− 10	0	90					
− 15		0	100				
− 20		0	96	100	100		100
− 25			29	90	100	90	83
− 30			0	3	80	60	40
− 35				0	0·5	0	0
Eutectic point (°C)	− 2·9	− 11·1	− 18·5	− 21·8	− 33·6	− 54·9	—

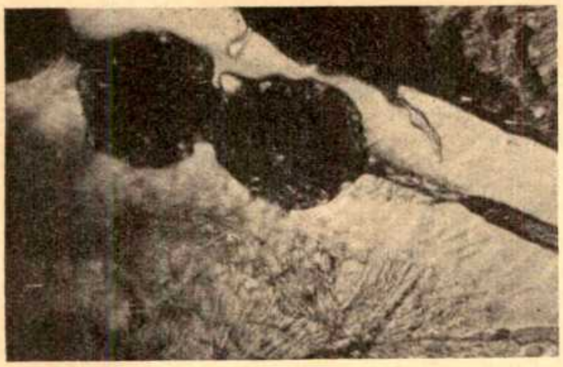

Fig. 1. Intracellularly frozen egg cells in potassium nitrate solution at the eutectic point. Fibre-like potassium nitrate crystals can be seen below on the underlying ice mass (× 180)

cells frozen with sodium chloride or magnesium chloride solution, the occurrence of an intracellular freezing at a definite low temperature was not clear; but in the process of re-warming from the critical temperature shown in Table 1 the yellow colour of the egg cells invariably turned to dark brown, showing a denaturation of the cytoplasm.

From the results described here, it is reasonable to suppose that at the eutectic point of the suspending medium or at least at a definite temperature below that point the egg cell is promptly damaged. Such damage seems to occur as a result of a destruction of protoplasmic structure of cell surface, perhaps by a very rapid change in the interactions between protoplasm surface and surrounding water, because a complete removal of water from the contacting medium suddenly occurs at the time of the formation of cryohydrate. The fact that in solutions of any single solute other than potassium salt the suspended egg cells are killed at a freezing temperature lower than the eutectic point may be explained as a consequence of easy attainment of metastable state in these solutions. This seems reasonable because in these solutions the apparent crystallizing out of the solute was scarcely observed at least at about their eutectic temperatures. The damage at the cell surface may certainly cause a decrease in the capacity of living protoplasmic membrane to serve as a barrier against ice inoculation into the cell[3]. Therefore, if freezing is rapid, an ice seeding from the injured spot into the cell may result, especially in the cells suspended in potassium salt solutions in which the eutectic temperature is high, due to which fact the cells have still a lot of easily freezable water within them. In the case of freezing cells suspended in calcium chloride solution another cause of frost injury should be considered, because the eutectic point is far lower than their danger-point temperature.

In any event, the process of occurrence of frost damage reported here is very characteristic in instantly occurring on a cell at a definite low temperature according to the kind of the suspending solution. In this respect the nature of this type of frost damage seems to be different from the hitherto known effect of increased salt concentration at the freezing[4], which is also found in freezing sea urchin egg cells even at a high sub-zero temperature if the freezing is prolonged.

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MEIOSIS IN OVARIAN OOCYTES OF ADULT MAMMALS

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MANY of the chromosomal anomalies in man and animals arise through non-disjunction or lagging chromosomes during meiosis in the oocyte. Investigations of the origin and primary incidence of such anomalies would be greatly facilitated if meiotic stages in the mammalian oocyte were easily available. Unfortunately, this has not been the case. Oocytes undergo the prophase stages of meiosis until diplotene just before or shortly after birth in the majority of mammalian species[1,2]. DNA synthesis occurs in the oocytes before birth, no further synthesis occurring in the oocyte until after fertilization[3]. The total number of oocytes per female is thus determined at birth in most species, no oocytes being formed thereafter. After diplotene, oocytes enter the dictyate stage and remain nucleated for the long period from diplotene until shortly before ovulation. In most mammalian species the stages of meiosis between diakinesis and the metaphase of the second maturation division occur in the brief period between the stimulation of the Graafian follicle by luteinizing hormone and ovulation a few hours later. Anaphase and telophase of the second meiotic division occur after penetration of the egg by the fertilizing spermatozoon. Thus, to find the early stages of meiosis, oocytes must be taken from late fœtuses or newly-born animals, for example, mouse and man[4]. The later stages of meiosis will be found in oocytes taken from adult ovaries.

Much fundamental information on non-disjunction, lagging chromosomes, crossing-over, and associations between normal and structurally altered chromosomes will be provided by studies on meiotic stages after diplotene. The present article is primarily concerned with methods designed to obtain these stages. Oocytes which are naturally in diakinesis or later stages of their meiotic divisions can be recovered from the ovaries just prior to ovulation[5,6]. But the number of such oocytes recovered is meagre, and the stage of meiosis can vary widely between different animals. Under appropriate endocrinological conditions the dictyate stage in oocytes of adult mammals can be terminated, and the resumption of meiosis can be induced. The exact stage of meiosis at a particular time can often be predicted with considerable accuracy.

In the rabbit, an injection of a luteinizing hormone or the coital stimulus initiates the resumption of meiosis, and ovulation occurs 8–9 h later[7]. In man and other species, a single or multiple injection of a luteinizing hormone, with or without previous follicular priming by a follicle-stimulating hormone, induces the maturation of many oocytes and ovulation. Hormonal treatments leading to the resumption of meiosis or to ovulation are known in the mouse[8,9], rat[8,10], cow[11], monkey[12], man[13], and many other species. The timing of each stage is very precise in the rabbit[7] and the mouse[14], and any stage of meiosis between diakinesis and the second metaphase can be obtained with precision. Such timing could presumably be extended to other species, for example, pig and sheep[15]. Metaphase of the second maturation division should be especially vulnerable for study, since in the majority of mammalian species the oocyte remains in this stage for some hours after ovulation. Anaphase and telophase of the second division will be more difficult to obtain, since they occur rapidly after sperm penetration, and anomalies at these stages could perhaps best be inferred from the examination of chromosomes at the first cleavage.

In the investigation of meiosis in human oocytes, such endocrinological techniques are obviously difficult to apply. Fortunately, there is an alternative technique which promises to supersede the endocrinological control of ovulation in most species. This is to stimulate the oocytes directly *in vitro* after their liberation from the follicle. In addition to being a most attractive technique in that it avoids the use of hormone injections *in vivo* and permits the use of small pieces of ovary, it has also proved to be an unusually simple technique when used in several species. All that is necessary is to liberate the oocytes into a simple culture medium by puncturing the follicles; many oocytes then immediately resume their maturation. Pincus and Enzmann[7] were among the first to use this technique in the rabbit. Pincus and Saunders[16] extended this work to human ovarian oocytes, and claimed that approximately 50 per cent of the oocytes displayed meiotic chromosomes or first polar bodies. Chang's[17] detailed analysis of the maturation of rabbit oocytes *in vitro* is perhaps the most extensive report. We have induced large numbers of mouse, rat and hamster oocytes to resume their maturation in this manner (Table 1). Pituitary hormones are clearly unnecessary to provide the stimulus to the oocyte; indeed, mouse oocytes resume meiosis when placed in 0·85 per cent saline or when taken from pregnant or non-pregnant animals. An inhibitory influence within the follicle rather than a factor in the oocyte evidently causes the persistence of the dictyate stage, and rupture of the follicle releases the oocyte from the inhibitor. If oocytes within their follicles are cultured *in vitro*, no evidence of maturation is found (Table 1). Mouse oocytes liberated from their follicles are excellent material for squash preparations, many of them having few or no cumulus cells attached. The rate of maturation of oocytes from the rabbit[17] and mouse is approximately the same *in vitro* and *in vivo*. Care is necessary in evaluating the stage of meiosis, for many oocytes are in metaphase of the second maturation division when liberated from their follicles (Table 1). These oocytes are usually free from corona cells, and are presumably atretic[1,18]. Although the ooplasm of rabbit oocytes matured *in vitro* differs in some respects from those stimulated *in vivo*, many of the former are capable of being fertilized after transfer to the Fallopian tubes of recipient rabbits[17]. Squash preparations of two stages in the maturation of mouse oocytes *in vitro* are shown in Figs. 1 and 2.

Unfortunately, human, baboon, monkey and dog oocytes have not responded, in our hands, as well as those of rabbits and rodents after liberation from their follicles. No chromosomes were found in 48 dog oocytes after culture for up to 12 h. It must be stressed, however, that the dog oocyte is one of the exceptions among mammalian oocytes in that its meiotic divisions are resumed after ovulation[1]. In *Cynus* monkeys 9 + 1? out of 56 living or squashed ovarian oocytes possessed chromosomes after 4–12 h in culture (Table 2). The three oocytes which were squashed appeared to be in metaphase of the second maturation division. A baboon autopsied near the time of ovulation[19] yielded 90 ovarian oocytes. They were cultured for periods up to 24 h. Altogether, 13 oocytes possessed meiotic chromosomes of the second

Table 1. PROPORTION OF MOUSE, RAT AND HAMSTER OVARIAN OOCYTES WITH CHROMOSOMES OR POLAR BODIES AFTER 6–8 H IN CULTURE

		Mouse				Rat	Hamster
Condition of animals		In œstrous cycle			2·5–8 days pregnant	In œstrous cycle	In œstrous cycle
No. of animals		10			4	3	2
Type of oocyte		With corona cells	Without corona cells	In follicle	With corona cells	With corona cells	With corona cells
Proportion of oocytes with chromosomes or polar bodies	At recovery	1/52	13*/30	0/7	0/18	0/15	0/12
	After 6–8 h in culture	68/79	29/42	0/14	27/39	19/23	11/13

*At least 11 of these oocytes were in metaphase of the second meiotic division.

Table 2. PROPORTION OF *Cynus* MONKEY AND BABOON OVARIAN OOCYTES WITH CHROMOSOMES OR POLAR BODIES AFTER VARIOUS PERIODS IN CULTURE

Species	No.	Stage of menstrual cycle	Period of culture (h)				
			0 (controls)	4–6	12	21–24	Total 4–24
Cynus	1	No cycle. Offspring weaned one week previously. Follicular. (No corpus luteum)	0/7	1/5	4/13	—	5/18
	2	Follicular phase (no corpus luteum)	0/8	0/3	2/15	—	2/18
	3	Fresh corpus luteum	0/5	1/1	1+1 ?/19	—	2+1 ?/20
Baboon	1	Recently ovulated	3/11	1/10	1/12	8/57	10/79
	2	Follicular	0/21	1/9	—	2/19	3/28
	3	Follicular	0/6	1/11	0/12	0/18	1/41
	4	Immature	2/19	0/11	—	0/17	0/28

The stage of the menstrual cycle in *Cynus* monkeys was determined from examination of the ovaries; baboons from the condition of the sex skin.

maturation division, the chromosomes appearing to be somewhat degenerate (Fig. 3). Meiosis had evidently not been induced by liberation of the oocytes from the follicles and subsequent culture, since the 13 oocytes occurred randomly in the control and culture groups (Table 2). Moreover, these oocytes had few or no corona cells attached, and at least seven of them had a polar body. These oocytes had evidently been stimulated *in vivo* before removal of the ovary, and had probably been liberated from atretic follicles. The great majority of oocytes from four baboons, one in the luteal phase, two in the follicular phase, and the fourth immature, were all nucleated at recovery or after culture for up to 24 h. Seventy-six human eggs were cultured for periods up to 20 h. Three of them appeared to contain chromosomes and two a polar body, as judged by phase-contrast microscopy of 58 living eggs, although none of the 18 that were squashed had either chromosomes or polar body. Chromosomes could not be seen in the oocytes containing polar bodies, which indicates that the 'polar bodies' were probably cytoplasmic extrusions from degenerating eggs. A difficulty with ovarian oocytes from many species is that the corona cells adhere very tightly to the oocytes. Hyaluronidase fails to remove these cells, although Shettles[20] removes them from human oocytes by culturing small pieces of the Fallopian tube with the oocytes.

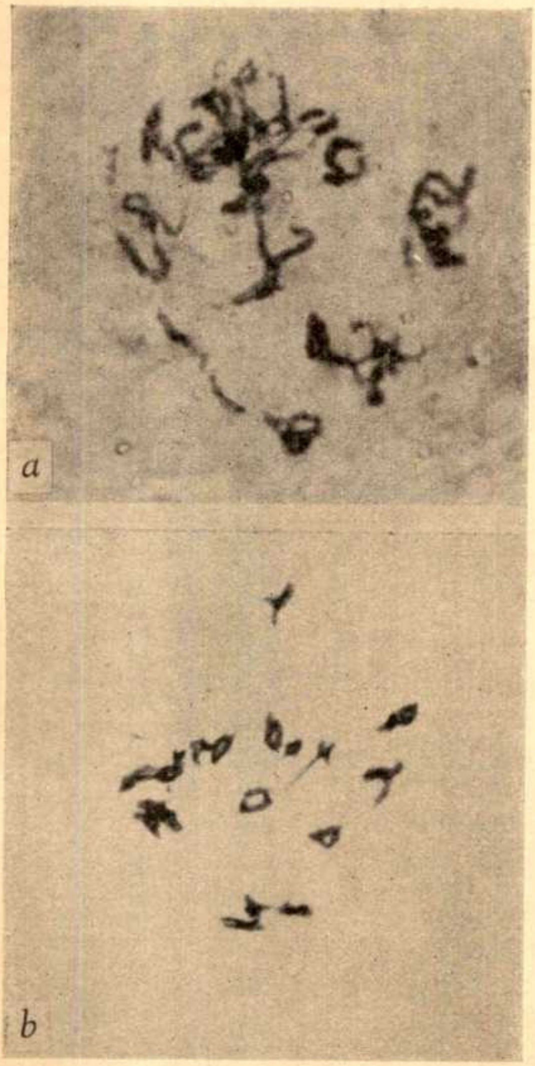

Fig. 1. Prophase of meiosis in mouse oocytes. *a*, Late diplotene, in the only oocyte so far found in this stage, after culture for 2 h (× 1,510); *b*, diakinesis-early metaphase in an oocyte cultured for 2¼ h (× 1,500)

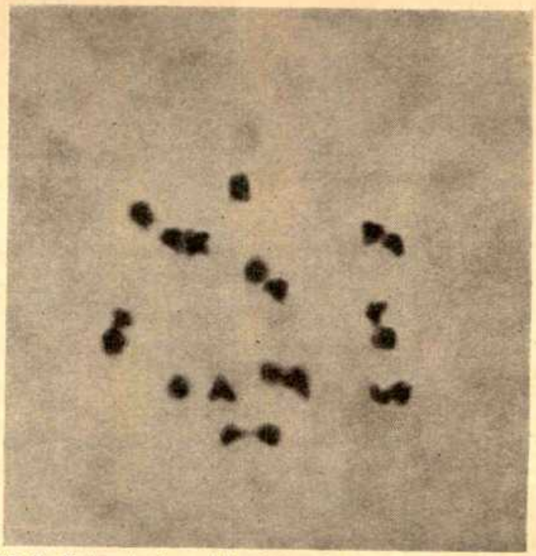

Fig. 2. Late metaphase of the first meiotic division in an oocyte cultured for 6¾ h. Colcemid (0·5 μg/ml.) added to medium during last 30 min (× 1,280)

Because the incidence of stimulated human eggs is so low it is clearly possible that the three human oocytes with chromosomes were stimulated *in vivo* before removal of the ovary. Shettles[20] observed tetrads in human ovarian oocytes in culture, but the inference from his wording is that the proportion of such oocytes was also low. Our results conflict with those of Pincus and Saunders[16]. We suspect that the oocytes classified by them as "with polar body" were either degenerate or had been stimulated *in vivo*, for they found some of these after only one or two hours in culture. Such a brief interval between dictyate and polar body extrusion appears to be improbable. Failure to stimulate the human oocyte *in vitro* might be one of the factors involved in the low incidence of fertilization *in vitro* using ovarian oocytes[21], for rabbit ovarian oocytes are fertilizable only after maturation has occurred[17,22].

The results from the baboons also indicate that the oocytes with chromosomes had been stimulated *in vivo*. On the other hand, the ratio in the *Cynus* monkeys seems to be sufficiently high to indicate some stimulation *in vitro*, although the possibility of atresia cannot be ruled out since no oocytes were seen to be actually in their first maturation division. The prospects of obtaining primate meiotic stages in the oocyte by this method thus appear to be slight. However, since oocytes from several species matured *in vitro* so easily, it might not prove too difficult to activate human and other primate oocytes. Neither the addition of gonadotrophins, steroid hormones or phytohæmagglutinin to the culture medium, nor the use of widely differing culture media, for example, various mixtures of medium 199, Gey's saline, fœtal calf serum, autologous (not human) and homologous serum, has so far induced the resumption of meiosis in human, monkey or baboon oocytes. Most of the human ovaries used in the work reported here were taken from women near to their menopause or from

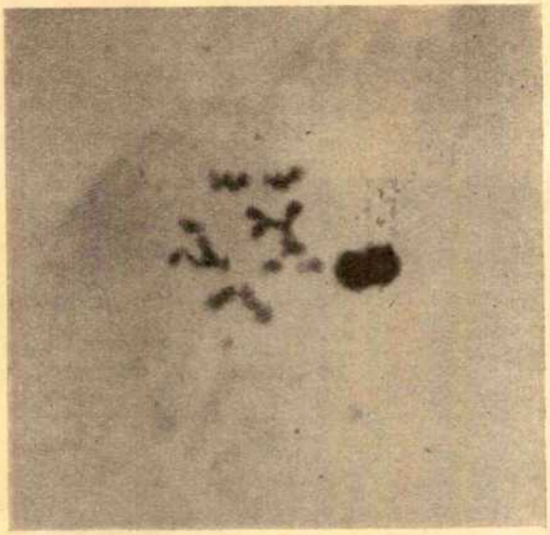

Fig. 3. Chromosomes in a baboon oocyte after 24 h in culture (× 1,050)

patients with gynæcological disorders, and there was a delay of up to 1 h before the oocytes were liberated from the follicles. Oocytes from the ovaries of younger women removed near the time of ovulation might yield meiotic figures as did those from the baboon, provided that some follicular atresia also occurs in man at ovulation[1,19]; but the value of such atretic oocytes in the analysis of chromosome behaviour is debatable.

Alternative techniques may be necessary to obtain meiotic stages in human ovarian oocytes. One alternative method is to cannulate the ovarian artery in patients undergoing surgery, and then perfuse the ovary with hormones after removal. Such a direct endocrinological stimulus in the absence of pituitary secretions might induce maturation of the oocytes in the excised ovary; in the intact mouse, for example, a single intraperitoneal injection of human chorionic gonadotrophin in various stages of the œstrous cycle or pregnancy will induce ovulation *in vivo*[23]. Moreover, many oocytes in secondary follicles, in addition to those in Graafian follicles, are found in meiosis[24]. So far, however, we have found no meiotic stages in human ovarian oocytes after the excised ovaries had been perfused for 4 h with H.C.G.[25] though the perfusion technique was not good in that the arteries were cannulated after removal of the ovaries. It may be necessary to perfuse a priming hormone in man, since luteinizing hormones alone evidently fail to induce ovulation[13]. Another alternative technique for stimulating the ovarian oocyte is to inject hormone directly into the follicle. Moricard[26] claims that this technique is successful in the rabbit. One suspects, however, that puncture of the follicle has released or diluted the inhibitor discussed above[17], and that the hormones played little or no part in stimulating the oocytes.

Some preliminary observations have been made in the present work. Large numbers of oocytes can be recovered from mammals; we obtained 38 from one human ovary, and 90 from both ovaries of a baboon. In living mouse oocytes, a close association can be seen in early metaphase between the nucleolus and some of the meiotic chromosomes[14]. Elucidation of these associations might yield information on the relationship between trisomy and the chromosomes with nucleolus organizers[27]. No evidence has so far been seen of precocious terminalization of chiasmata during the dictyate stage in the mouse; all 25 oocytes examined in detail have possessed 20 tetrads (see also refs. 6 and 8). We have observed the result of secondary non-disjunction in mouse oocytes. Three secondary oocytes were recovered after induced ovulation from a mouse classified genetically as *XO*. One oocyte possessed only 19 chromosomes (Fig. 4), the others possessing 20 ($2n=40$). This observation should be considered *sub judice*, since the mitotic complement of the donor female was not analysed and can only be inferred from the genetic evidence and from previous cytological examination of similar types of mouse[28]. It would obviously be of great

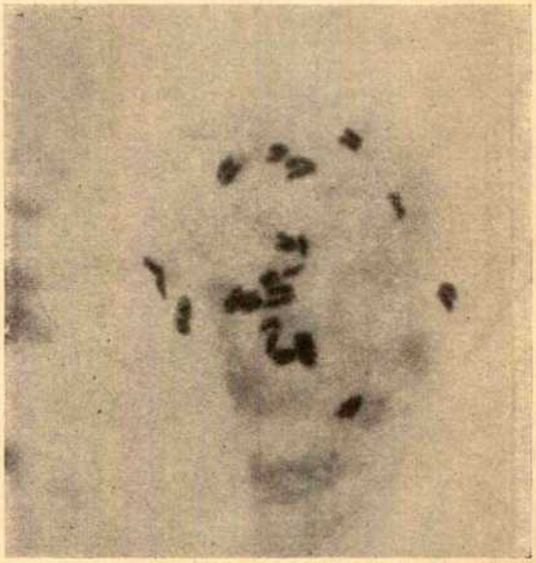

Fig. 4. Nineteen chromosomes after secondary non-disjunction in a secondary oocyte of an *XO* mouse. Ovulation induced by gonadotrophins. Mouse injected with 0·5 ml. of 'Colcemid' (1/50,000 w/v) 2 h before autopsy; cumulus cells removed by hyaluronidase (× 1,500)

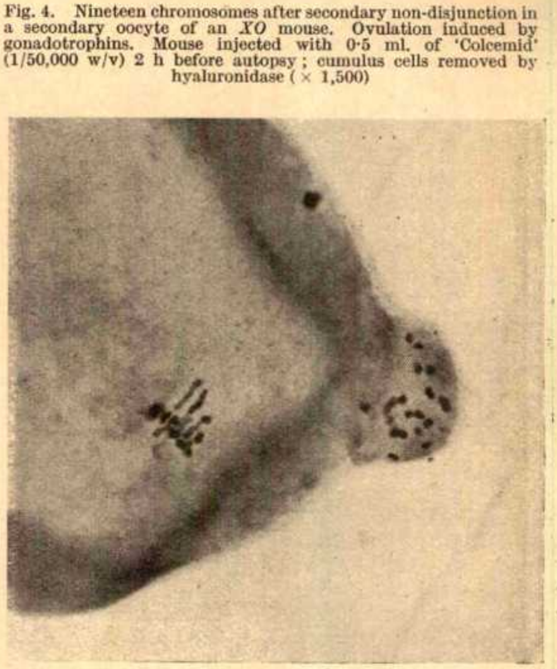

Fig. 5. Chromosomes in the egg and in the first polar body of a mouse oocyte. Treatment as Fig. 4. Light squashing pressure (× 720)

interest to study the behaviour of this chromosome during the first meiotic division. In many oocytes, the meiotic chromosomes in the first polar body persist for a length of time and can be seen clearly (Fig. 5), despite the smallness of this polar body. Direct studies of the reciprocal products of the first meiotic division should be feasible. The clear separation of the chromosomes in the first polar body may be due to the absence of a spindle in many of them.

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GLUTAMIC AND ALANINE DEHYDROGENASE DETERMINED BY ONE GENE IN *NEUROSPORA CRASSA*

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IT has been shown that crystalline beef L-glutamic dehydrogenase possesses L-alanine dehydrogenase activity[1,2]. Experiments are reported here which show that the triphosphopyridine nucleotide (TPN)-specific glutamic dehydrogenase of *Neurospora crassa*, which is determined by a single gene[3], also has alanine dehydrogenase activity. The two reactions catalysed by the enzyme are:

$$\text{L-glutamate} + TPN^{+} + H_2O \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^{+} + TPNH + H^{+}$$

and

$$\text{L-alanine} + TPN^{+} + H_2O \rightleftharpoons \text{pyruvate} + NH_4^{+} + TPNH + H^{+}$$

All the experiments described in this communication refer to the TPN-specific glutamic dehydrogenase and not the DPN-linked enzyme that also occurs in *Neurospora*.

Amination deficient, *am*, mutants of *N. crassa* lack the TPN-specific glutamic dehydrogenase which can be demonstrated in wild-type, am^{+}, strains. Cell-free extracts of am^{+} and *am* strains were assayed for both alanine and glutamic dehydrogenase activity. The enzyme activity was measured by following the oxidation of reduced TPN (TPNH), in the presence of appropriate substrates, by optical density measurements at 340 mμ. A series of results obtained from such enzyme assays is given in Table 1. The results show that wild-type *N. crassa* possesses both glutamic and alanine dehydrogenase. The *am* strains do not possess either normal glutamic or alanine dehydrogenase activity since their specific activities (Table 1) are not significantly different from zero.

Table 1

Strain	Specific glutamic dehydrogenase activity	Specific alanine dehydrogenase activity
am+	2,176	200
am-1	11	9
am-2	8	6
am-3	22	4

The unit of specific enzyme activity is: change in optical density at 340 mμ/min × 10^3/mg protein.

In addition the alanine dehydrogenase activity of cell free extracts of *am* and am^{+} strains was measured by their ability to synthesize alanine when incubated with pyruvate, ammonia and TPNH. The presence of alanine in the reaction mixtures was shown by paper chromatography. Incubation of am^{+} extracts with the complete system resulted in the formation of alanine; alanine was not formed if any one of the substrates was omitted. Incubation of *am* extracts with the complete system did not result in the formation of alanine. Since the *am* mutants are known to be the result of mutation in a single gene locus[3] it is evident that this locus also determines the production of alanine dehydrogenase in *N. crassa*.

The glutamic dehydrogenase of an am^{+} strain of *N. crassa* was purified by the method given by Fincham[4]. The method consists essentially of ammonium sulphate fractionation, adsorption on calcium phosphate gel and finally fractionation on a diethylaminoethyl cellulose (DEAE) column. During the final stage, 5-ml. fractions were collected from the DEAE column. Each of the fractions was assayed for glutamic and alanine dehydrogenase activity and the optical density at 280 mμ taken as a measure of the protein concentration. The results of the enzyme

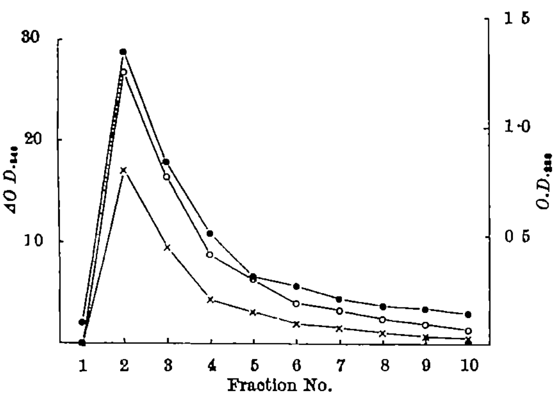

Fig. 1. DEAE chromatography of glutamic and alanine dehydrogenase. After initial purification 875 ml of enzyme extract in 0·02 M phosphate buffer pH 7·4 containing 0·001 M EDTA was applied to a 3-cm column of diethylaminoethyl cellulose. The column was eluted with 0·05 M phosphate buffer pH 7·4 and 5 ml. fractions collected.

The standard substrate mixture for glutamic dehydrogenase assay by glutamate deamination was 0·066 M sodium L-glutamate, $1·7 \times 10^{-4}$ M TPN and 0·1 M pH 8·3 *tris*-HCl buffer. The standard substrate mixture for alanine dehydrogenase assay by pyruvate amination was 0·008 M sodium pyruvate, 0·04 M ammonium chloride, $2·0 \times 10^{-4}$ M TPNH, and 0·1 M pH 8·6 *tris*-HCl buffer. Glutamate deamination (○) is expressed as $\Delta O.D._{340}$/min/μl. of column effluent. Pyruvate amination (×) is expressed as $\Delta O.D._{340}$/min/10 μl. of column effluent. (●) shows protein concentration as measured by $O.D._{280}$

assays of the various fractions are shown in Fig. 1. The ratio of the two specific enzyme activities is similar in all the fractions. The parallel behaviour of the two enzymes during DEAE chromatography is strong evidence that the same protein molecule is responsible for both glutamic and alanine dehydrogenase activity in *N. crassa*.

The best enzyme fractions obtained after DEAE cellulose column chromatography showed a 30–40-fold increase in specific activity compared with the initial cell-free extracts. They were used for a preliminary investigation of the properties of the alanine dehydrogenase. The enzyme is TPN specific and has a pH optimum of 8·8 in 0·1 M *tris*-HCl buffer at 35° C with respect to pyruvate amination. The Michaelis constants for the various components of the pyruvate amination reaction were estimated at pH 8·8 in 0·1 M *tris*-HCl buffer at 35° C. They were 29 mM for NH_4^+; 14 mM for pyruvate and 14 μM for TPNH. A comparison of the maximum rate of the α-ketoglutarate amination at pH 8·3 with the maximum rate of pyruvate amination at pH 8·8 showed that pyruvate was aminated about 3 per cent as rapidly as α-ketoglutarate by the enzyme. The incubation of the enzyme with L-alanine and TPN resulted in the reduction of the TPN as shown by an increase in optical density at 340 mμ. The maximum rate of L-alanine oxidation was about 0·4 per cent of the maximum rate of glutamate oxidation.

The current state of knowledge concerning the determination of protein specificity suggests that a single structural gene determines the specificity of a single polypeptide chain. The amination deficient mutants are known to be due to mutation within such a single structural gene, the *am* locus. Therefore the fact that the *am* mutants are deficient in both glutamic and alanine dehydrogenase activities suggests that a single protein mediates both catalytic activities. The parallel behaviour of both enzyme activities on purification also indicates that a single protein is involved. Therefore on the basis of both lines of evidence it is concluded that in *N. crassa* a single protein, determined by a single gene, is responsible for both TPN linked L-glutamic dehydrogenase and TPN linked L-alanine dehydrogenase.

The most thoroughly investigated glutamic dehydrogenase is the crystalline enzyme from beef liver[1,2,5]. The *N. crassa* glutamic dehydrogenase is like the beef enzyme in that the same protein shows both glutamic and alanine dehydrogenase activities to a similar degree and at a similar pH. In beef liver the two enzyme activities depend on the degree of association of the protein which is affected by certain agents, notably some steroid hormones[1,4]. The glutamic dehydrogenase of *N. crassa* probably consists of a number of polypeptide units, but the molecular weight is about 200,000 compared with about 1,000,000 for the beef enzyme[1,4]. It will be of interest to see if any of the agents mentioned differentiate between the two enzyme activities of the *Neurospora* protein.

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USE OF THE TENTACLES, SWIMMING AND BUOYANCY CONTROL IN THE PEARLY NAUTILUS

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THE pearly nautilus has been an object of interest to zoologists since the descriptions of the eighteenth-century naturalist Rumphius[1] and of Owen[2]. The only accounts of the living animal apart from those of Rumphius were given by Bashford Dean[3] and Willey[4]: all three observers were restricted to observations made, either from a canoe, or on a dying animal in a pail or small tank.

During a recent visit to the West Pacific under a grant from the Nuffield Foundation, I was able to make some relevant observations on living *Nautilus*. Most of these were made on *Nautilus macromphalus* Sowerby, while I enjoyed for three months the hospitality of Dr. Catala-Stucki's aquarium at Noumea in New Caledonia, and valuable observations were also made on a specimen of *Nautilus pompilius* L. immediately after it was brought to the surface from about 100 fathoms in Rabaul, New Britain, where I received assistance from the Department of Agriculture, Stock and Fisheries of the Australian Government.

In Noumea, *Nautilus* was caught on the outside of the Barrier Reef, in 60–100 m, in traps of lobster-pot type, baited with dead fish and set on the bottom. The laborious work of setting and hauling up the traps was carried out by Madame Catala-Stucki and Dr. L. Merlet. Thanks to their efforts, about 40 animals were made available, many of which lived for up to 8 weeks in the aquarium. The repeated observations thus made possible produced a mass of interesting information out of which three topics are here selected: the use of the tentacles, the method of swimming, and the buoyancy-control.

The Tentacles

Since Owen's[2] classical description of *Nautilus pompilius* L., it has been known that the pearly nautilus has numerous tentacles which can be divided into 3 groups: (1) pre- and post-optic tentacles, one in front of and one behind each eye; (2) 19 pairs of digital tentacles and, within these, (3) a close-set circle of numerous buccal tentacles (the 'labials' of Owen) which, as their name implies, surround the powerful beak and buccal mass, and some of which are modified, in both the male and the female, to form secondary sexual organs.

Each of the optic and digital tentacles can be completely retracted into a muscular sheath; the buccal tentacles are carried on two lateral and one ventral pad, and are less fully retractile[4]. The tentacles carry no suckers or hooks, but are ridged, particularly on the inner side, and the ridges of both digital and buccal tentacles are adhesive: Willey[4] describes adhesive powers so strong that a tentacle attached to an object can be torn from its base and remain attached to the object.

From observations made on the animals at rest, swimming, and feeding, it emerged that there were not only marked differences in the reactions of the three types of tentacles already enumerated, but also that three groups of digital tentacles could also be distinguished by the different circumstances in which they were extended.

When the animal was at rest or quietly swimming, all tentacles were withdrawn into their sheaths, although the tips were sometimes visible. When the animal was in some way alerted, the pre- and post-optic tentacles and the four most lateral digital tentacles on each side might be extended, but there was considerable variation in the degree of extension and in the number of tentacles involved. The pre-optic and the second and third lateral digitals appeared to be more readily extended than either the post-optic or the dorsal and ventral lateral pairs.

When food (lumps of fish or crab) was brought within a certain distance, a further group of median lateral digitals, together with any of the four pairs of outer laterals not already extended, would be simultaneously thrust out to form a spreading 'cone of search' and would be held, so extended, while the animal circled round, forwards or backwards, until the food was found. The cone of search might also be formed when the animal was hungry and searching for food, or when it was exploring a new environment.

As soon as a tentacle of the cone of search touched food, an inner ring of digital tentacles, consisting of the innermost lateral and the dorsal digitals, would be extended, grasp the food and draw it under the hood, while the tentacles of search generally let go of the food. The buccal tentacles were rarely seen extending beyond the hood, but apparently held the food while the beak was biting. The three groups of digital tentacles may thus be differentiated functionally into an 'alert', a 'search' and a 'feeding' group.

A pre-optic tentacle and an outer lateral have each been drawn out by a piece of food held near, but not touching, the tentacles, showing a direct chemosensory response; the post-optics were never induced to extend in this way. The inner ring of digitals seem never to be extended during the search for food, but only when food is touched; they can also be stimulated to extend by lightly brushing the front of the hood. When an animal was held by the shell, under water, the optics and all the digitals would quickly be extended, but still in the order already described: first the pre- and post-optics and the outer laterals, then the median laterals and lastly the inner ring of laterals and dorsals. During the day the animal would often rest, attached by some of the tentacles to the glass of the tank. Sometimes only two or three tentacles were used for this, sometimes a dozen. Despite the functional differentiation already described, any combination of digital tentacles might be used for attachment, which was sometimes at the tip, sometimes by a point mid-way along the length, and which sometimes involved only one ridge, sometimes a number.

The most striking feature of a nautilus swimming with extended tentacles, whether the 'alert' or 'search' group, was the constant angle at which the tentacles were held, regardless of the direction of movement. This was well described by Moseley[5] in his account of the single living *Nautilus* dredged up during the *Challenger* Expedition.

The animals were never seen to use the tentacles in creeping or crawling, either along the bottom or over the glass of the tank.

Swimming and Respiratory Movements

Nautilus is a swimmer, and swims by jet-propulsion; but the method of propulsion differs from that of other living cephalopods (Coleoidea). In these, the shell is internal and reduced, or totally lost, and the mantle has become strongly muscular; its contractions drive the water from the mantle-cavity through the funnel, the narrow dimensions of which produce the jet. The funnel is passive in this propulsion; its muscles serve largely to anchor it against the force of the jet: it is also capable of turning the jet in various directions. In *Nautilus*, the shell is external, the mantle lines and secretes it, is only slightly muscular, and plays no part in locomotion, the muscular force for which is provided by the funnel alone. The funnel is not a tube, but a triangular muscular sheet, massively muscular in the middle, thinner at the margins, which roll over and overlap at the narrow anterior end; this can project beyond the margin of the shell, and forms, effectively, a tube. Within the mantle-cavity, the two halves of the funnel diverge so as to include the gills and only overlap when contracted together. Within the tip of the tubular portion is a valve, resembling that of the squids and cuttlefishes (Decapoda).

The funnel valve was first described by Rumphius[1] and the non-muscular character of the mantle by Owen[2], but Owen regarded the funnel simply as a vent for waste-products. Valenciennes[6] was the first to recognize that *Nautilus* is a jet-swimmer, and to perceive that the funnel was an organ of propulsion and that the ventral wrap-over, in contradistinction to the closed tube of the Coleoidea, played a vital part in this distinctive habit of funnel jet-propulsion. This intelligent reconstruction appears to have been overlooked or forgotten, and Griffin[7] thought the funnel insufficiently muscular to be used in vigorous swimming and postulated withdrawal of the head into the shell as the propulsive force, a view which has since been frequently quoted. Moseley[5], Bashford Dean[3] and Willey[4] all actually observed *Nautilus* swimming, and implied that it swam by jet-propulsion, but none of them, not even Willey, who described and figured the funnel, gave consideration to the mechanism involved.

In captivity, *Nautilus* swims chiefly at night and spends most of the day at rest on the bottom, poised on the outer curve of the living chamber with the tip of the funnel projecting 1–2 cm beyond the rim of the shell. The respiratory jet may be sufficient to rock the animal gently, or it may be so slight that there is no detectable movement except that of the posterior wings of the funnel. These may be seen flapping gently just inside the inhalent aperture, that is, the posterior angle of the shell aperture, close to the umbilicus of the shell on each side. The resting animal is partly withdrawn into the shell, with the pupil of the eye just clear of the optic notch, and only withdraws completely when handled or otherwise disturbed. When an animal hangs on to the glass of the tank, it is often possible to look straight down the funnel and watch the two pairs of gills waving to and fro in the respiratory current.

From time to time during the day an animal becomes restless and swims about until a new resting-place is found. When the animal is swimming the head is held clear of the shell; the funnel projects freely below it. Normally the animal swims backwards with the curve of the shell leading, but the tip of the funnel is very flexible, and can direct the jet so that the animal can turn about and swim in any direction. In slow swimming, the rhythmic ejections of water by the funnel are reflected in an undulating swimming movement which largely disappears when the animal swims fast.

The roll-over of the flap can change, in the same animal, from right-over-left, to left-over-right; the actual change was not observed. The funnel-valve could occasionally be seen, but always pressed against the dorsal wall of the funnel; it was never observed to project across the lumen, either at rest or in swimming Its function is thus a matter for conjecture : it may play a part in the rapid swimming which I have not seen.

A peculiar feature of the jet-propulsion of a cephalopod with an external shell is due to the fact that, when the animal is swimming with the head held steady, the capacity of the mantle cavity is constant, unlike the situation in, say, *Sepia*, in which the muscular mantle drives out water by contraction and thus diminishes the mantle-cavity's capacity. Inhalation takes place as the mantle expands again. In *Nautilus*, it is the internal volume of the funnel which changes : when the two halves of the funnel separate, water is drawn into the funnel from the surrounding mantle-cavity; when they come together and expel this water through the funnel tip, the water expelled must be simultaneously replaced and is drawn into the mantle-cavity, as already described, through the posterior angle of the shell. Expiration and inhalation are thus achieved by one and the same movement The roll-over form of the funnel allows the water to pass in readily as the two halves separate. Water is ejected by a broad band of transverse muscles, the 'compressors' of Owen[2]; the musculature of the funnel is complex, and the mechanism by which the two halves are drawn apart is not yet clear. The compressor muscles appear to consist of two layers, and it is possible that the funnel is closed by a contraction of the inner, opened by a contraction of the outer layer; a mechanism comparable to that of the decapod fin. A lightly anæsthetized animal, removed from its shell, showed vigorous flapping movements of the funnel, too rapid for their mechanism to be analysed.

In the restricted water of the tank, *Nautilus* never swims fast, but a Fijian woman told me that she had seen it in a lagoon swim 'as fast as a fish'.

Buoyancy Control

The chambered shell of *Nautilus* has long been a subject of interest to both zoologists and palæontologists, and speculation has especially been focused on the nature and condition of the contents of the enclosed chambers, and on the manner of buoyancy control. The recent work of Denton and Gilpin-Brown[8–11] on the buoyancy mechanism of *Sepia officinalis* L. has added point to these speculations.

The only existing record of the gaseous nature of the shell-contents is an oft-quoted statement by Vrolik[12] that the gas contained a higher proportion of nitrogen than is found in atmospheric air. This statement was based on tests made on an animal caught in the Bay of Amboina, preserved in alcohol, brought back to Holland by sea to the Academy of Sciences at Haarlem where gas from the chambers was examined. Oxygen is not mentioned; no trace of 'carbonaceous gas' was found. The technique used in examining this rather ancient material is not specified.

In the aquarium, healthy animals were near neutral buoyancy, and, when observed during the day, were usually slightly heavier than water, resting easily on the bottom. Changes in buoyancy were observed in individual animals. Newly captured animals were sometimes of positive, sometimes of negative buoyancy, but the light animals (positive buoyancy) would usually become heavy in a few hours.

A change in buoyancy was also observed several times on animals which were resting attached to the glass of the tank: such an animal might at one moment be seen to hang down from its tentacles, and be found, an hour or two later, straining up from them like a captive balloon. More than once, an animal was seen resting 'on the bottom' with clear water between the base of its shell and the bottom of the tank. At a later stage of captivity, the power to adjust buoyancy diminished, and the animal became permanently light, cruising round in the surface waters of the tank with the shell breaking surface. It is not known yet whether the diurnal changes in activity already described are related, as in *Sepia*, to diurnal changes in buoyancy[9].

The chambers contained both liquid and gas. X-ray photographs of a living animal, and samples drawn from the shells of live and newly-killed animals both showed a much greater volume of liquid in the newest chambers than in any other. The X-ray photographs showed that the chambers next to the newest, those which have, in fact, an apical position in life, contained little or no fluid, but that small amounts were present in the chambers overhanging the back of the hood. The floating animal, whether dead or alive, has stable equilibrium, which would seem consistent with this distribution. It is closely comparable with that found by Denton and Gilpin-Brown[11] for fluid in the cuttlebone of *Sepia*. The fact that the quantity of fluid changes with age in each chamber suggests that liquid can move out of, and probably also into, the chambers; this is also consistent with the situation in *Sepia*. The control of liquid in *Sepia* is through the 'siphuncular membrane', which closes the chambers; in *Nautilus* it must be through the siphuncule, which is drawn out from the mantle, contains a vein and an artery[4] and runs through the siphuncular tube in the shell. This tube is formed of calcareous spicules[13] which surround a 'horny' sheath (the nature of which has not been determined) secreted by the siphuncle itself: there is thus a considerable barrier between the gas and liquid in the chamber, and the blood of the siphuncle. The histology of the siphuncular epithelium closely resembles but does not appear to be exactly the same as that of *Sepia*, suggesting rather a condition from which the *Sepia* membrane could have evolved.

The buoyancy mechanism must be closely similar to that in *Sepia*, but, even if the physiology of the two siphuncular membranes proves to be the same, and an 'osmotic pump' similar to that found in *Sepia* is found[10], there are mechanical differences between the two animals. On one hand, the cuttle-bone is a small fraction only of the total weight, whereas nearly the whole of the buoyancy of *Nautilus* is utilized in supporting the shell. On the other hand, the chambers of the *Nautilus* shell are much larger than those of the cuttle-bone, and the last 6 or 8 chambers make up a large proportion of the shell's capacity, so that changes in the contents of one of these chambers would have a greater effect on the buoyancy than would be the case for a single chamber of the cuttle-bone.

The examination of the liquid from the chambers was inconclusive ; the small quantity of fluid found in the older chambers tended to be viscous ; the liquid contains organic material as well as dissolved salts.

The gas appeared to be at a pressure of about 1 atmosphere, both in an animal which had lived for some time in the aquarium, and in the animal brought up, at Rabaul, in 6–8 min from about 100 fathoms (180 m). In each case, when the shell was punctured under water, no gas emerged until the meniscus within the hole was pricked with a needle, when one small bubble sluggishly emerged. Two samples of gas from a single animal were scanned, through the kindness of Prof. Odell, in the mass-spectrograph in the Chemistry Department of the University of Auckland. The scannings suggested that the proportion of oxygen to carbon dioxide changes, as might be expected, with the physiological condition of the animal, but showed that the proportion of argon to nitrogen, in each sample, was three times that of the atmospheric air of Auckland, scanned at the same time.

Speculations on the buoyancy of fossil cephalopods have sometimes included the hypothesis of 'pre-septal' gas between the body of the animal and the newest septum. No evidence was obtained in support of this hypothesis.

It should be noted that the animal is at all times found firmly fixed in its shell, and the possibility of either air or sea-water being included in a new-formed chamber must be rejected.

In conclusion, it may be said that *Nautilus* does indeed conform to the cephalopod pattern known in the Coleoidea in that it is a predatory animal, catching food by prehensile tentacles which surround beak-like jaws and swimming by jet propulsion ; yet the form and use of the tentacles and the manner of jet-propulsion are basically different from the coleoid pattern. The difference in the swimming mechanism and the special problems of buoyancy control are intimately associated with the presence of an external shell, but the peculiar form of the tentacles must be taken as evidence of the plasticity of this region and allows free speculation about the form of the tentacles in other shelled cephalopods.

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TEMPERATURE AND LIFE-SPAN IN POIKILOTHERMOUS ANIMALS

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ALTHOUGH ageing is a complex phenomenon physiologically, it may stem from a relatively simple physico-chemical one. Many theories of the past have supposed it to result from the accumulation of toxic substances.

A more modern hypothesis is that the accumulation of nuclear damage is responsible. DNA is particularly suspect as the crucial substance as it controls all metabolism. A theory of damage to DNA, phrased more in terms of somatic mutation, has been elaborated by Szilard[1].

The purpose of this article is to consider the effect of temperature on life-span in poikilothermous animals. This bears on the question of whether ageing may be reasonably interpreted as due to a single or simple molecular process.

Rates of chemical reactions are described by an equation of the type:

$$-\frac{dy}{dt} = ky^n \qquad (1)$$

where y = concentration of substance, = time, and n, called the order of the reaction, generally refers to the number of species of molecules participating.

If death is assumed to be due to a longevity substance being reduced to some final value y_f, then t at the time of death may be represented by l, the life-span.

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If one plots log $1/l$ against reciprocal of absolute temperature, T, of certain invertebrate animals some surprising approximations to straight lines are obtained. This is shown in Figs. 1, 2 and 3. The organisms are respectively *Daphnia*, *Drosophila* and *Moina*. The sources are MacArthur and Baillie[2], Loeb and Northrop[3], Terao and Tanaka[4]. The only points that are very far off occur at extreme temperatures which may be unphysiological for the organism.

Such plots are equivalent to the Arrhenius plot, which yields a straight line when the logarithm of reaction rate constant is plotted against reciprocal of absolute temperature. The $1/l$ in the ageing plots corresponds to the rate constant k in the Arrhenius plot. That k is proportional to $1/l$ can readily be seen for the zero order equation. If y_f is the value of y at $t = l$, $y_f = c - kl$, so $k \propto 1/l$.

Also for first order $y_f = e^{c-kl}$

$$\ln y_f = c - kl$$
$$k \propto 1/l$$

This proportionality holds for higher orders.

Not only does the Arrhenius relationship appear to hold for ageing, but temperature-change experiments with *Drosophila* give results compatible with the idea that ageing can be represented by the reaction rate equation (1).

What will be the life-span of a fly kept for a while at 25° C and then for the rest of its life at 29°? Let us at first suppose that the process responsible for ageing and death is describable by a function $f_1(t)$ at 25° and a function $f_2(t)$ at 29°. We will not say for the moment what the function is, but it is represented by smooth curves in A and B of Fig. 4. For reasons which will appear shortly, a function $t = g_2(y)$ is also given there. This is $f_2(t)$ expressed with t as the dependent variable.

Diagram C shows the compound curve obtained if the change in temperature is made at time $t = x$. The life-span for a fly treated in this way will be l_3 rather than the l_1 or l_2 corresponding to the two temperatures used separately.

The question on which the experiment hinges is this: If the time of change, x, is varied in the experiment, how will a plot of l_3 against x appear? We will take the hypothesis that functions $f_1(t)$ and $f_2(t)$ in Fig. 4 correspond to an integral form of the rate equation (1). They differ only in the rate constant, which is k_1 at temperature 1 and k_2 at temperature 2.

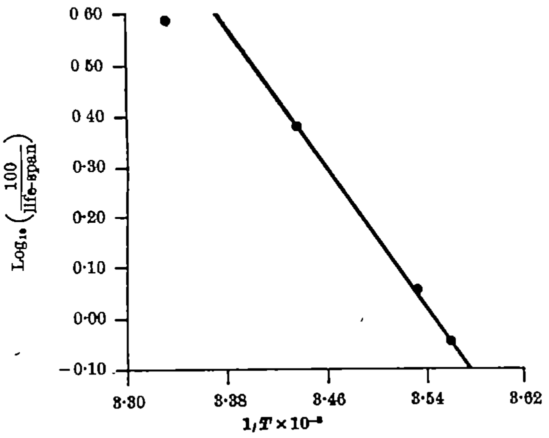

Fig. 1. Arrhenius plot on *Daphnia* from MacArthur and Baillie

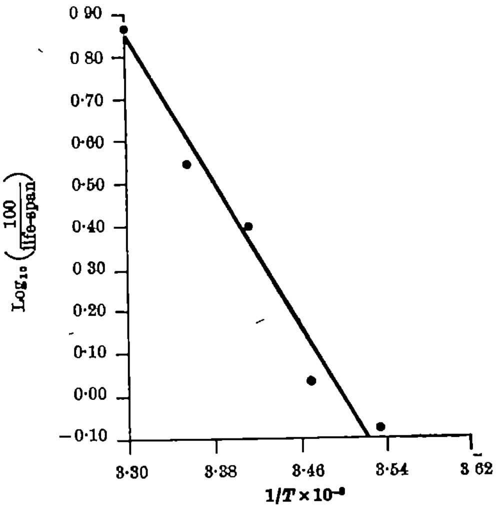

Fig. 2. Arrhenius plot of data on *Drosophila* from Loeb and Northrop

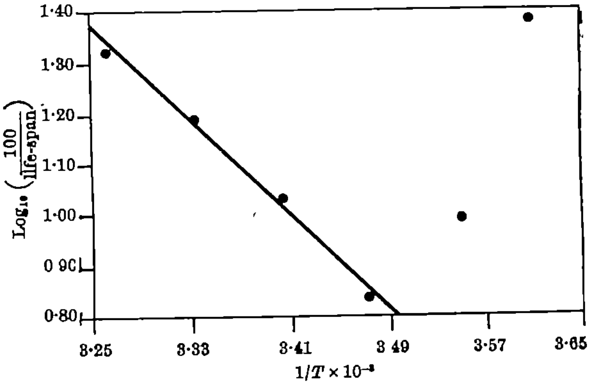

Fig. 3. Arrhenius plot of data on *Moina* from Terao and Tanaka

Under these conditions l_3 and x have a straight-line relationship as shown in D of Fig. 4.

The reason is as follows. As shown in C, the predicted value of l_3 for any x is given by x plus the distance from x to l_3. This distance is called Δt and appears in diagram B. It can be seen there to equal l_2 minus an initial amount of time given by $g_2(y_x)$.

$$\text{Thus } l_3 = x + \Delta t$$
$$= x + l_2 - g_2(y_x)$$

Since y_x is given by $f_1(t)$ when $t = x_1$ the above equation becomes

$$l_3 = x + l_2 - g_2[f_1(x)] \qquad (2)$$

It now becomes possible to demonstrate the linear relationship of l_3 and x for reactions described by equation (1).

For a first-order reaction $f_1(t) = y = e^{-k_1 t - c}$, and when $t = x$:

$$y_x = e^{-k_1 x - c}$$

Since it can be shown that $g_2(y) = -\frac{\ln y - c}{k_2}$, it follows that $g_2(y_x) = -\frac{\ln y_x - c}{k_2}$

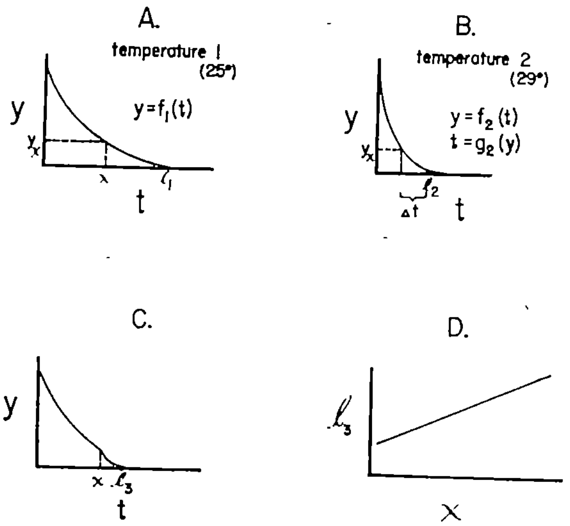

Fig. 4. The hypothesis being tested in the experiment on temperature change with *Drosophila*. *A*, Physiological age-state, *y*, of the organism is given as a function of time. At time x the age-state is y_x. At time l_1 the fly dies; *B*, time function of the age-state at a higher temperature. The life span is shorter; *C*, time function of the age-state for a fly kept at first at temperature 1 and later at temperature 2. The temperature is changed at time $t = x$. The curve is a composite of *A* and *B*; *D*, relationship of life-span to time of temperature change. It is shown in the text that if the ageing process follows the rate equation of chemical kinetics the relationship must be linear

If we substitute $y_x = e^{-k_1 x - c}$ in the above, the expression simplifies to give $g_2(y_x) = \dfrac{k_1 x}{k_2}$.

It is shown earlier that k is proportional to the reciprocal of l. So $g_2(y_x) = \dfrac{l_2 x}{l_1}$.

Substituting this in equation (2) yields:

$$\begin{aligned} l_3 &= x + l_2 - \frac{l_2 x}{l_1} \\ &= l_2 + x\left(1 - \frac{l_2}{l_1}\right) \end{aligned}$$

so demonstrating the linear relationship.

For $n \neq 1$ the general integral of the rate equation (1) is $\dfrac{y^{1-n}}{1-n} = -kt - c$.

We again evaluate $g_2\,[f_1(x)]$ in order to substitute in equation (2).

Solving for y allows us to write $f_1(t)$ as:

$$f_1(t) = y = [(1-n)\ (-k_1 t - c)]^{1/1-n}$$

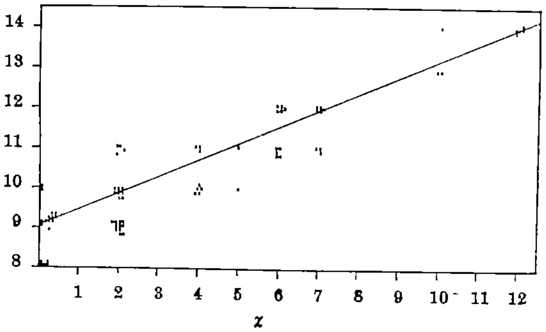

Fig. 5. Findings of the *Drosophila* experiment. A straight line appears to fit the points better than any other simple curve

Alternatively solving the general integral for t allows us to write $g_2(y)$ as $g_2(y) = t = -\dfrac{1}{k_2}\left[\dfrac{y^{1-n}}{1-n} + c\right]$.

When $t = x$:

$$f_1(x) = y_x = [(1-n)\ (-k_1 x - c)]^{1/1-n}$$

$$\text{and } g_2(y_x) = x = -\frac{1}{k_2}\left[\frac{y_x^{1-n}}{1-n} + c\right]$$

Substituting the expression for y_x into the above equation gives:

$$\begin{aligned} g_2[f_1(x)] &= -\frac{1}{k_2}\left[\frac{\left[(1-n)\ (-k_1 x - c)\right]^{\frac{1-n}{1-n}}}{1-n} + c\right] \\ &= \frac{k_1 x}{k_2} \end{aligned}$$

As before this is the same as: $g_2[f_1(x)] = \dfrac{l_2 x}{l_1}$. Substitution into equation (2) gives the same linear relationship as was found in the first-order process.

The experiment was carried out on newly emerged males of stock Oregon-*R* raised to adulthood at 25° C. The flies were put into shell vials with corn meal, agar, and molasses medium. They were observed every three and one-half days when transferred to fresh food. The life-span measured is in fact the life-span of the imago. It was also found convenient to put twelve flies in each vial. The experimental unit was thus a dozen flies rather than the individual. The measure of life-span was the time required for a dozen to be reduced to four.

The results were obtained in two different experiments done at different times. The slopes and intercepts of these were calculated by the method of least squares. The slopes of the two plots were the same as judged by t test ($P > 0.05$). But the intercepts differed by 1·05. This was probably because of a slight difference in thermostat adjustment from one experiment to the next in one or both incubators.

Since it did not appear that pooling the data could introduce any bias favouring the hypothesis, the results of the two experiments were pooled after being adjusted to the same intercept. They are presented together in Fig. 5. The line shown there was also fitted by least squares.

For the degree of variability around the regression line a better approximation to linearity could not be expected. It must be concluded that the result is that predicted by the hypothesis—the hypothesis being that ageing has a simple time function and so perhaps has a correspondingly simple molecular basis.

The results from the Arrhenius plots are also compatible with this conclusion. Some years ago there was a large school of biologists led by Crozier who supposed that a straight-line Arrhenius plot, when found for physiological processes, indicated the dominance of a single, simple, rate-limiting chemical reaction. The criticism of Burton[5] shows that the Arrhenius plot is not so powerful a tool as those workers thought, and that they may have often been misled.

It is interesting, however, that the method has, to our knowledge, never been applied to ageing except recently by Strehler[6]. The results of Figs. 1, 2, and 3 do approximate linearity. Strehler found the same for *Drosophila*. The findings are thus compatible with the view of determination of life-span by a simple rate-limiting reaction. But they are far from adequate to prove the presence of such a reaction However, by even the most sceptical view they have some significance. The nature of the ageing process is not known and the form that these plots would take could not have been predicted.

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HETEROGENEITY OF THE PRODUCTS OF TOBACCO MOSAIC VIRUS BIOSYNTHESIS

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THE characteristics of intermediate stages in virus biosynthesis are descriptive of the mechanism of replication. For example, some investigators[1-4] propose that in tobacco mosaic virus (TMV) replication the virus ribonucleic acid (RNA) fibre serves as a germinal component, providing a structural model (or 'template') which governs the specificity of new RNA biosynthesis. According to this view the resultant product is a replica of the germinal RNA fibre, which in turn regulates the specificity of subsequent protein biosynthesis, yielding thereby the characteristic protein sub-units of the virus.

Intermediates expected from such a course of events are free entire RNA fibres, free protein sub-units, and, possibly, intermediate stages in the subsequent assembly of the virus ribonucleoprotein rod from the former. Since TMV contains a single RNA fibre, but some 2,000 protein sub-units, intermediates that consist of virus RNA and protein in the proportions characteristic of the final TMV rod would appear to be specifically excluded from this scheme. A contrasting view is that TMV replication is indirect[5,6], the entering inoculum instigating a sequence of processes in which an exact replica of the virus or of its component parts appears only in the final product, the fully formed TMV ribonucleoprotein rod. This view admits of the possible occurrence of a wider range of products of TMV biosynthesis, including, for example, ribonucleoproteins which contain an incomplete RNA fibre.

These considerations suggest the importance of investigating the heterogeneity of the ribonucleoprotein products of TMV biosynthesis. There is some evidence that forms of TMV which differ from the predominant product of infection—a ribonucleoprotein rod about 3000 Å long—occur in infected leaf. Bawden and Pirie[7] have directed attention to the presence of rods of various lengths in all virus preparations. A small concentration of a specific form of the virus which, unlike the bulk virus, is closely associated with buffer-insoluble leaf components has been reported from this laboratory[8]. Zeitlin and Boardman[9] find, following incorporation of aspartic acid labelled with carbon-14 into infected tobacco leaf for periods of 2–67 h, that a form of the virus closely associated with the chloroplast exhibits a significantly higher specific activity than does ordinary TMV.

In order to investigate the heterogeneity of TMV biosynthesis we have analysed the incorporation of carbon-14, introduced into TMV-infected leaf by brief periods of photosynthesis in the presence of $^{14}CO_2$, into fractions of TMV which are separable on a chromatographic column. Leaf-blade tissue from 7-day infected plants of *N. tabacum* was exposed to $^{14}CO_2$ (150 μc. of carbon-14; 0·07 per cent carbon dioxide in air) under illumination for 3–20 min periods. The leaf chamber was then flushed with 5 per cent unlabelled carbon dioxide in air for 1 min, the tissue removed and frozen in liquid nitrogen. TMV was purified from the frozen tissue by a standard procedure which has been described elsewhere[10]. The purified virus was fractionated on an 'Ecteola' column (see legend for Fig. 1 for details) and the separate fractions analysed for carbon-14 content, protein content, infectivity, serological reactivity and ribonucleotide composition by methods given elsewhere[10].

Fig. 1 summarizes the results of a typical chromatographic separation of purified TMV isolated from infected leaf after a 20-min period of $^{14}CO_2$ incorporation. The elution pattern is similar to one observed previously on the basis of a discontinuous process[11]. An extremely small amount of ultra-violet-absorbing material is eluted at concentrations of less than 0·025 M sodium chloride. This appears to represent free virus protein sub-units which tend to become dissociated from TMV rods during purification procedures. This material does not precipitate on ultracentrifugation; its ultra-violet spectrum is that of an RNA-free protein; it forms a specific precipitate with anti-TMV serum; it is uninfectious.

Beginning at a concentration of about 0·025 M sodium chloride, increasing amounts of ultra-violet-absorbing material appear in the eluted fractions, reaching a peak in fraction 13 (about 0·06 M sodium chloride). The curve then declines gradually and measurable amounts of material are eluted up to a concentration of 0·3 M sodium chloride (fraction 60). About 90 per cent of the total material placed on the column is eluted up to this point. The remainder is removed from the column by elution with 2 M sodium chloride, followed by 0·5 M sodium hydroxide. In all fractions between 13 and 35 the ratio of absorbancies at 260 and 280 mμ is 1·20, the value characteristic of highly purified TMV. The ratios exhibited by later fractions tend to increase somewhat, and approach 1·30; however, the accuracy of the latter values is necessarily limited by the relatively low total concentrations of material in these fractions. Similar relationships are shown by the ratios of the Folin values and absorption at 260 mμ, which are also diagnostic of the ribonucleoprotein composition of TMV.

Fig. 1 shows that the fractions past No. 8 exhibit substantial infectivity toward two test plants,

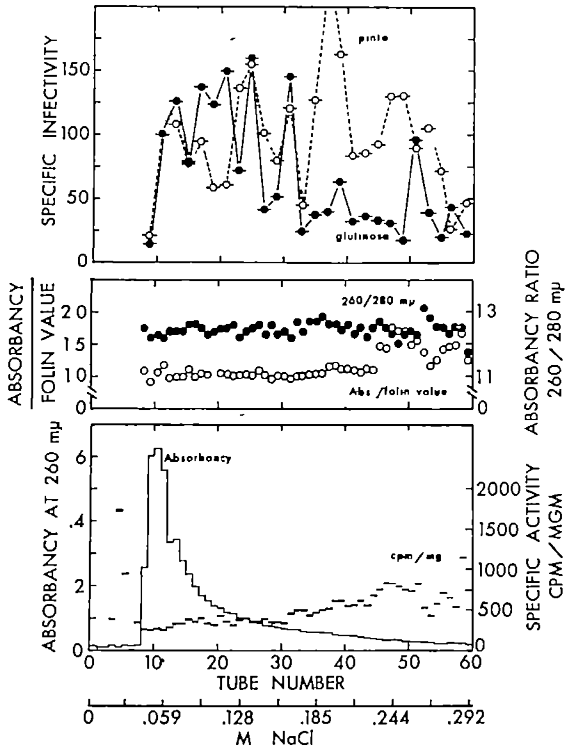

Fig. 1. Results of typical chromatographic fractionation of ^{14}C-labelled TMV on an 'Ecteola' column. The TMV was labelled by incorporating $^{14}CO_2$ photosynthetically into infected tobacco leaf (7 days after inoculation) for a 20-min period, followed by a 1-min period of unlabelled carbon dioxide. The virus was isolated from the leaf and purified by standard procedures before being applied to the chromatographic column. Successive 4-ml. fractions were collected during elution with a continuous gradient of increasing concentrations of sodium chloride. Specific activities were determined on mixtures of two successive fractions. The half-leaf method was used to determine specific infectivities, the values being expressed as per cent of the lesion count due to an equal concentration of standard unfractionated TMV.

N. glutinosa and pinto bean. The specific infectivities toward pinto bean (that is, relative number of lesions produced per unit mass of inoculum as compared with lesions yielded by a standard TMV preparation inoculated on the opposite leaf-halves) are equal to that of bulk TMV for most fractions between 10 and 50. Infectivity toward *N. glutinosa* is about equivalent to the infectivity of ordinary TMV in fractions 10–30, and declines somewhat in later fractions. The infectivity of the first 2 fractions in the leading edge of the main TMV peak is relatively low.

Specific activity is constant only in the first few fractions of the main TMV peak (fractions 10–13). Specific activity rises gradually in succeeding fractions; the carbon-14 levels in fractions 30–33 are about 75 per cent greater than those observed in fractions 10–13. Larger but more variable increases in specific activity are observed in fractions 40–55. These effects reflect the carbon-14 content of the virus protein which comprises 95 per cent of the preparation.

In order to obtain comparable data regarding the virus RNA a series of pooled chromatographic fractions were formed from material eluted from the chromatographic column between 0·10 and 0·14 M sodium chloride. RNA nucleotides were isolated from these preparations by the methods described earlier[10], and their carbon-14 specific activities determined. The results of this analysis are given in Table 1 in the form of the average values for material eluted with 0·10–0·13 and 0·13–0·14 M sodium chloride respectively. The nucleotide compositions of the RNA components from both of the composite TMV fractions are nearly identical, and correspond to the RNA composition observed in bulk TMV. The specific activities of the RNA nucleotides from fraction 2 are higher than those derived from fraction 1, and the difference is proportional to that observed in the virus protein. These results show that the substance responsible for the difference in carbon-14 levels between these successive chromatographic fractions consists of a ribonucleoprotein with the RNA composition characteristic of TMV.

Table 1. CARBON-14 SPECIFIC ACTIVITIES IN VIRUS PROTEIN AND IN VIRUS RNA RIBONUCLEOTIDES FROM SUCCESSIVE CHROMATOGRAPHIC FRACTIONS OF TMV*

Component	Fraction 1 (0·10–0·13 M NaCl)			Fraction 2 (0·13–0·14 M NaCl)		
	Molar ratio	Specific activity c.p.m./mg	% of TMV specific activity	Molar ratio	Specific activity c.p.m./mg	% of TMV specific activity
Cytidylic	0·74	†	—	0·76	†	—
Adenylic	1·12	97	43	1·12	109	41
Guanylic	1·00	25	11	0·97	34	13
Uridylic	1·16	30	13	1·16	36	14
TMV (protein)	—	223	—	—	266	—

* The values given represent averages of 2 successive 4 ml. chromatographic fractions in the case of fraction 1, and of 3 such fractions in the case of fraction 2. RNA was isolated by heating in 0·13 M NaCl; it was then hydrolysed in alkali, purified on charcoal and the ribonucleotides separated by paper electrophoresis.

† Carbon-14 content too low for accurate determination. The cytidylic acid content of TMV RNA is relatively low and the free ribonucleotide achieves relatively low levels of carbon-14 in the plant (ref. 10).

To determine whether the isotope effect is due to specific TMV protein, the serological analyses reported in Table 2 were carried out. Column fractions were pooled into two groups representing the 0·06 M–0·10 M sodium chloride and the 0·22 M–0·27 M sodium chloride eluates respectively, the specific activity of the group of late fractions being significantly greater than that of the earlier fractions. The carbon-14 recovered in the precipitate formed by treating the earlier fractions with anti-TMV serum represented 97 per cent of the carbon-14 content of the starting material. In the second group of fractions, about 87 per cent of the carbon-14 in the starting material was recovered in the antiserum precipitate. Thus, nearly all the excess carbon-14 present in the later chromatographic fraction is precipitable by anti-TMV serum, and is therefore associated with a component that contains TMV protein.

Table 2. PRECIPITATION OF CHROMATOGRAPHIC FRACTIONS WITH ANTI-TMV SERUM

	Early fractions (0·06 M–0·1 M NaCl)	Late fractions (0·22 M–0·27 M NaCl)
Amount of antigen (μg)	43	46
^{14}C in antigen (c.p.m.)	72	146
^{14}C in antigen–antibody precipitate (c.p.m.)	70	127
Per cent of original ^{14}C recovered	97	87

To evaluate the kinetic behaviour of the various chromatographic fractions relative to isotope incorporation from $^{14}CO_2$, a series of experiments similar to that described in Fig. 1 were carried out in which the time of $^{14}CO_2$ incorporation was varied between 3 and 20 min. In each case the virus found in successive fractions between 10 and 30 exhibited the gradual increase in specific activity shown in Fig. 1.

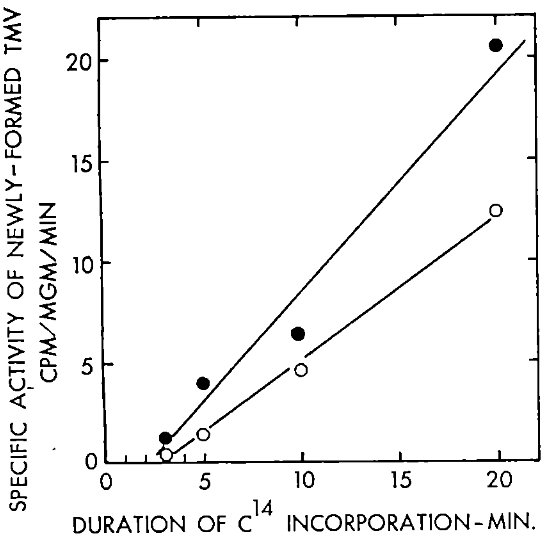

Fig. 2. Relative carbon-14 specific activity of newly formed TMV (specific activity of preparation/duration of $^{14}CO_2$ incorporation) in two groups of 'Ecteola' chromatographic fractions after various periods of $^{14}CO_2$ incorporation. Open circles: fractions eluted with 0·050 M–0·065 M sodium chloride. Closed circles: fractions eluted with 0·165 M–0·175 M sodium chloride

In such experiments the relative specific activity of the TMV newly formed during the period of isotope incorporation is given by $A/\Delta t$, where A is the specific activity of the TMV isolated from the leaf and Δt is the duration of incorporation of carbon-14. This is evident from equation (3), it being noted that V, the total amount of TMV, is so large compared with the amount synthesized during the brief experimental period (ΔV) as to be essentially invariant, and that the latter value is proportional to Δt (see also ref. 10). If $A/\Delta t$ is plotted against Δt, the intercept on the time axis represents the least time required for ^{14}C introduced as $^{14}CO_2$ to become incorporated into virus substance.

Fig. 2 shows this relationship for the TMV present in two different chromatographic fractions. The relative specific activity of newly formed TMV in the later fraction is consistently greater than that of the earlier fraction. In both cases the specific activity of the newly formed virus increases with the time of incorporation of $^{14}CO_2$, and both intercepts indicate a transit time of carbon-14 of 2–3 min for passage of carbon-14 from $^{14}CO_2$ to virus substance.

This result shows that the two chromatographic fractions represent forms of the virus that are synthesized concurrently from metabolic precursors. Their consistently different specific activities show that they are not identical materials. The increase in this difference with time of incorporation of carbon-14 suggests that the two fractions are not in the relation of precursor and final product.

Apart from the foregoing observations, there is some evidence in our experiments that purified preparations of TMV labelled with carbon-14 are associated with extremely small amounts of material that is rather high in specific activity. In the experiments described here it was found that the specific activity of unfractionated TMV remained constant when the purification process was increased from the standard 3 stage procedure to 6 stages. Nevertheless, although more than 90 per cent of the ultra-violet-absorbing material placed on the columns was recovered in the eluted fractions, the total recovery of carbon-14 was only 30–50 per cent. The remaining carbon-14 is bound to the column but can be eluted from it by extensive washing with 0·25 M sodium chloride. In the chromatographic procedure described in Fig. 1, the relative amount of carbon-14 eluted with 0·25 M sodium chloride, while quite variable from run to run, tends to be relatively high, especially when the preparation has been exposed to $^{14}CO_2$ for 5 min or less. The chromatographic fractions that exhibit this effect (40–55) tend to be moderately infectious toward pinto bean, but rather uninfectious toward *N. glutinosa*. Although this suggests a possible connexion between the high carbon-14 levels and a component related to the virus, this is by no means certain. The amounts of material involved are extremely small, and the effects rather variable. More detailed studies are required before any possible association of these effects with the process of TMV biosynthesis can be elucidated.

The foregoing experiments show that TMV preparations can be separated chromatographically into fractions that are composed of serologically specific TMV protein, that exhibit an RNA content and ribonucleotide composition characteristic of TMV, and that possess the infectivity associated with the virus. Despite these similarities the fractions do differ in carbon-14 specific activity, proportional differences being observed in the protein and in the separate RNA nucleotides of the virus. These results are evidence of heterogeneity in the ribonucleoprotein products of TMV biosynthesis. In what follows we consider the possible origins of this heterogeneity.

In these experiments all the carbon-14 found in the TMV isolated from the labelled leaf must be present in virus newly synthesized during the course of $^{14}CO_2$ incorporation, since pre-existing virus is metabolically inert. The specific activity of the newly formed virus ($A_{\Delta V}$) is given by:

$$A_{\Delta V} = \frac{C}{\Delta V} \qquad (1)$$

where C is the carbon-14 content of the newly-formed virus, the amount of which is given by ΔV. The observed specific activity of the total isolated virus, A, is given by:

$$A = \frac{C}{V} \qquad (2)$$

where V is the total amount of TMV isolated from the leaf.

Hence:

$$A = A_{\Delta V} \cdot \frac{\Delta V}{V} \qquad (3)$$

The observed differences in specific activity of carbon-14 between the TMV fractions that are separated on the chromatographic column (that is, the value of A for each fraction) must be due to corresponding variations in one or more of the foregoing parameters. The possibility that the quotient $\Delta V/V$ is significantly different for the various chromatographic fractions of TMV seems rather limited. The value of ΔV is proportional to the duration of incorporation of carbon-14, and to the rate at which the virus is synthesized from its metabolite precursors. As we have shown previously[10] this rate is reflected by the intercept on the time axis, of the line relating the specific activity of newly-formed TMV to the time of incorporation of carbon-14, since the intercept represents the minimum time required to convert $^{14}CO_2$ into TMV substance. Fig. 2 shows that two chromatographic fractions of TMV that differ

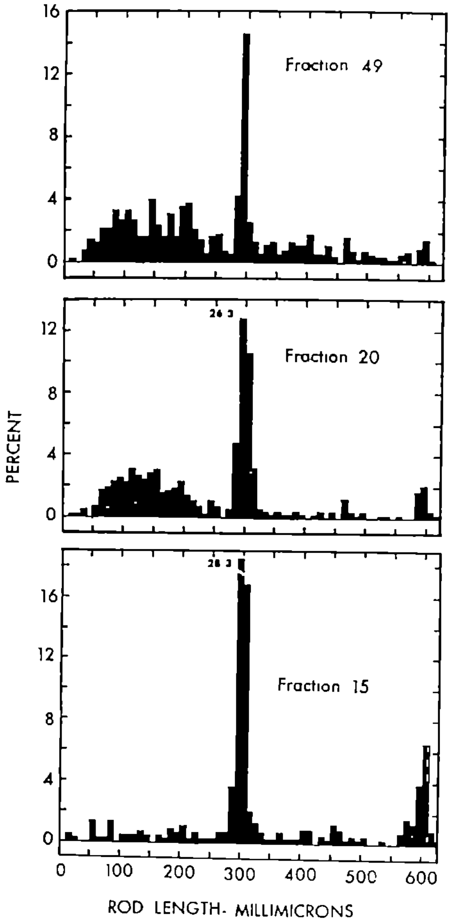

Fig. 3. Frequency distribution of the lengths of rods observed in electron micrographs prepared from several chromatographic fractions of TMV obtained by elution from an 'Ecteola' column with a continuous gradient of increasing concentrations of sodium chloride. Fraction 15 was eluted at 0·045 M NaCl; fraction 20 at 0·060 M NaCl and fraction 49 at 0·160 M NaCl Per cent of rods shorter than 3000 Å ± 300 Å: fraction 15, 13 per cent; fraction 20, 39 per cent; fraction 30 (eluted at 0·095 M NaCl, but not shown above), 43 per cent; fraction 49, 52 per cent Rods longer than the predominant 3000 Å class result from end-to-end polymerization during purification

significantly in specific activity of carbon-14 exhibit essentially identical intercepts. This result appears to preclude the possibility that the observed differences in specific activity are due to comparable variations in the rates of synthesis of different TMV fractions. The total virus content of the leaf, *V*, is essentially equal to the amount of pre-existing unlabelled virus, for the time during which labelled virus is synthesized is very short (3–20 min) compared with the time during which the pre-existing unlabelled virus is formed (7 days). Since the value of *V* for successive chromatographic fractions is a function of the relative rates of synthesis of the different fractions, this factor is also an unlikely source of the observed differences in the values of *A*. It is therefore unlikely that the observed variations in the specific activities of successive chromatographic fractions of TMV are due to corresponding differences in the ratio of newly formed virus to total virus $(\Delta V/V)$ in these fractions.

These considerations indicate that the levels of carbon-14 observed in our experiments are the result of differences, among successive chromatographic fractions, in the specific activity of the virus particles that are newly synthesized during the course of isotope incorporation ($A_{\Delta V}$). A possible explanation of these differences is that the various fractions of TMV are formed from separate metabolite pools which maintain unlike specific activities of carbon-14. Such an effect might, for example, result from variable rates of $^{14}CO_2$ incorporation into the metabolic pools of different classes of infected cells in the tobacco leaf. However, this would also require that the TMV formed in various types of infected cells be sufficiently unlike in physico-chemical properties to be distinguishable on a chromatographic column. There is no evidence in the relevant literature to support such a conclusion.

If it is assumed that the separable fractions of TMV are formed from the same metabolite pool at the same overall rate of synthesis, then the observed variations in specific activity can only mean that these fractions differ with respect to the relationship, within newly formed virus particles, between the amounts of non-isotopic carbon and of carbon-14. The simplest example of such an effect would be the addition of equal amounts of carbon-14 to the ends of pre-existing rods of TMV which differ significantly in length. In this case the specific activity of a TMV rod would be expected to vary inversely with the mass and therefore with the length of the TMV rod.

As a preliminary test of the possible association of the specific activity of TMV fractions with rod-length, we have determined the frequency distribution of rod lengths observed in electron micrographs prepared from a series of 'Ecteola' fractions of TMV. The results, which are illustrated in Fig. 3, show that the proportion of rods shorter than the predominant length of 3000 Å increases significantly in the later chromatographic fractions. Since TMV specific activity also increases in successively later fractions (Fig. 1), the empirical relationship between specific activity and rod-length corresponds to that suggested by the foregoing considerations.

Experiments designed to elucidate in detail the relationship between TMV biosynthesis and the lengths of the resultant ribonucleoprotein rods have been carried out and are to be reported elsewhere. These support the conclusion, proposed earlier on theoretical grounds[6,12], that the TMV rod is formed by a process of linear growth, and that ribonucleoprotein rods of various lengths are alternative products of TMV biosynthesis.

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LETTERS TO THE EDITOR

ASTRONOMY

Orbits of Natural Satellites

Bode's law, a three-parameter formula giving the distances of the planets from the Sun, is well known. In a recent communication, Woolfson[1] discussed whether Bode-type laws also applied to the innermost satellites of Jupiter, Saturn and Uranus and arrived at the conclusion that agreement was then less precise. It is the object of this communication to point out that the distances of all known satellites from the centre of the parent body can be given reasonably accurately by a simple two-parameter formula, subject to the assumption that vacant positions sometimes occur.

Fig. 1 shows a plot of the logarithm of the orbit radius of the five known satellites of Uranus against the satellite number; these five points are nearly colinear, as shown, indicating that the orbit radii of the satellites follow an exponential relationship reasonably accurately.

Consider now the satellites of Jupiter in similar manner. The four points corresponding to the satellites discovered by Galileo are effectively colinear (Fig. 2). The seven satellites exterior to these are very much smaller in size and fall into two asteroid-like groups at around 12 million and 23 million km orbit radius, respectively. These two groups then fit well the projected line through the Galilean satellites provided three positions (at about 3·1, 4·8 and 7·7 million km) are regarded as vacant. Similarly, the innermost satellite of Jupiter can be accommo-

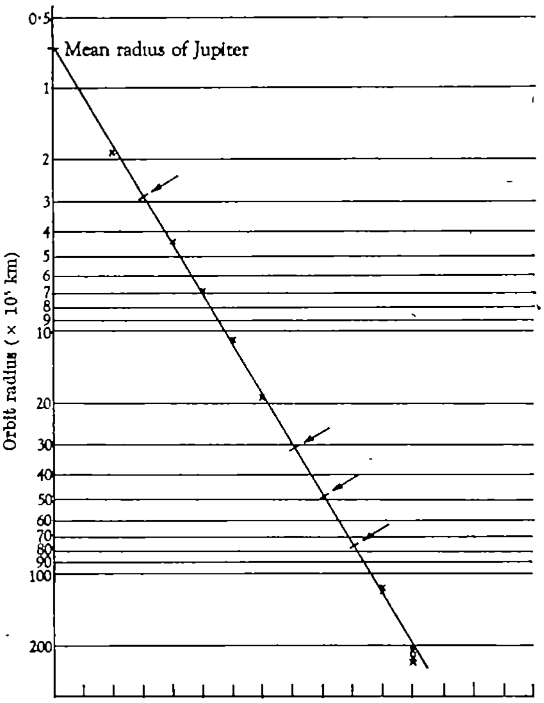

Fig. 2. Satellites of Jupiter. ×, Known satellite; arrow indicates vacant position

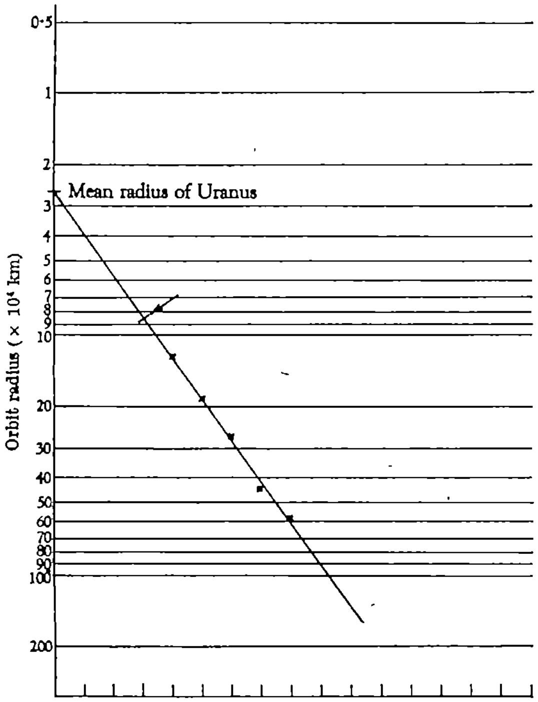

Fig. 1. Satellites of Uranus. ×, Known satellite, arrow indicates vacant position

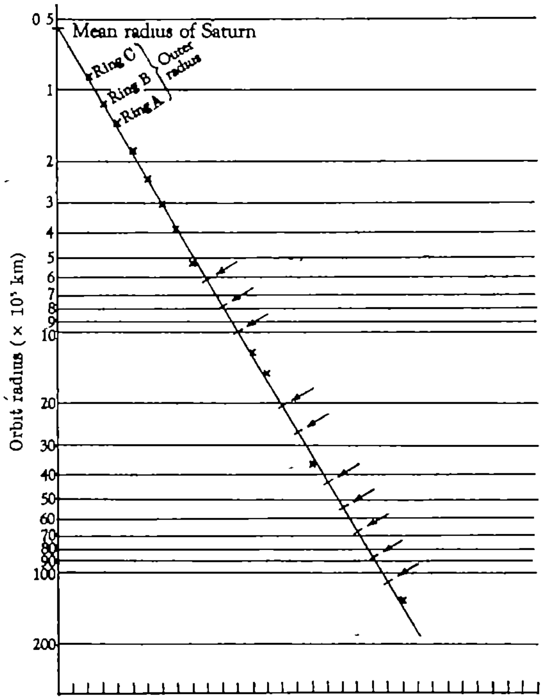

Fig. 3. Satellites of Saturn. ×, Known satellite; arrow indicates vacant position

dated provided a vacant position at 0·29 million km is allowable.

Fig. 3 shows a possible way of accommodating the satellites of Saturn according to the exponential law. It is seen that the exponential relationship is obeyed quite well by the five innermost satellites—by the innermost four in particular. As these satellites are, relatively speaking, closer together the number of vacant positions is more open to conjecture, but would seem to be larger than in the case of Jupiter; 10 vacant positions are shown in Fig. 3. It might be noted that the outer radii of Saturn's three rings lie close to vacant positions within the Roche limit while the problematical 10th satellite of Saturn, Themis, announced by Pickering in 1904 and the existence of which is now usually rejected, does not correspond to one of the vacant positions.

Possible further evidence for an exponential relationship between the orbit radii is the fact that from Mars to Uranus inclusive (including the mean asteroid) the orbit radii of the planets accurately follow such a relationship, with no vacant positions.

It is now suggested that some, in any event, of the vacant positions noted in the cases of the satellite orbits are filled either by one satellite or by asteroid-like groups of satellites. In the cases of both Jupiter and Saturn there are no vacant positions between the innermost known satellite and the Roche limit, whereas there is one such position in the case of Uranus—at about 85,000 km orbit radius. In the absence of any limit to the maximum possible orbit radius for a satellite with respect to a given central body one might also speculate on the possibility of the occupation of apparently vacant positions exterior to the outermost known satellites. Indeed, in the case of Mars this would seem to be the only possibility for the existence of additional satellites—the first available exterior position occurring at about 63,000 km orbit radius.

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ASTROPHYSICS

A New Estimate of the Strength of the Magnetic Field of the Planet Venus

IN some earlier publications[1], attention was directed to the behaviour of the geomagnetic activity around the dates of lower conjunction of Venus. It appeared that, especially at times of weak solar activity and with conjunctions, where Venus passed the Sun at a small angular distance, seen from the Earth, a screening effect of Venus on the solar corpuscula was found from 6 days before up to 1 day after the date of conjunction. An estimate of the strength of the magnetic field of Venus met with difficulties.

Since 1956 the effect has been further and more quantitatively examined by us, the results of which will shortly be published in more detail. First it was found that the screening effect can be considered as statistically significant. Secondly, the relation between the amount of screening and the geocentric latitude of Venus β(♀) was investigated. The result is given in Fig. 1, in which the geomagnetic activity during the period of 6 days before up to 1 day after conjunction with respect to the mean activity over a longer period has been plotted against the angle β(♀). In this graph all lower conjunctions of Venus, since 1890, have been included which occurred in a period where the yearly sunspot-relative-number (Wolff number) was smaller than 50. The difference in position of the points at β(♀) < 4·5° and β(♀) > 4·5° is striking. At the small angles all conjunctions show a decrease of the geomagnetic activity, whereas at the bigger angles deficiencies and surpluses show up equally; the change at β(♀) = 4·5° is rather sharp. This effect seems to indicate that also in a direction perpendicular to the orbit of Venus, an estimate of the extent of the screening can be made.

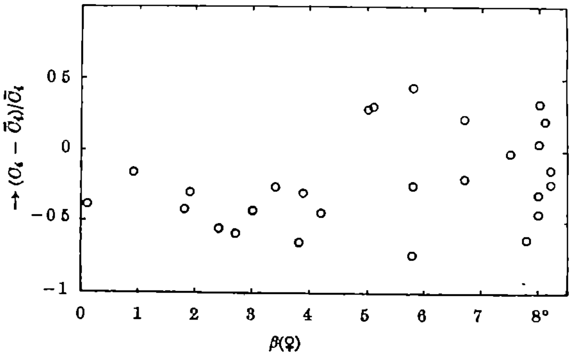

Fig. 1. Geomagnetic activity in the period of 8 days around conjunction date for 27 inferior conjunctions of Venus, plotted against geocentric latitude of Venus at the moment of conjunction

The period of 8 days in the direction of the orbit gives an extensiveness of the perturbing part of the Venus orbit, which is seen from the Earth under an angle of 8 × 0·63° = 5°. In a direction perpendicular to the orbit one finds from Fig. 1 an angle of 9°. A larger extension of the screening in this direction has to be expected, if the magnetic axis of Venus is more or less perpendicular to the orbital plane (for a dipole the difference amounts to a factor 1·26 = $\sqrt[3]{2}$).

For a calculation of the strength of the magnetic field of Venus from the distance over which the screening is active, a knowledge of the nature and velocity of the corpuscular stream seems necessary. Assuming that the screening action is equal to that on free protons, which are ejected from the Sun with a speed of 500 km/sec, it has previously[1] been calculated that the magnetic field of Venus is about 5 times as strong as that of the Earth. In case the corpuscula come from the Sun in electrically neutral clouds (which seems much more plausible and which would result, as was already remarked, in a much larger strength of the magnetic field of Venus) computational difficulties prevented a calculation at the time.

It may be said, however, that the screening of Venus will extend to where the dipole field of Venus has the same strength as the interplanetary magnetic field, of which we now know, thanks to space research, that in the neighbourhood of the Earth it has a value of 1–10 × 10^{-5} oersted[2,3].

If we assume a value of 3 × 10^{-5} oersted for the strength of this field in the neighbourhood of Venus, this would then be also the strength of the dipole field of Venus, in the direction of the poles at a distance of

$$\frac{7\cdot5^{\circ}\ \text{(average of polar dimension of screening)}}{1'\ \text{(geoc. diam. of Venus)}} = 450\ \text{Venus radii} = 2\cdot5\ \text{million km}$$

With a space probe a well-measurable field strength could be shown, therefore, to be present at a distance of 1 million km from Venus.

It follows that for the magnetic field strength at the surface of Venus near the poles we might expect a value of $450^3 \times 3 \times 10^{-5}$ oersted = 3×10^3 oersted; this is 5,000 times that of the geomagnetic field.

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Errors in the Measurement of the Temperature of the Moon

Measurement of the temperature of the Moon is generally done by absolute radiometry assuming the emissivity of the lunar surface to be independent of wave-length and to be that of a 'black-body', namely, ε = 1·00, and calculating the temperature from Stefan's law[1]. This 'black-body' assumption is based on the 1930 interpretations of Pettit and Nicholson[2] of radiometric data they obtained with crude filter monochromators. The basis of these deductions was that the ratio of the radiant energy between 8–10μ to the radiant energy between 8–14μ observed was nearly identical to the ratio calculated for a 'black-body' at 400° K. The temperature of the sub-solar point (400° K) was calculated in the usual manner (assuming the emissivity to be unity) after relating all the measurements to the radiometric magnitude of a comparison star. From these paradoxical assumptions they concluded that the emissivity of the lunar surface in the region 8–10μ is unity and is unaffected by the restrahlen of any silica content it may have; this is contrary to observations made on the emissivity of silica or silicates[3,4]. In order to reconcile this dissimilarity with their original conclusion Pettit and Nicholson decided the lunar crust was finely divided, like sand, or porous, like pumice, and that its radiating properties would therefore be nearly those of a 'black-body'.

Considering the technology and knowledge of the Moon and Earth's atmosphere in 1930 Pettit and Nicholson's work was truly classic. However, in the past few years, with refined instrumentation, the radiative and transmissive characteristics of the Earth's atmosphere have been clearly defined and reveal that there is sufficient ozone in the atmosphere to absorb 25 per cent of the radiant energy in the 8–10μ region[5]. The fact that Pettit and Nicholson did not observe ozone in the atmosphere makes us sceptical of the validity of their work. Even if they had had modern instrumentation and observed this phenomenon, they would not have been able to come to any conclusions concerning the nature of the lunar crust from these measurements because the low emissivity from silicates occurs in a wave-length range in which all Earth-bound instruments are not responsive because of total absorption by ozone. Needless to say, significant temperature measurements and compositional assignments of the lunar crust can be made only from above the Earth's atmosphere; from high-altitude balloon investigations, or better yet from an orbiting lunar vehicle.

To aid in interpretation of results to be obtained above the Earth's atmosphere, we have investigated the effect of composition on the emissivity of several smooth surfaces as a function of wave-length and have calculated the emittance, $\mathfrak{R}(\lambda, T)$, at several temperatures. For these calculations:

$$\mathfrak{R}(\lambda, T) = \epsilon(\lambda) W(\lambda, T)$$

where $\epsilon(\lambda)$ is the emissivity of the surface which is dependent on wave-length, and $W(\lambda, T)$ is the energy of a black-body at wave-length, λ, and temperature, T. The $\epsilon(\lambda)$ curve was obtained indirectly from reflectance measurements on polished, opaque specimens. In this manner $\epsilon(\lambda)$ was calculated readily without having to make absolute radiometric measurements; the polished and opaque requirements negate diffusely reflected (scattered) radiation and make the transmittance negligible, respectively, hence:

$$\rho(\lambda) + \alpha(\lambda) = 1$$

where $\rho(\lambda)$ is the reflectivity and $\alpha(\lambda)$ is the absorp-

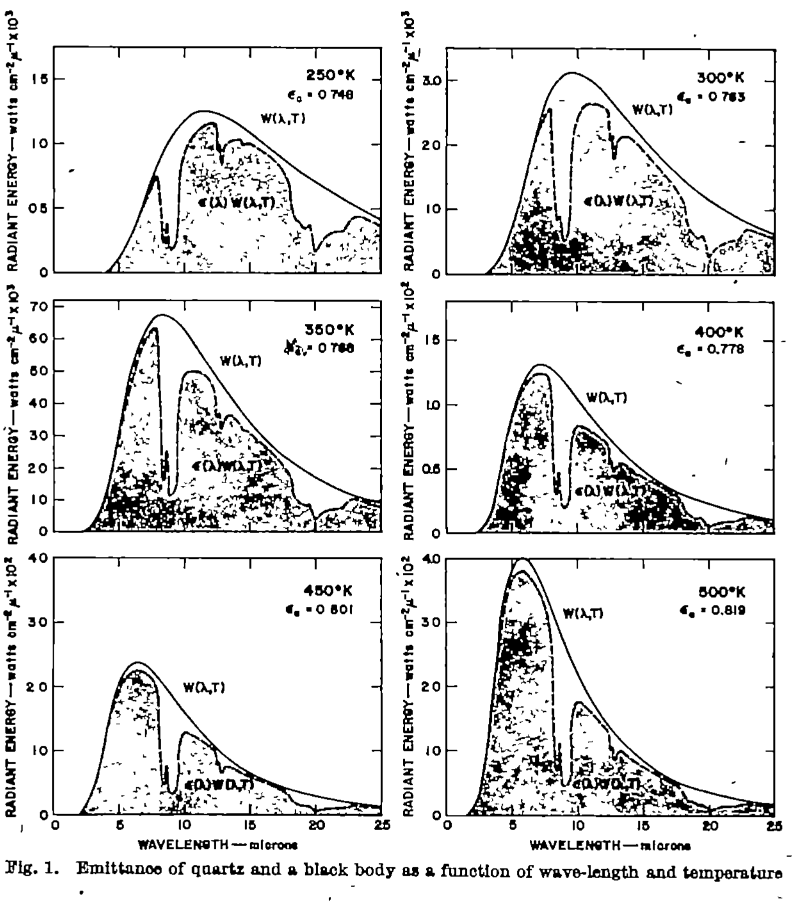

Fig. 1. Emittance of quartz and a black body as a function of wave-length and temperature

tivity. At thermal equilibrium, the absorptivity equals the emissivity, hence:

$$\varepsilon(\lambda) = 1 - \rho(\lambda)$$

Comparisons of $\Re(\lambda, T)$ curves of quartz with the corresponding $W(\lambda, T)$ curves at six temperatures are shown in Fig. 1. These curves dramatically demonstrate why the wave-length of maximum energy, λ_m, cannot be used to evaluate the temperature from the Wien law:

$$\lambda_m T = 0{\cdot}2896 \text{ cm } ^\circ \text{ K},$$

for at 300° K an error of about − 45° K would result.

Temperature determination by absolute radiometric measurements will also be in error when the emissivity is a function of wave-length. A prerequisite for the applicability of this method is that the average emissivity, ε_a, is independent of temperature; however, because of its dependence on wave-length, it is also a function of temperature.

The average emissivity, ε_a, over the wave-length interval 2–25μ is defined as:

$$\varepsilon_a = \frac{\int_2^{25} \varepsilon(\lambda)\, W(\lambda, T)\mathrm{d}\lambda}{\int_2^{25} W(\lambda, T)\mathrm{d}\lambda}$$

for any temperature, T.

It can be shown that the dependence of ε_a on temperature is a second order effect, hence measuring temperature changes of the same material by radiometric methods is valid. On the other hand, absolute values of the temperature can be significantly in error.

Because of the predominance of the strong Si–O vibrational bands near 9–10μ for most minerals, it is reasonable to conclude that a situation similar to that presented for quartz would exist for lunar surfaces. Table 1 lists ε_a for typical proposed lunar materials—granite, obsidian, dunite, and chondritic meteorite—at 350° K. Details of these measurements will be published elsewhere[6]. A variation in ε_a of about 10 per cent is obtained between granite and dunite, which would correspond to an error of about 9° K at 350° K when the temperature was calculated by use of Stefan's law. Hence, the lunar temperature changes reported by Shorthill and Saari[7] could instead be caused entirely, or in part, by compositional changes.

Table 1. AVERAGE EMISSIVITY OF TYPICAL LUNAR MATERIALS AT 350° K

Material	ε_a
Granite	0·86
Obsidian	0·83
Meteorite 'Haven' (19-13) chondrite	0·81
Dunite	0·78

If the surface of the Moon is finely divided or porous, the deviation of the emissivity curve from ideality may not be as dramatic as that obtained from our reflectance measurements on polished surfaces. On the other hand, Bell has obtained such deviations from absolute radiometric measurements of gypsum sand at White Sands, New Mexico[8], and quartz in the beach sands at Cocoa Beach, Florida[9]. Because the exact nature of the surface of the Moon is uncertain and may indeed be a slag-type material, the proposed application of lunar infra-red radiation spectrometry for compositional identification and precision temperature estimation cannot be ruled out at this time.

We are at present conducting investigations along these lines to further establish the validity of approach.

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RADIOPHYSICS

Apparent Relation between Solar Activity and the 440-Mc/s Radar Distance of Venus

A CURIOUS correlation (Fig. 1) has been found between the systematic variations in the astronomical unit, as derived from radar observations of Venus at 440 Mc/s (68 cm)[1], and the solar 20-cm flux as measured at the Heinrich Hertz Institut in Berlin-Adlershof[2]. The 20-cm flux is known to be a good indicator of solar activity, and there is evidence that solar decimetre radiation (slowly varying component) is related to both the solar extreme ultra-violet flux and the solar wind intensity[3,4].

From a first glance at Fig. 1 one would suppose that the systematic variations in the radar data are due to changes in the group velocity or ray path curvature of the radar signals caused by variations in the solar wind electron density between Venus and the Earth. But the correlation is in antiphase and therefore cannot offer an immediate explanation for the observed

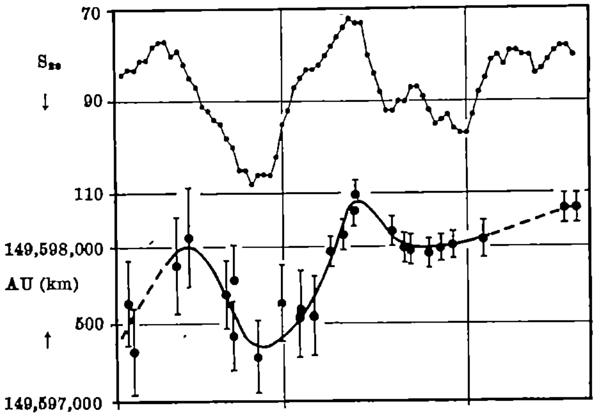

Fig. 1. Astronomical unit (AU) as derived from Venus radar measurements at 440 Mc/s reduced with Duncombe's orbit (lower part of the figure) and solar 20-cm flux (upper part) in units of $10^{-22} \frac{\text{W}}{\text{m}^2\text{c/s}}$. In order to demonstrate the inverse relation between both quantities the ordinate scale of the 20-cm flux has been drawn increasing downwards

variations in the radar distance of Venus. Furthermore, the interplanetary electron density required for this explanation is, however, of the order of 5×10^4 cm^{-3}, with variation of up to 2×10^4 cm^{-3}. These figures are unacceptably high[5,6].

A possible explanation for the correlation might be that Venus has an ionosphere with electron densities of the order of 10^9 cm^{-3}. If so, the 440 Mc/s radar signal would be reflected from this ionosphere. Then the correlation in Fig. 1 would be explained by a further assumption that the height of the Venus ionosphere changes with solar activity in a manner similar to that found for the terrestrial upper atmosphere. The scale must be somewhat larger, however, with maximum height variations of about 100 km. This figure is derived from the maximum amplitude of 600 km in Fig. 1, divided by 2, accounting for the reflexion of the signal, and reduced by a factor of 0·35, the ratio of the mean distance of Venus during the measurements to the astronomical unit.

An electron density of 10^9 cm^{-3} would imply difficulties in understanding the rather high intensity of the reflected radar signal observed at 2388 Mc/s (12·5 cm)[7,8]. Assuming an equivalent thickness of 30 km for a peak electron density of 10^9 cm^{-3} the ionosphere would have an optical thickness τ of the order of 1 at this frequency. Therefore at 12·5-cm wavelength we would have to account for an attenuation factor of $e^{-2\tau} \approx 0{\cdot}14$ in the signal reflected from the surface of Venus.

This difficulty could be removed by assuming inhomogeneities in the ionosphere, where the required peak electron density of the order of 10^9 cm^{-3} occurs only in regions small compared with the entire surface. In this case the 12·5-cm radar signal will pass the ionosphere with an appreciably smaller attenuation, whereas the 68-cm signal would be reflected from the dense regions.

The difference of 650 km between the mean value of the astronomical unit obtained by the Jet Propulsion Laboratory group at 12·5 cm : 149,598,500 ± 500 km, and the value obtained by the Massachusetts Institute of Technology group at 68 cm : 149,597,850 ± 400 km, is compatible with the assumption that the latter wave-length is reflected from an ionosphere in an altitude of about 200 km above the surface of Venus. The above difference and its entire range of uncertainty yield a value of 110 ± 150 km.

In our calculations we used the following crude model of an ionosphere: a layer with an electron density of 5×10^8 cm^{-3} containing 'clouds' with electron densities of 2×10^9 cm^{-3} which cover one quarter of the surface. The temperature was assumed to be 800° K and the equivalent thickness 5×10^6 cm, yielding an attenuation factor of 0·25 for the radar signal at 12·5-cm wave-length.

The model is in satisfactory agreement with the observed radio spectrum of Venus in the wave-length-range 0·4–21 cm (refs. 9–11), since it yields the following brightness temperatures: 470°, 590° and 730° K at 3·3-, 10- and 20-cm wave-length, respectively, if we take additionally to the model already mentioned a surface temperature of Venus of 400° K (ref. 12).

A second, but remote, possibility would be that the correlation is completely fortuitous and that the variations in the derived values for the astronomical unit are due to remaining cosine terms in Duncombe's orbit calculation, causing a spurious 30-day period; but it seems unlikely that the errors in the periodic terms can be responsible alone for the observed residuals (R. L. Duncombe, private communication).

The striking correlation between the variations in the observed value of the astronomical unit and the solar 20-cm flux urgently needs proof or disproof by further measurements, especially at 440 Mc/s, before any definite conclusion can be made.

We thank Drs. Albert Arking and Alan T. Moffet for helpful discussions. We also thank Drs. R. L. Duncombe and J. V. Evans for their communications.

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METEOROLOGY

A Cyclic Change in Possibilities of Precipitation at the Plateau Rosa Meteorological Station, and the Subjacent Valtournanche Valley

One of us has recently shown[1] that although necessary temperature conditions for a transition of phase from liquid water to ice often existed in southern, cloud-bearing air masses at the Plateau Rosa in the Italian Alps, the actual periods of precipitation did not appear to follow such conditions in a concomitant manner, if considered on an annual basis. This was further substantiated by extending the analysis from the original period of 5 years to the interval covering 1952–61 inclusive[2].

Data on annual volumes of precipitation, collected during the years 1926–43 inclusive, at about 765 rain-gauge stations distributed throughout Italy south of Rome, as well as in Sardinia and Sicily, appear, on the other hand, to indicate the existence of a phenomenon cyclic with time which governs the precipitation there. Anomalies of precipitation which were computed individually for 5 different categories of station altitudes (ranging between 0 and 1,200 m in elevation) are consistently indicative of a periodic phenomenon which lags about 1–3 years behind the sunspot cycle, as monitored by the average annual Zurich sunspot numbers[3].

Since it was pointed out recently[4] that short-wave radiation may have bearing on the activation towards nucleation of water of some components of atmospheric aerosol, the possibility of correlating the 'alpine supersaturation' data quoted here with the sunspot cycle appeared to be of immediate interest. Data on total possible percentage 'precipitation increments' associated with the southern air-flow

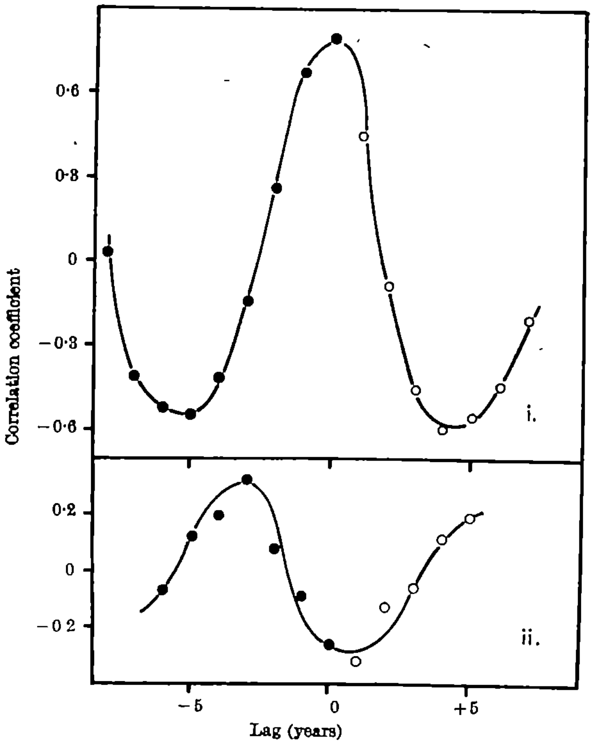

Fig. 1. Plot of linear correlation coefficients between: (i) relative percentage of possible precipitation (h); (ii) precipitation occurred (h); and the relative Zurich annual average sunspot number, against the phase, in years, by which the sunspot numbers used were shifted with respect to the year of computation. Open circles stand for interpolated Zurich numbers

were taken from refs. 1 and 2 while data on Zürich annual sunspot numbers until 1961 inclusive were obtained from pertinent Zurich publications[5]. Tentative Wolf numbers for the years 1962–68 were calculated assuming approximate minimum and maximum values of solar activity for the years 1964 and 1968 respectively, and interpolating the data in proportion and on the basis of the 2 previous cycles. Table 1 illustrates the data tentatively used for this period of time, while Table 2 gives data both on effective precipitation and the 'total probable precipitation increments' in the Plateau Rosa zone.

Linear correlation coefficients between 'possible relative percentage increments of precipitation' and the Zurich sunspot values were computed following the well-known technique of phase lagging. Results obtained on the basis of this calculation are shown in Fig. 1. A pronounced cyclic pattern of precipitation possibilities can be seen, while actual precipitation values seem to follow an opposite trend, with indications of a maximum at about 3 years behind the maximum of sunspots, and in line with more extensive data previously obtained[3]. Since the curve dealing with situations favourable towards precipitation appears to indicate reasonably encouraging consistency, especially as compared with the actual precipitation diagram, it is thought that the technique used may be profitably applied, wherever necessary data are available, to gain better understanding of natural precipitation phenomena in zones of interest for this type of study, and to indicate suitable timings of artificial cloud-nucleation.

Table 1

Year	Zurich No.	Year	Zurich No.	Year	Zurich No.
1962	34	1964	12	1967	102
1963	18	1965	24	1968	110
		1966	67		

Table 2

Year	Precipitation (h)	Possible relative percentage precipitation associated with southern air flow
1952	2,405	15·34
1953	1,956	14·62
1954	2,459	7·65
1955	2,132	10 60
1956	2,163	16·41
1957	1,980	21·88
1958	2,114	18·11
1959	2,116	18 71
1960	2,503	18 06
1961	1,987	15·70

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PHYSICS

Pre-freezing Effects in Ionic Aqueous Solutions

VARIOUS workers have reported that for some liquids the dependence on temperature of certain physical properties have special features in the neighbourhood of the normal melting points. We have begun an investigation of phenomena of this type, which are potentially important for the molecular physics of liquids, and in exploratory work have found a somewhat similar effect in the electrical conductivity of an ionic aqueous solution.

Some authors, working with pure compounds, have reported instances in which a plot of the logarithm of the viscosity against the reciprocal absolute temperature appears to take the form of two straight lines which intersect at the normal melting point. Analogous effects have been reported for the dielectric constant and ultrasonic velocity of some organic liquids and the electrical conductivity of some non-aqueous liquids. Greenwood and Martin[1] reviewed this work and directed attention to some unsuspected further cases in published data. More recently, Urazovskii and Chernyavskii[2] have reported similar effects at the melting points of metastable crystal forms of the solid as well as the normal melting point. The results of Parthasarathy and Bindal[3], which show discontinuous ultrasonic velocity values at the melting point, require confirmation. Some of the foregoing results are marginally significant, and could be just as well represented by smooth curves as by curves with a discontinuity in the slope.

On the other hand, Hunter[4,5], working with three organic liquids for which discontinuities of slope in the logarithmic-viscosity and dielectric constant curves had already been reported, found that ultrasonic absorption increased rapidly but smoothly as the melting point was approached from above. McLaughlin and Ubbelohde[6,7] found analogous smooth effects in the viscosities of an organic substance and

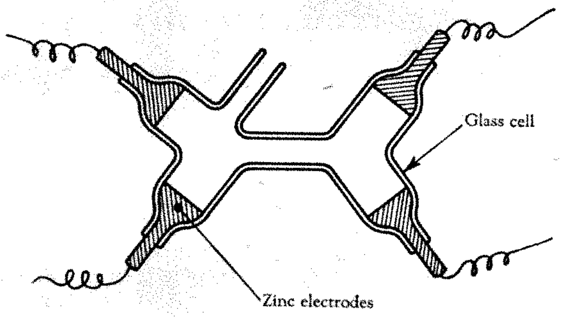

Fig. 1

two metals. They assumed clustering was involved and made deductions about the amount of clustering, using the theory of the viscosity of suspensions.

The reports of discontinuities in slope are remarkable, for they suggest a change in the structure of the liquid occurring or beginning abruptly at the melting point, even though this point is defined by equality of the thermodynamic potentials of the liquid and normal solid phases, and so depends on the structure of both. On the other hand, the rapidly increasing, smooth deviations of the second group of results have the general character of 'pre-transition' effects[8].

The true forms of the temperature plots in the neighbourhood of the melting point must be regarded as rather uncertain at present, especially for liquids for which discontinuities of slope have been claimed. A need exists for measurements of higher accuracy on highly pure liquids, of which the most amenable to preparation with high purity include water and dilute aqueous solutions.

We have obtained accurate exploratory data for the ionic conductivity of dilute aqueous solutions of potassium chloride using a four-terminal cell with platinum black electrodes, and of zinc chloride using both a similar cell and one with zinc electrodes. In one case the phenomenon observed was as follows.

A solution containing approximately 1 per cent by weight of the salt (anhydrous) was prepared from zinc of 99·99 per cent purity. Resistance measurements were made to an accuracy of 3 parts in 100,000 at a frequency of 170 c/s using the cell with four zinc electrodes (Fig. 1). A special double-bridge circuit was used. The temperature was held constant to ±0·001° C at each point, and measured by platinum resistance thermometry. Two sets of data are shown in Fig. 2, plotted as deviations from a relationship of the form:

$$\log_{10} R/R_0 = -A + B/(C+T)$$

where R/R_0 is the ratio of the resistance at T° C to the value at 0° C, and the values of B and C are approximately 144° and 104° C respectively. Fig. 2 shows a smooth deviation of approximately exponential form, rising to significance below the freezing point (−0·36° C). There is no suggestion of any sort of discontinuity.

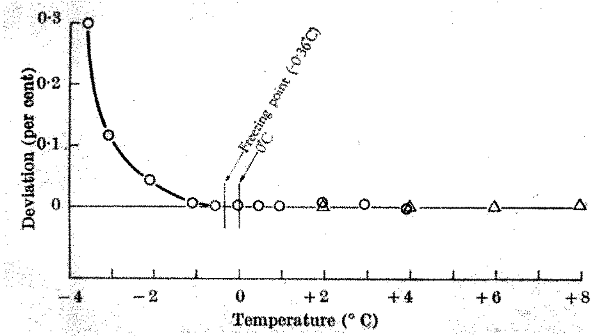

Fig. 2. Percentage deviation of the electrical conductivity of a 1 per cent aqueous solution of zinc chloride from an empirical equation relating conductivity and temperature

In our other experiments, including one made with zinc chloride solution under apparently identical conditions, no significant deviation from the above equation was found down to −3·5° C, the lowest temperature used. The root-mean-square deviations of the results from smooth curves approximate 4 parts in 100,000.

The close proximity of the phenomenon of Fig. 2 to the freezing point suggests that the normal-ice structure is involved. This view is reinforced by the results of Urazovskii and Chernyavskii showing effects corresponding to the melting points of metastable as well as stable solid forms. However, our experiments show no evidence that a change in the structure of the basic liquid develops below the normal freezing point. The phenomenon of Fig. 2 appears to be a manifestation of suspended heterogeneous phase nucleation.

As well as continuing these measurements we are obtaining accurate results for the viscosities of the solutions and for pure water.

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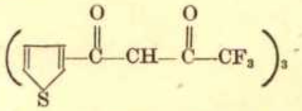
Fluorescent Properties of Rare Earth Chelates in Vinylic Hosts

Both single crystals and glasses[1] have been successfully used as hosts for rare earth laser materials. We wish to report the observation of sharp line fluorescence of f^n orbital transitions resulting from rare earth chelates dissolved in organic vinylic monomers which were polymerized to solid solutions. These materials are more easily and accurately doped than either glasses or single crystals. The simplicity of preparation and workability of these materials make them particularly attractive for possible laser applications. The observed sharp fluorescent lines may be attributed to uniform distribution and equivalent surroundings of the rare earth ion as found in single crystals.

A large number of organic chelates, co-ordinative compounds and organic salts of the rare earth elements such as neodymium, samarium, gadolinium, dysprosium and holmium have been synthesized. Four β-diketone chelates of 1,1,1-trifluoroacetylacetone; 1,3-diphenyl-1,3 propanedione; 1-phenyl-1,3-butanedione and 4,4,4-trifluoro-1,2-thienyl-1,3 butanedione have been selected out of many other chelates investigated because they satisfy the solubility requirement in monomers and polymers, and in addition exhibit good optical properties and stability. They were prepared from the hydroxides or chlorides of the rare earths and the chelating agent in boiling toluene or xylene. Monomers and comonomers such as methyl methacrylate, styrene and acrylonitrile giving macromolecular materials with known optical transparency have been used. Polymerization with peroxides as catalysts and/or thermal or ultra-violet radiation has been accomplished at normal or high pressures, under inert atmosphere. The method is general and offers a reliable procedure to obtain clear, solid solutions of individually isolated atoms of rare earths, of controllable concentrations.

Rare earth salts or chelates have also been dissolved in vinylpyridine type monomers or co-monomers with styrene and methyl methacrylate, followed by polymerization in absence of oxygen. Other olefinic monomers capable of forming co-ordinative bonds with transition elements are now being investigated in order to test the feasibility of bonding the metal atom directly to the polymeric chain.

In the case of



samarium in polymethyl methacrylate, fluorescent lines were found at 645 mμ, 598 mμ, and 562 mμ at both 300° K and 77° K with excitation into a broad absorption band centred at approximately 360 mμ. These results are in good agreement with those calculated by Wybourne[2] and observed by others[3,4]. The fluorescent lines are respectively attributable to the $A \rightarrow Z$, $A \rightarrow Y$, and $A \rightarrow X$ transitions as designated by Magno[3] and suggested by Crosswhite[5] for anhydrous samarium chloride-doped lanthanum chloride. They also coincide to within 50 Å of those observed by Adams and Millichamp[6], who investigated the electro-fluorescence of samarium-doped α-aluminium-oxide. However, they attributed the spectra to the presence of divalent samarium ion rather than the trivalent ion. The three lines exhibited widths of approximately 15 Å at 300° K for concentrations of 0·02 g/cm^3 samarium chelate in methyl methacrylate. Temperature-dependent line-widths are being investigated.

Broad band luminescence centred at 5200 Å from all the β-diketone chelates in polymethylmethacrylate was excited at an absorption band centred at 4100 Å. This luminescence is attributed to the resonating chelated rings.

Thorough spectroscopic analyses of various rare earth chelate combinations in plastic hosts are being made at liquid helium temperatures.

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CRYSTALLOGRAPHY

Structure of Graphitic Oxide

GRAPHITIC oxide is the pale, non-conducting product formed in treating graphite in suspension with strong oxidizing reagents. The composition is not well defined but is usually given as approximately $C_4O(OH)$ for the most highly oxidized form. The structural information which can be obtained by X-ray methods is limited because the structure is heavily disordered. One strong ring in X-ray patterns indicates an inter-layer *c*-spacing of about 6·2 Å for the dehydrated compound. Two or three weak *hk* bands may be interpreted to indicate a hexagonal structure of the layers with a spacing of 2·45 Å. This and some chemical evidence have suggested that the layers are made up of puckered graphite-like sheets. The various proposals which have been made concerning the bonding of oxygen and OH groups are summarized in recent reviews by Hennig[1] and Croft[2].

The only prospect for obtaining further structural information appears to be offered by electron diffraction techniques; in particular, the electron microprobe diffraction techniques developed in this laboratory and successfully applied to several substances of comparable disorder[3-5]. By this means we obtained single crystal patterns, such as shown in Fig. 1, from small flat platelets of graphitic oxide (diameter 1μ: thickness < 50 Å). This indicates a hexagonal layer structure with spacing ($a_0 = 2{\cdot}43$ Å) and intensities not very different from those of graphite ($a_0 = 2{\cdot}455$ Å). Single-crystal and ring patterns from tilted specimens show that no appreciable ordering exists in the stacking of the layers.

The intensities of the spot patterns and of ring patterns were measured photographically and by the electronic method of Goodman[6]. The structure was investigated by Fourier methods. Fig. 2 is a difference Fourier map of the projected layer structure obtained after subtraction of carbon atoms, at the points indicated, in a hexagonal net. Since the absence of any indication of a superlattice implies

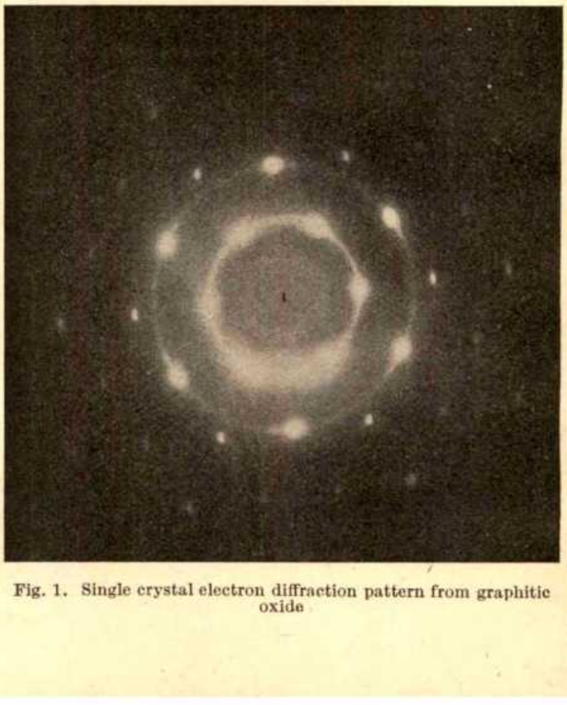

Fig. 1. Single crystal electron diffraction pattern from graphitic oxide

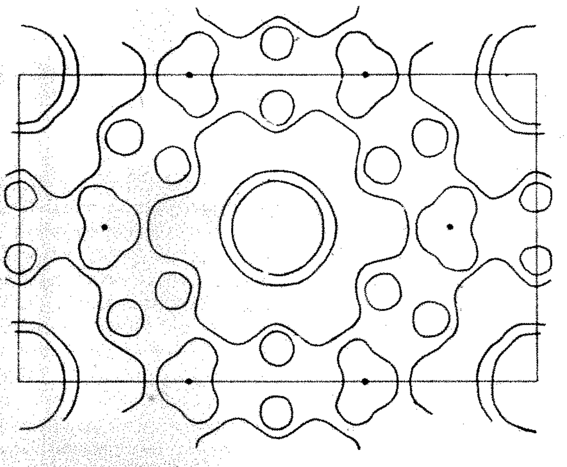

Fig. 2. Difference Fourier map of graphitic oxide layer structure after subtraction of carbon atoms at the points indicated by dots

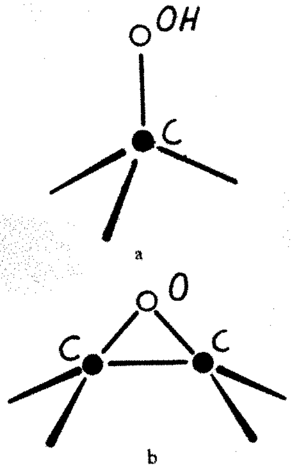

Fig. 3 *a* and *b*. The types of oxygen-bonding deduced from Fig. 2

that the oxygen positions show no long-range ordering, this map indicates the averaged distribution of oxygen atoms in the unit cells. It is seen that, in the projection, the oxygen peaks lie almost at the carbon positions or else half-way between adjacent carbon positions. It is implied that the oxygen atoms are present either as OH groups attached to single carbon atoms with the C–OH bond almost perpendicular to the plane of the layer, as in Fig. 3*a*, or else as oxygen atoms forming a bridge between adjacent carbon atoms, as in Fig. 3*b*.

Fig. 3*a* and 3*b* represent ideal symmetrical cases in which all bonds in equivalent positions relative to the oxygen atom make the same angle with the plane of the layer. This will not necessarily apply in practice because of the lack of order in the layer structure. As a result the C–O bonds will often be tilted relative to these ideal configurations, and the oxygen peaks will be spread out, as observed.

Weak broad maxima occur in Fig. 2 at positions near the centre of the carbon hexagon. These may represent some slight tendency for ordering between adjacent layers, or they may indicate the positions of occasional absorbed water molecules.

The absence of any peak in Fig. 2 on the lines joining alternate carbon atoms suggests that no appreciable fraction of the oxygen atoms is involved in ether linkages between carbon atoms in meta positions, as in the structures proposed by Ruess[7] or by Hofmann *et al.*[8]. The epoxy linkage between adjacent carbon atoms suggested in Fig. 3*b* is not ruled out by chemical evidence and does seem to be favoured by the results of infra-red spectroscopic studies[1,2], although the interpretation of these results is uncertain.

A similar electron diffraction study has been made in methylated graphitic oxide. The single-crystal and ring patterns were almost identical with those of graphitic oxide, the only difference being a weakening of the innermost ring or set of spots. The corresponding difference made to Fig. 2 is a considerable increase in the strength of the peak at the centre or the hexagonal carbon array. This suggests that when a C–OH linkage is replaced by a C–O–CH_3 side-chain, the CH_3 group tends to take up a position above or below the centre of the carbon ring.

One of us (F. A.) thanks the Australian Academy of Science for the award of a Selby fellowship.

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Diamond Replication of Freezing Metal Structures

Manufactured diamond exhibits a wide variety of surface detail which permanently preserves an impression of the immediate environment during the last moments of diamond growth.

In particular, diamonds grown within a molten metal sheath, surrounded by graphite[1], show the freezing pattern of the metal on the diamond faces[2]. These patterns are particularly strong on the diamond cube faces. Fig. 1 illustrates the topography seen on the (100) faces of diamond grown within a nickel–iron alloy catalyst sheath. Examination of similar diamond surfaces in an interference microscope indicates that the main stems of the dendritic pattern are round-bottomed grooves about 1500 Å deep, while the side stems are less deep. The irregular lines separating the dendrites are about 1500 Å above the average surface.

It is suggested that this surface topography is formed as follows. When a diamond-growing experiment is terminated by switching off the heating power to the diamond-growing cell (press force remains unchanged), the metal catalyst sheath cools and eventually freezes. During the metal-freezing process, diamond growth continues in those areas where molten metal remains. The dendrite main stems freeze first, stopping growth locally and hence form the deepest grooves in the diamond surface. Diamond growth continues in other areas until stopped by the freezing of the dendrite side-stems and, finally, by the metal solidification in the dendrite boundaries. This mechanism is consistent with the observed surface contours.

The crystal axis orientation of the diamond determines the dendrite direction and, I presume, crystal orientation in the metal sheath. Dendrite orientation is parallel with the diamond crystallographic axes. In Fig. 1 the diamond crystal sides are at 45° to the long edges of the (100) face photographed. Inclusions in the diamond surface will interfere locally with the dendrite orientation.

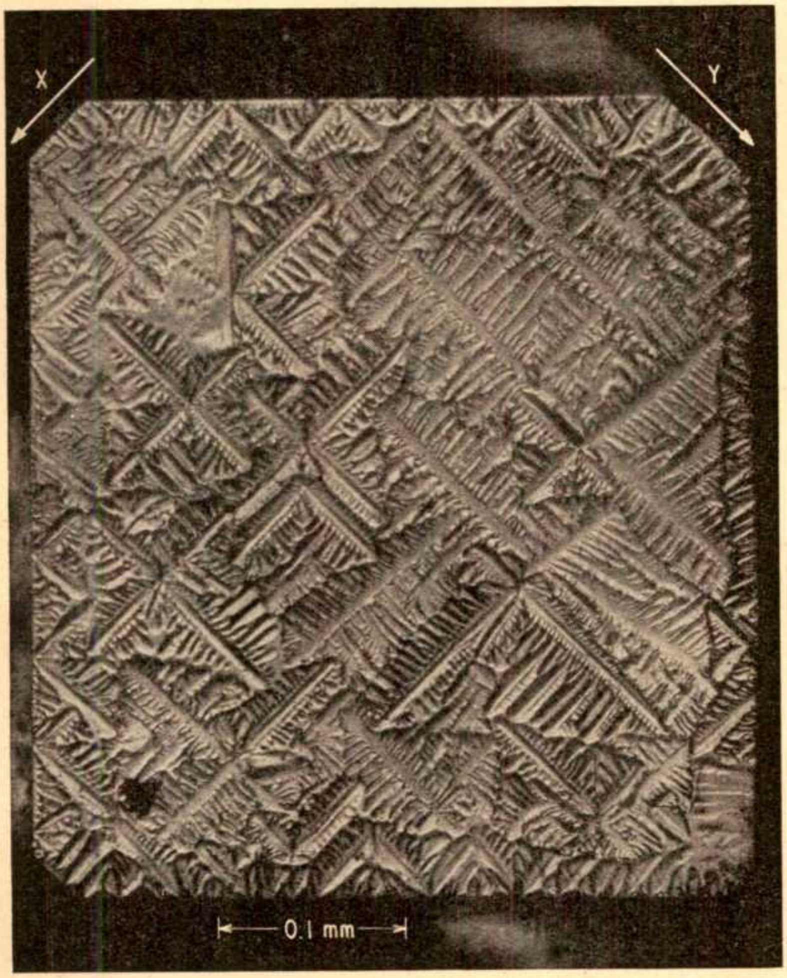

Fig. 1. Dendrite replication on diamond (100) face. Two cubic axes of the diamond are shown

The dendritic pattern is not intrinsic to diamond. When a diamond showing this pattern on a cube-face is etched in a mixture of hot sodium carbonate and nitrate, the surface dendrite pattern disappears. Square etch pits oriented parallel to or at 45° to the crystal axes are formed at the new surface.

The diamond shown was grown by K. A. Darrow of this Laboratory.

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CHEMISTRY

Explosive Sensitivity of Cobalt Ammine Azides

THE correlation of the explosive sensitivity of simple metal azides with the metal's ionization potential has been related to the control of the azide's electrons by the metal and a consequent influence on the activation energy of the decomposition reaction[1]. Gray has stressed the importance of both kinetic and thermodynamic factors in the problem of sensitivity, and pointed out that, in addition to influencing the activation energy, the ionization potential is a major factor in determining the heat of formation of simple metal azides[2]. Thus a correlation with sensitivity may be predicted on both kinetic and thermodynamic grounds and no simple indication of the importance of electron control is possible.

Co-ordination compounds permit some insight into this problem. Very energetic compounds[3] exist with the azides bonded either directly to the metal, $Co(NH_3)_3(N_3)_3$, or isolated from it by the ligand sphere, $[Co(NH_3)_6](N_3)_3$. In the latter the metal cannot directly influence the azide ion's electrons. The azides are distinguishable, the co-ordinated azides having the asymmetric character of covalent azides (HN_3, CH_3N_3) while the ionic azides are symmetric as in simple metal azides[4,5].

Impact testing, despite well-recognized drawbacks, can indicate relative labilities. Our tests used a 2-kg falling hammer and 25 mg samples loosely coned on sand-paper. Crystalline samples (crystal sizes of about 0·1 mm) and powder (0·1–10μ) were prepared as previously reported[6,7]. The 50 per cent points (the drop height which on the basis of 20 tests produces ignition in 50 per cent of the samples) are reported for independent preparations.

The triammine, with only co-ordinated azides, is a very sensitive explosive as expected on both

Table 1. IMPACT SENSITIVITIES OF COBALT AMMINE AZIDES

Compound	50 per cent points (cm)	Character of ignition
$[Co(NH_3)_6](N_3)_3$	22 25 35 33*	Weak to moderate, 5–75 per cent of sample consumed in explosion
$[Co(NH_3)_5N_3](N_3)_2$	17 21 34 18*	Violent. 90–100 per cent complete
trans$[Co(NH_3)_4(N_3)_2]N_3$	13 22 41	Violent, complete
cis$[Co(NH_3)_4(N_3)_2]N_3$	7 43 55	Violent, complete
$Co(NH_3)_3(N_3)_3$	2·8* 3·2*	Violent, complete

* Powders.

thermodynamic grounds and arguments on the control of electrons. The tetraammines and pentaammine show a considerable range of 50 per cent points. It is suspected that the 7-cm *cis*-tetraammine preparation may have been sensitized by contamination with the triammine.) It is possibly of interest that these compounds are less sensitive than the triammine and comparable with the hexaammine despite the presence of co-ordinated azides.

The hexaammine with only ionic azides is particularly important. This compound does ignite at a low drop height, thus establishing that an azide may be quite sensitive even though the metal is isolated from the azide ion. Presumably, reaction begins in impact generated hotspots and by means of a high heat of reaction propagates to visible proportions in spite of an apparently unfavourable structure.

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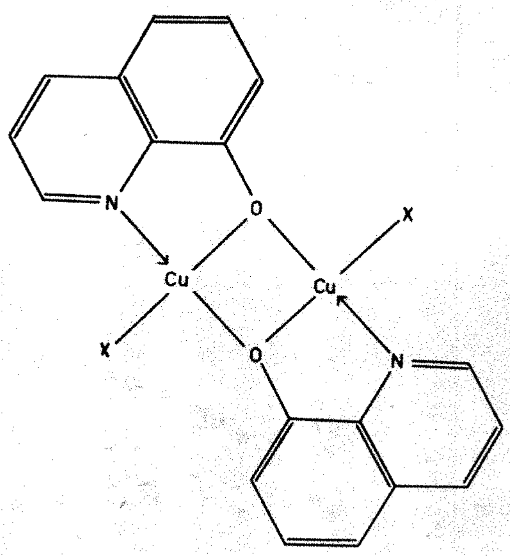
Anomalous Paramagnetism and Binuclear Structure of some Copper(II) Complexes of 8-Hydroxyquinoline

Fanning and Jonassen[1] reported that the reaction of copper(II) halide with 8-hydroxyquinoline in methanol or acetone yields compounds of the empirical formula $Cu(C_9H_6NO)X$ ($X = Cl, Br$) which possess magnetic moments in the range 1·0–1·1 Bohr magnetons at room temperature. They proposed an unusual tetrameric structure to explain the properties of these compounds and suggested that their anomalous paramagnetism and the presence of an absorption band at ~ 375 mμ in their reflectance spectra are indicative of 'δ bonds' between copper atoms.

We have synthesized the chloro-complex (found: C, 44·1; H, 2·5; N, 6·0; Cu, 25·9 per cent. Calc. for $C_9H_6ONCl\ Cu$: C, 44·4; H, 2·5; N, 5·8; Cu, 26·1 per cent) by reacting copper(II) chloride with *bis*-(8-hydroxyquinolinato)copper(II) in boiling ethanol and have confirmed its low magnetic moment (see table). The reaction of excess copper(II) nitrate with 8-hydroxyquinoline in warm 98 per cent ethanol has been found to produce the reddish-brown nitrato-complex $Cu(C_9H_6NO)NO_3.H_2O$ (found: C, 37·9; H, 3·0; N, 9·9; Cu, 21·9 per cent. $C_9H_8O_5N_2Cu$ requires C, 37·6; H, 2·8; N, 9·7; Cu, 22·1 per cent). This compound is a non-electrolyte in nitrobenzene solution and possesses a room temperature magnetic moment (see table) which is less than the 'spin-only' value for one unpaired electron.

The anomalously low paramagnetism of these complexes may be interpreted in terms of the binuclear oxygen-bridged structure I ($X = Cl, Br, NO_3$) in which electron-exchange demagnetization occurs between pairs of oxygen-bridged copper(II) atoms. This type of electron exchange occurs in the copper(II) complex of acetylacetone-mono-(*o*-hydroxyanil). This compound possesses the binuclear oxygen-bridged structure II and its magnetic properties are similar to those of binuclear copper(II) acetate[2].



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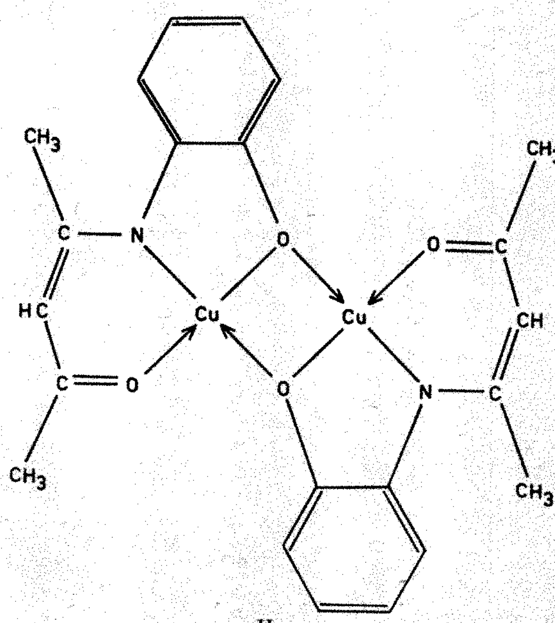


II

Such a structure precludes the possibility of 'δ-bond' interactions between the pairs of copper atoms. Electron exchange demagnetization between pairs of oxygen-bridged copper(II) atoms has also been recently observed in the pyridine- and quinoline-N-oxide complexes $Cu(C_5H_5NO)X_2$ and $Cu(C_9H_6NO)X_2$ ($X = Cl, Br$)[3].

The postulate of a binuclear structure such as I for the complex of copper(II) nitrate with 8-hydroxyquinoline is supported by magnetic susceptibility measurements of the nitrato-complex over the temperature range of 95°–363° K. The μ–T and χ_M–T curves (Fig. 1) are in agreement with those calculated from Bleaney and Bowers's[4] expression:

$$\chi_M = \frac{g^2N\beta^2}{3kT}[1 + 1/3 \exp (J/kT)]^{-1} + N\alpha$$

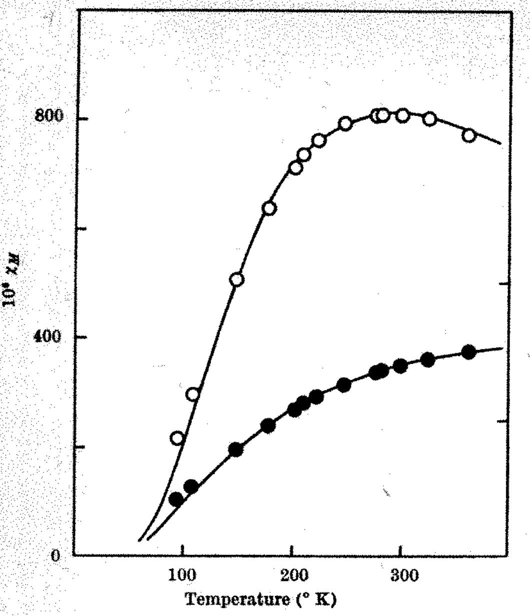


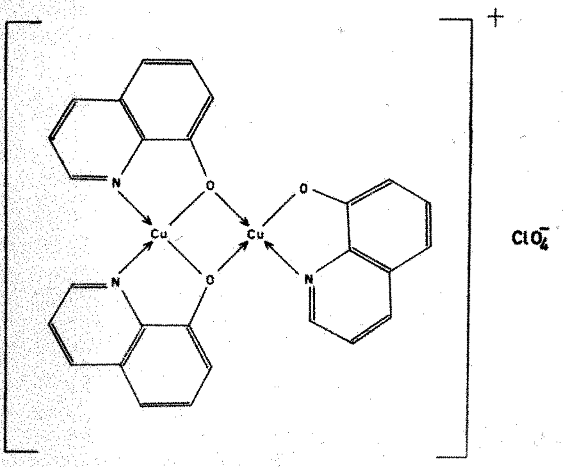
Fig. 1. ○, χ_M observed; ●, μ observed ; —, curves calculated from Bleaney and Bowers's expression:

$$\chi_M = \frac{g^2 N\beta^2}{3kT}\,[1 + \tfrac{1}{3}\exp(J/kT)]^{-1} + N\alpha$$

and closely resemble those for copper(II) acetate[5] and compound II (ref. 2).

The presence of an absorption band at ~375 mμ together with its Z-axis polarization property has been associated with the binuclear copper(II) acetate type of structure[6]. However, the occurrence of a band at ~375 mμ in the above chloro- and bromo-complexes[1] is not diagnostic of the presence of either anomalous paramagnetism or a copper(II) acetate type of structure since it is known to occur in compounds such as *bis*(acetylacetonato)copper(II)[7] which are monomeric and possess normal magnetic moments.

The reaction of *bis*(8-hydroxyquinolinato)copper(II) with hexaquo-copper(II) perchlorate in ethanol gives brown crystals of the perchlorate $[Cu_2(C_9H_6NO)_3]$-$ClO_4.0{\cdot}5C_2H_5OH$ (found: C, 49·7 ; H, 3·3 ; N, 6·2 ; Cu, 18·5 per cent. $C_{27}H_{18}O_7N_3ClCu.0{\cdot}5C_2H_5OH$ requires C, 49·3 ; H, 3·1 ; N, 6·2 ; Cu, 18·6 per cent). This compound has a molecular conductivity of 24·2 mho in $3{\cdot}3 \times 10^{-4}$ M nitrobenzene solution and undoubtedly possesses the oxygen-bridged structure (III) in keeping with its low magnetic moment (see Table 1).

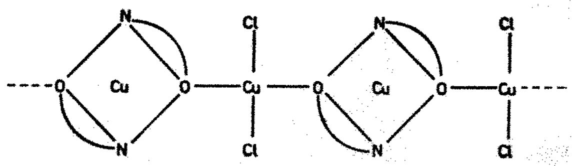


III

Table 1. MAGNETIC MOMENTS OF SOME 8-HYDROXYQUINOLINE (C_9H_6NOH) COMPLEXES OF BIVALENT COPPER

Compound	Temp. (°K)	Magnetic moment per Cu atom (in Bohr magnetons)
$Cu(C_9H_6NO)_2$	300	1·89
$Cu(C_9H_6NO)_2.2H_2O$	297	1·92
$Cu(C_9H_6NO)Cl$	293	1·14
$Cu(C_9H_6NO)NO_3.H_2O$	296	1·39
$[Cu_2(C_9H_6NO)_3]ClO_4$	295	1·60

The magnetic moment of the chloro-complex $Cu(C_9H_6NO)Cl$ was found to vary from 0·5 Bohr magnetons at 113° K to 1·4 Bohr magnetons at 372° K. However, it was not possible to fit its susceptibility curve to one calculated from Bleaney and Bowers's expression (see foregoing). It is possible that this compound possesses the polymeric structure IV rather than a dimeric one similar to I.



IV

Such a structure is compatible with the compound's preparation from *bis*(8-hydroxyquinolinato)copper(II) since in this compound the oxygen atoms would occupy trans-positions in a planar arrangement about the copper atom.

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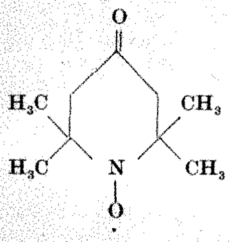
Free Radical Reactions involving no Unpaired Electrons

REACTIONS between free radicals and molecules have been extensively investigated. These involve numerous reactions of hydrogen abstraction and substitution with the contribution of unpaired electrons[1-3]:

$$A\cdot + BH = AH + B\cdot$$

In studying the effect of various factors on the stability and reactivity of free radicals, we first succeeded in observing a fundamentally new type of radical reactions with persisting free valence of the initial radical.

Investigations involved a very stable nitrogen oxide of triacetoneamine, the stability of which is accounted for by purely steric factors:



This stable free radical of tetravalent nitrogen may be readily obtained by careful oxidation of triacetoneamine with hydrogen peroxide in the presence of a tungsten, molybdenum or vanadium salt. By chromatographic separation of reaction products on aluminium oxide, we succeeded in obtaining an individual free radical of tetravalent nitrogen.

The triacetoamine-*N*-oxide is a crystalline paramagnetic substance melting at 36° C, soluble in water and ether to form red-coloured solutions.

Anal. calc. for $C_9H_{16}NO_2$: C, 63·49; H, 9·47, N, 8·23; spin/mole 6·2 × 10^{23}. Found: C, 63·53; H, 9·60; N, 8·25, spin/mole, 6·1 × 10^{23}.

The solid substance displays electron spin resonance singlet. On dissolving the substance in benzene it becomes a triplet with 13·3 gauss splitting and a *g*-factor of 2·0064 (Fig. 1). The triplet nature of the spectrum due to coupling of the unpaired electron magnetic moment with that of the nitrogen nucleus is evidence that considerable spin density is localized on nitrogen.

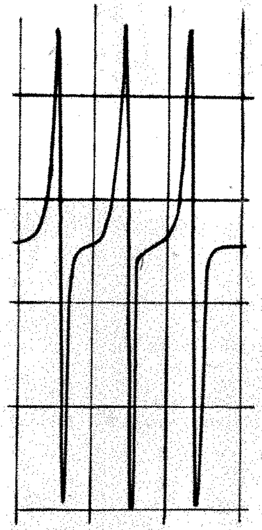


Fig. 1. Electron spin resonance spectrum of triacetone nitrogen oxide in benzene solution

On one hand, catalytic reduction of the radical in the presence of palladium on barium sulphate yields a diamagnetic hydroxylamine derivative identical to that obtained from phoron and hydroxylamine[4] (I).

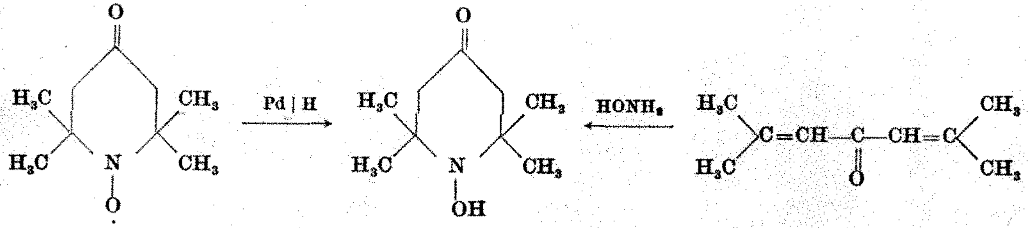
On the other hand, due to considerable steric hindrance close to the heteroatom carrying the free valence, this radical may enter into characteristic reactions with respect to the carbonyl group, without affecting the free valence. For example, the oxime radical (II) is formed by interaction between triacetoneamine-*N*-oxide and hydroxylamine.

On recrystallization from alcohol this oxime radical is in the form of well-defined rose-coloured crystals with a melting point of 155° C, readily soluble in water and yielding red-coloured solutions.

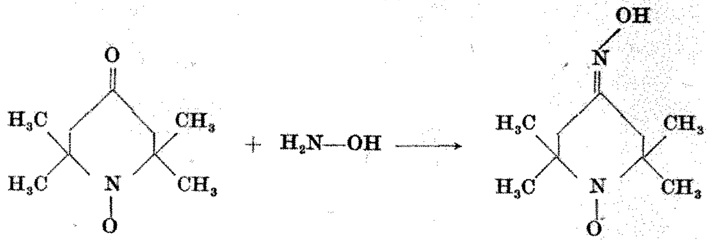
Anal. calc. for $C_9H_{17}N_2O_2$: C, 58·51; H, 9·25; N, 15·12; spin/mole, 6·2 × 10^{23}. Found: C, 58·45; H, 9·37; N, 15·25; spin/mole, 6·0 × 10^{23}.

The reactions of this radical with semicarbazide and 2·4-dinitrophenylhydrazine are similar. A yellow semicarbazone radical and an orange 2,4-dinitro-

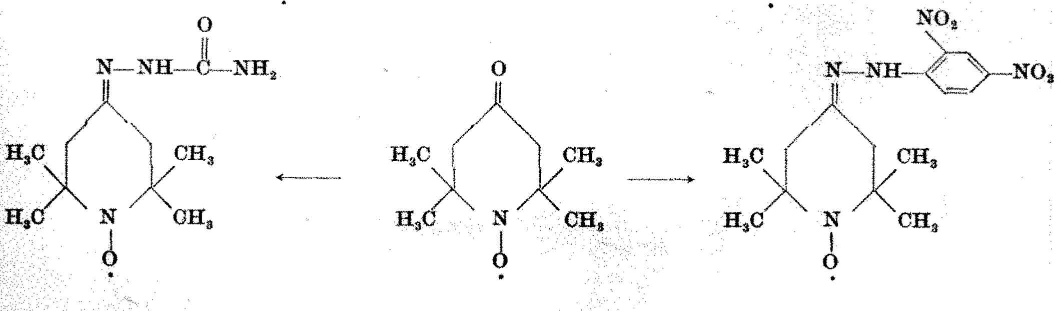
(I)



(II)



(III)



phenylhydrazone radical (III) are formed by these reactions.

On dissolving in benzene, electron spin resonance singlets displayed by solid radicals become triplets similar to that of the initial ketone radical.

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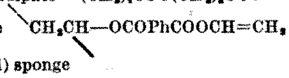
Triboelectricity in Polymers

It must be emphasized that the reality of a triboelectric series implies a single determinant property acting at both donor and acceptor surfaces. An 'abundance effect'[1], if real, may be a cause of minor anomalies, but it cannot be dominant since metals, which may be inserted in the series, contain incomparably more conduction electrons than do insulators. The Frenkel mechanism[2] of thermal excitation alone is inadequate, since pyrolysis would occur, in the 100 μsec suggested by Frenkel, below 500° C, at which the population of conduction electrons might well be only 100 per c.c. Since it is known that calendering may reduce the molecular weight of polymers, I suggest that free radicals, produced by mechanical stress, form a source of relatively high-energy electrons, easily excited to the conduction-level. The much-discussed charge transfer by non-rubbing contact remains improbable except between distant members of the series.

My triboelectric series of well-defined polymers shows no correlation with dielectric constant, with dipole moment, nor indeed with the colour or intensity of ultra-violet-excited luminescence, which renders unlikely the proposed role of electron traps[3]. Members tend to be grouped by chemical nature, pointing to a determinant property linked to molecular constitution, perhaps more particularly to that of the surface[4]. The Fermi-level remains the most reasonable series-determining property[5].

TRIBOELECTRIC SERIES

(positive end)
Silicone elastomer with silica filler
Borosilicate glass, fire-polished
Window glass
Aniline-formol resin (acid catalysed) $—CH_2—Ph—NH—$
Polyformaldehyde $—CH_2—O—$
Polymethylmethacrylate $—CH_2—CMe(COOCH_3)—$
Ethylcellulose
Polyamide 11 $—(CH_2)_{10}CO—NH—$
Polyamide 6-6 $—(CH_2)_4CO—NH(CH_2)_6NH—CO—$
Rock salt Na^+Cl^-
Melamine formol
Wool, knitted
Silica, fire-polished
Silk, woven
Poly-ethylene glycol succinate $—(CH_2)_2COO(CH_2)_2OCO—$
Cellulose acetate
Poly-ethylene glycol adipate $—(CH_2)_4COO(CH_2)_2OCO$
Poly-diallyl phthalate 
$\diagdown CH_2CH—OCOPhCOOCH{=}CH_2$ $\diagdown$
Cellulose (regenerated) sponge
Cotton, woven
Polyurethane elastomer $—NHPhNHCOOROCO—$
Styrene acrylonitrile copolymer
Styrene butadiene copolymer
Polystyrene $—CH_2—CHPh—$
Polyisobutylene $—CH_2—CMe_2—$
Polyurethane flexible sponge
Borosilicate glass, ground surface
Polyethylene glycol terephthalate $—COPhCOO(CH_2)_2O—$
Polyvinyl butyral
Formo-phenolique, hardened $—CH_2—PhOH—CH_2—$
Epoxide resin $—OPhCMe_2PhOCH_2CHOHCH_2—$
Polychlorobutadiene $—CH_2CCl{=}CHCH_2—$
Butadiene acrylonitrile copolymer
Natural rubber $—CH_2CMe{=}CHCH_2—$
Polyacrylonitrile $—CH_2—CH(CN)—$
Sulphur
Polyethylene $—CH_2—$
Polydiphenylol propane carbonate $—OPhCMe_2PhOCO—$
Chlorinated polyether $—CH_2—C(CH_2Cl)_2CH_2O—$
Polyvinylchloride with 25 per cent D.O.P.
Polyvinylchloride without plasticizer $—CH_2—CHCl—$
Polytrifluorochlorethylene $—CF_2CFCl—$
Polytetrafluoroethylene $—CF_2—$

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Complexity of Hop Oil

Progress in instrumental methods of analysis during the past 10 years, particularly in the new field of gas chromatography, has provided the chemist with powerful tools for the separation and identification of complex mixtures. Nevertheless, it is often advantageous to fractionate the mixture prior to gas chromatography analysis in order to simplify the task of isolation and identification of single components. The oil of the hop (*Humulus lupulus* L. var. Bullion) isolated by the method of Wright and Connery[1] has been fractionated as follows: hydrocarbons were selectively separated from oxygenated compounds through alumina or silica-gel columns using light petroleum ether as eluant[2]. Esters and ketones were separated from alcohols by selective elution from a neutral alumina column using absolute ether as eluant. Esters were subjected to methanolysis using mineral acid as catalyst, and the resulting methyl esters separated from the freed alcohols, as described here. Compounds having a straight chain of seven carbons or more were separated from shorter or branched chain compounds by formation of crystalline adducts with urea in methanol[3]. Carbonyls were extracted from mixtures containing other oxygenated compounds by the Girard method[4,5]. Unsaturated compounds were separated from saturated compounds by bromination in carbon tetrachloride[6]. Unsaturated compounds having a conjugated double-bond system were separated from others by the Diels–Alder reaction[7].

Mishandling of essential oils may result in the destruction of some components and the formation of artefacts. Flash-evaporation of compounds in the injection chambers of gas chromatographs has often led to the pyrolysis of some esters and the dehydration of some terpene-alcohols[8].

The following hop oil fractions were obtained.

(1) A hydrocarbon fraction representing 70 per cent of the total oil was analysed by gas chromatography and found to contain 21 and possibly 26 compounds. The three largest components isolated and identified

by mass spectral analysis were myrcene (30 per cent), caryophyllene (20 per cent), and humulene (40 per cent). The remaining 10 per cent of this fraction was tentatively identified as a mixture containing pinene, limonene, farnesene, several other terpenes, and sesquiterpenes.

(2) The total oxygenated fraction representing 30 per cent of the whole oil was found to contain a mixture of esters, carbonyls, and free alcohols.

(3) In the carbonyl fraction, methyl nonyl ketone was the main component; but the entire homologous series of methyl ketones from acetone to hexadecanone was tentatively identified. There was also strong evidence of the presence of eight-branched ketones. Several saturated, unsaturated, and branched aldehydes were tentatively identified, including citral and the homologous series of normal aldehydes from propyl to undecyl.

(4) The alcohol fraction was also found to be a complex mixture. Isoamyl alcohol and linalool represented the major constituents, followed by geraniol, nerolidol, nerol, terpineol, and many others. Tentatively identified were *n*-amyl, hexyl, heptyl, octyl, nonyl, decyl, and undecyl alcohols.

(5) The ester fraction, analysed by gas chromatography, was a mixture of compounds having a wide boiling range[6,9]. Tentatively identified were linalyl acetate, geranyl formate, geranyl acetate, and geranyl isobutyrate. Upon methanolysis and separation from the freed alcohols, the methyl esters were analysed by gas chromatography. Approximately 60 peaks were recognized. The methyl esters were then separated into three sub-fractions: (*a*) the straight-chain, saturated esters; (*b*) the branched esters; (*c*) the unsaturated esters. Thus nine straight-chain normal acids from hexanoic to tetradecanoic were identified, plus six-branched unsaturated acids, fourteen saturated branched isoacids which did not complex with urea, and two-branched acids which did form urea complexes due to the presence of straight-chains of at least seven carbons.

Of the six branched and unsaturated acids already named, two were originally present in hop oil as methyl esters. Although results are still inconclusive, we agree with Roberts's[6] suggestion that these esters probably are isometric methyl geranates.

Positive identification of the most important components of hop oil is in progress at this laboratory, using infra-red, mass and nuclear magnetic resonance spectroscopy in conjunction with gas chromatography.

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BIOPHYSICS

Restricted-Diffusion Chromatography through Calibrated Columns of Granulated Agar Gel; a Simple Method for Particle-size Determination

RESTRICTED-DIFFUSION chromatography through columns of granulated agar gel, as introduced by Polson[1], has been used effectively for virus purification[2]. It is now proposed that the method may be satisfactory for size determination of cell components, viruses, and proteins, and will thereby supplement the existing methods of electron microscopy, sedimentation, X-ray diffraction, and light scattering. The advantage of this technique over other 'gel-filtration' methods for the fractionation and size determination of these larger particulates is based on the fact that the pores of most agar gels are so large that such particles diffuse into them[3] and become retarded as they pass through the column with the degree of retardation increasing as particle size decreases.

An accurate method for determination of the effective pore size of agar gels, described in a previous article[3], has proved very useful in selection of the proper concentration of agar to be used in columns for the fractionation of specific particles of known sizes. As we expanded our use of agar-gel columns the need for more thorough knowledge of their properties became evident. Therefore, we established, as described previously[2], standard columns (100 cm long and 1·5 cm in diameter) of 1, 2, 3, 4, 5, 6, 7, and 8 per cent agar granules and determined the volume of effluent liquid in which the peak concentration of a series of particulates of known size appears.

In these studies the samples were run at room temperature and a standard buffer (0·2 M sodium dihydrogen phosphate, 0·3 M sodium chloride adjusted to *p*H 6·8 with 5 N sodium hydroxide) was used throughout.

All columns were operated by gravity flow and were adjusted to give a flow-rate of 5–10 ml./h. A 2 ml. suspension of the desired particles was applied at the top of a column and 1·5–2 ml. samples were collected. Appearance of the particles in the eluate was followed with a recording spectrophotometer. With the information thus obtained we constructed calibration curves (Fig. 1) which show the behaviour of spherical or nearly spherical particles as they pass through the gel columns.

Particles used for calibration were:

		Approximate diameter (mμ)
(1)	Shope papilloma virus (ref. 4)	45
(2)	Southern bean mosaic virus (SBMV) (ref. 5)	28
(3)	Coliphage *f2* (ref. 6)	20
(4)	Ferritin (ref. 7)	9·4
(5)	Hæmoglobin (ref. 8)	6
(6)	Lysozyme (ref. 9) (estimated from molecular weight data)	4

Duplicate runs on identical suspensions gave readings within 2 ml. of each other. Duplicate columns gave somewhat greater variability with all points displaced in the same direction by 0–6 ml. as would be expected with slight differences in the agar gels or in the packing of columns.

Most samples were diluted approximately 10-fold on passage through each column; however, there was less spread of the smaller components on 7 and 8 per cent agar. Agar chips for all calibrated columns were between 40 and 60 mesh in size. A sample column containing 6 per cent agar, 60–80 mesh,

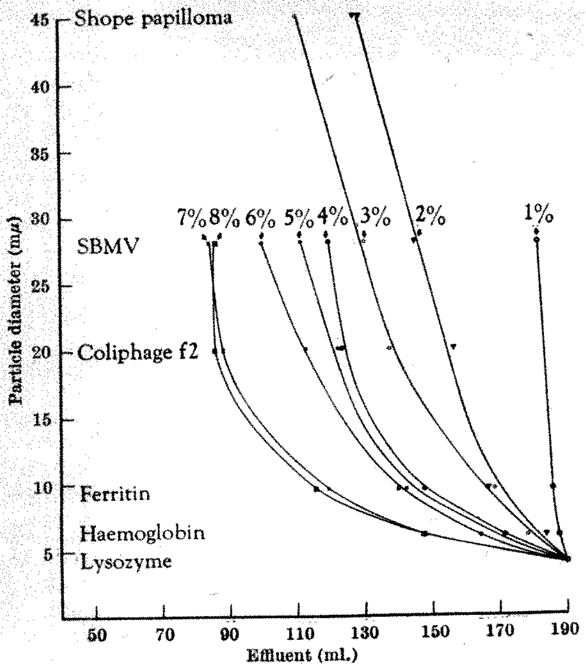

Fig. 1. Calibration curves for granulated agar columns. Note that the position of lysozyme for all curves is at 190 ml. For greater ease of comparison all curves which did not have lysozyme at this average point were shifted to right or left to make this one point coincide. Since the entire curve was shifted in each instance, relative positions of points on any one curve were retained. Each point represents the peak concentration of the component

gave nearly the same sample spread and position of points as did the corresponding column of 40–60 mesh size. Very fine agar granules were not tested for possible reduction in sample spread.

Once a column is calibrated within the proper size range it becomes a useful tool for the easy determination of particle size for unknowns. One need only introduce the test suspension at the top of the calibrated column, assay samples to determine effluent liquid volume to the peak of concentration of the test sample, and locate this point on the calibration curve. Furthermore, if a suitable assay method is available for determining the concentration of the desired particle and distinguishing it from contaminants one can even determine particle size in relatively impure preparations.

A standard buffer of *p*H 6·8 was used for the present work, but other buffers can be satisfactorily used and must be used if the columns are operated at 4° C, at which temperature our standard buffer crystallizes. We have had satisfactory separation of particulates in 0·01 M buffer and at *p*H 8, but the limitations in buffer and *p*H have not yet been throughly explored.

Our calibration curves are based on spherical or nearly spherical particulates, and any appreciable deviation from sphericity would tend to alter the position of peak concentration of a component. Thus noticeable errors in size determination could be experienced if the particulate being studied were not spherical.

Of interest are the facts that our 8 per cent agar membrane, reported earlier[3], had pore diameters of 18·6 mμ whereas the 7 per cent agar had pore diameters of 20·2 mμ, and that we have found the coliphage *f*2 particles, which are 20 mμ in diameter, to be slightly retarded in the 7 per cent agar-gel columns but not in the 8 per cent.

From a careful study of the calibration curves, one can obtain information useful in the purification and separation of large molecules, viruses, and cellular fragments. For example, one can pass a sample rapidly through 8 per cent agar and separate the large component, 20 mμ and over, from most of the smaller particulates. Agar gels with larger pore sizes can then be utilized for fractionation of these larger particles. Likewise 7 or 8 per cent agar columns can be useful in the fractionation of the smaller particulates.

The calibration curves presented in Fig. 1 are useful for determining the general properties of each gel concentration and for relating columns of one agar gel with those of another. However, one should not assume that any 100 cm × 1·5 cm column of a given per cent granulated agar gel will agree precisely with that presented here. Each column to be used for particle-size determination should be calibrated for this purpose with a series of particulates of known size and with the buffer to be utilized with the test sample.

We thank N. D. Zinder for supplying the coliphage *f*2 and C. A. Knight for supplying the Shope papilloma virus.

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Effect of Adsorbed Proteins on the Wettability of Hydrophilic and Hydrophobic Solids

WHEN barium stearate or other hydrophobic powders are exposed to blood plasma they remove certain coagulation factors such as factor V[1] and fibrinogen[2], and their surface becomes hydrophilic. Barium sulphate, aluminium hydroxide and other hydrophilic particles adsorb other coagulation factors preferentially (prothrombin, factors VII, IX and X), but changes of wettability at their surface are less obvious. To indicate if one protein may have areas of less polarity by which it can attach itself to less-wettable substrates, leaving its more polar sites exposed, and vice versa, the relative wettability of thrombin and fibrinogen adsorbed on to glass and lucite was observed as follows.

A pattern of three partially overlapping rectangles was outlined on paper (Fig. 1); a ⅛-in. thick 'Lucite' plate was placed on it and covered over area *ABC* with thrombin ('Topical, Bovine', Parke, Davis and Co., Detroit; 12·5 U. per ml. 0·85 per cent sodium chloride). The plate was tilted after approximately 1 min (edge *ABCEF* pointing down), rinsed with

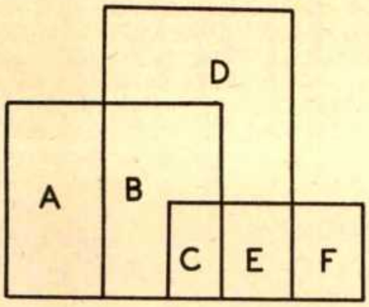

Fig. 1

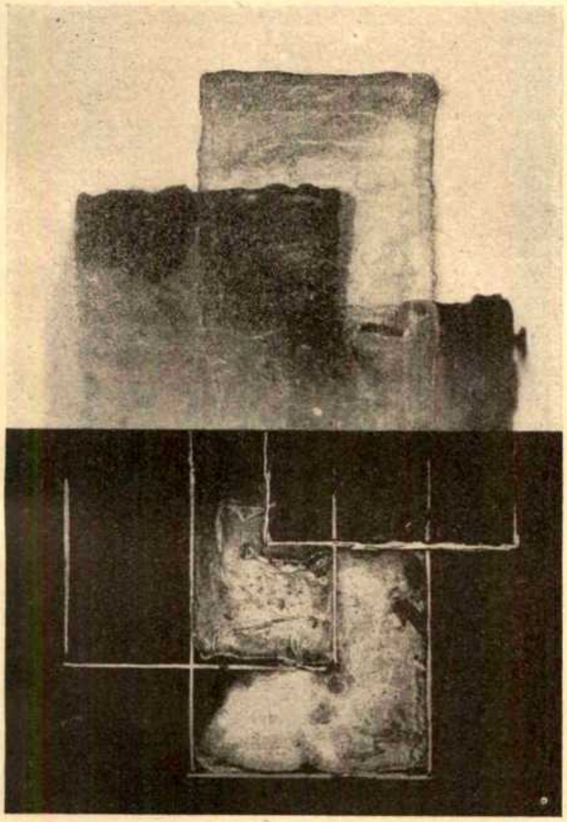

Fig. 2. Upper half, thrombin and fibrinogen pattern on lucite plate; lower half, same on glass plate with left and right position reversed to match pattern outline. Background on glass is black because water droplets (showing white on lucite background) fuse and have low contact angle. Both prints are positives

20 ml. 0·85 per cent sodium chloride followed by large amounts of cold running tap-water and finally with distilled water, and allowed to dry at room temperature. Next, area *DBCE* was covered with fibrinogen ('Human Fraction' I, Mann Res. Lab., New York; 2 mg per ml. 0·85 per cent sodium chloride) and the plate was rinsed and dried as before. Finally, area *CEF* was treated with the thrombin solution as had been area *ABC*. After drying, and cooling in a refrigerator, the plate was placed face down over a 50° C water bath; as soon as water had condensed on it sufficiently to reveal marked contrasts, the plate was held with the wet side facing, but not touching, a black velvet cloth and a photograph was taken using oblique flash. The more hydrophobic areas containing more droplets with high contact angle reflected more light.

Exactly the same procedure was followed using a ¼-in. thick plate of glass instead of 'Lucite'; wax crayon outlines were needed here to prevent random flow of reagents.

The combined photographs (Fig. 2) indicate the following: the surface of fibrinogen adsorbed either on glass or on 'Lucite' is less hydrophilic than that of adsorbed thrombin (area *D* is lighter than areas *A* and *F*). The wettability of thrombin adsorbed on to glass is lowered more after exposure to fibrinogen than is that of thrombin adsorbed on to 'Lucite' (area *B* is lighter than area *A* on glass but not on 'Lucite'. Similarly, the wettability of fibrinogen adsorbed on glass is increased more by exposure to thrombin than that of fibrinogen adsorbed on 'Lucite' (difference between areas *E* and *D* is greater on glass than on plastic).

Though fibrinogen does appear to have a less-polar surface on glass than on lucite, single observations on glass partly coated with a repellent were needed to avoid variations in technique; they confirmed our findings.

These results suggest that non-polar sites on fibrinogen will adhere to those on thrombin, and that adsorption of either molecule on an apolar substrate hinders this 'non-polar bonding'. This would be in agreement with the finding[3] that substrates are hydrolysed most by thrombin if they contain apolar side-chains.

To distinguish between change of polarity by removal of a single layer and by formation of a double layer, actual thickness measurements are needed. Ellipsometry[2,4] is used for this purpose.

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BIOCHEMISTRY

A New Variant of Human Transferrin in a Venezuelan Family

THE most prolific of the hereditary variations among the human serum proteins is that of the iron-transport protein transferrin. The initial demonstration of genetically determined transferrin heterogeneity[1] has been followed by numerous reports of additional variants, until at the present time thirteen transferrins have been described: B_0 (ref. 2), B_{0-1} (ref. 3), B_1 (ref. 4), B_2 (ref. 5), B_3 (ref. 6), C, D_0 (ref. 2), D_4 (ref. 7), D_{Montreal} (ref. 8), D_{Chi} (ref. 6), D_1 (ref 9), D_2 (ref. 4), and D_3 (ref. 2). Each of the molecular species of transferrin has been identified by its characteristic mobility in starch-gel electrophoresis. Transferrin C is common in all populations; variants of more rapid mobility than transferrin C have been named B, and slower-moving variants have been labelled D. Where convenient, subscripts have been chosen to define the relative electrophoretic mobilities of variants within the fast- and slow-moving categories. In certain cases, particular transferrins appear to be restricted to specific populations; thus, transferrin B_{0-1} occurs in 8 per cent of Navajo Indians, B_2 in 1 per cent of Caucasians, D_{Chi} in 6 per cent of Chinese, and D_1 in 10 per cent of American Negroes.

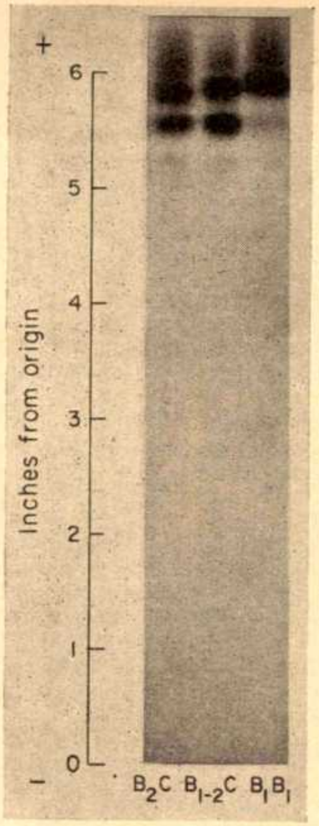

Fig. 1. Comparison by starch-gel electrophoresis of sera of transferrin phenotypes B_2C, $B_{1-2}C$, and B_1B_1. Electrophoresis for 6 h at 20 V/cm, 4° C. Faint iron-binding components migrating more rapidly and more slowly than the principal transferrins can be seen in the autoradiograph (ref. 8)

In the experiments described here, vertical starch-gel electrophoresis was carried out in the borate buffer system of Smithies[10], and transferrins were identified by autoradiography[2]. The examination of sera both from a hybrid Venezuelan individual of Italian and Negro descent and from one of her three sons revealed them to be heterozygous for transferrin C and a more rapidly migrating transferrin variant. When this variant was compared with known faster-moving transferrins by electrophoresis under conditions of high voltage and low temperature[8], it was found (Fig. 1) that this transferrin migrated slightly more rapidly than transferrin B_2 but more slowly than transferrin B_1. These electrophoretic differences were consistently observed in samples obtained from the individual at three different times over the course of two years. These experiments therefore provide evidence for the existence of another genetically determined transferrin variant, for which the name transferrin B_{1-2} is suggested. Population studies on more than one thousand Venezuelan Indians and hybrid individuals have revealed no further examples of the new transferrin.

Incubation of transferrin B_{1-2} with various concentrations of the enzyme neuraminidase[11-12] produced the characteristic stepwise pattern of four additional slower-moving components; at the highest enzyme concentrations, only the slowest-moving of these components was present. It appears, therefore, that the new variant, like the ten other transferrins which have been examined[8,12], contains four sialic acid residues accessible to the enzyme.

We thank Dr. Eloise Giblett, King County Central Blood Bank, Seattle, for providing sera of transferrin phenotypes B_2C and B_1B_1.

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Regulatory Mechanisms of Glycogen Deposition in Liver of Normal and Tumour-bearing Rat, and in Novikoff Ascites Hepatoma

THE lowering of glycogen store in normal animal tissues during the growth of a transplanted hepatoma and the absence of glycogen in neoplastic tissues are commonly observed phenomena. The complex processes which result in removal of glycogen from normal tissues during the growth of a transplanted tumour are not fully understood. However, evidence is available for low glycogen formation in tumours and is based mainly on the assumption of a defective system of glycogen synthesis[1,2].

In this communication emphasis will be placed on yet another factor, that is, the site of synthesis and degradation of glycogen in the cell as a determinant in glycogen storage. The changes that occur at the intracellular level in glycogen synthesizing and degrading enzymes have been examined and the interpretation of the results provides a possible explanation of the phenomena.

In the normal liver cell, the site of glycogen synthesis is believed to be the glycogen granules, where the synthesizing enzyme, glycogen synthetase, is present bound to the glycogen particles[3]. On the other hand, the glycogen-degrading enzyme, phosphorylase, is distributed in mitochondria, endoplasmic reticulum (microsomal fraction), glycogen granules (glycogen pellet) and the soluble part of cytoplasm (supernatant fraction). The phosphorylase in the glycogen pellet is not bound to the glycogen particles[4]. Amylase, which is also capable of removing glycogen store, is present in the liver in very low though estimable concentrations and is believed to be responsible for the formation of glucosyl-oligosaccharides[5]. Amylase activity in the Novikoff ascites hepatoma is considerably lower than in liver and no saccharides have been detected in the solid form of the tumour[2].

In our experiments, Sprague–Dawley rats weighing 150–200 g received transplants of Novikoff ascites

Table 1. INTRACELLULAR DISTRIBUTION OF GLYCOGEN SYNTHETASE AND PHOSPHORYLASE IN THE LIVER OF NORMAL AND HEPATOMA-BEARING RAT AND IN NOVIKOFF ASCITES HEPATOMA

	Glycogen synthetase						Phosphorylase					
	Normal liver		Tumour-bearing animals				Normal liver		Tumour-bearing animals			
			Liver		Transplanted hepatoma				Liver		Transplanted hepatoma	
	Protein content mg/ml.	UDP formed μmoles/min/mg protein	Protein content mg/ml.	UDP formed μmoles/min/mg protein	Protein content mg/ml.	UDP formed μmoles/min/mg protein	Protein content mg/ml.	Pi formed μmoles/min/mg protein	Protein content mg/ml.	Pi formed μmoles/min/mg protein	Protein content mg/ml.	Pi formed μmoles/min/mg protein
First supernatant (700*g*)	24	0·024	27	0·015	7	0·008	13	0·093	13	0·078	4	0·043
Mitochondrial fraction	25	0·016	27	0·002	5	0·015	10	0·087	10	0·035	2	0·021
Microsomal fraction*	28	0·027	31	0·011	6	0·016	14	0·072	12	0·070	2	0·061
Particulate glycogen fraction	18	0·050	20	0·003	4·5	0·016	8	0·206	6	0·120	6	0·009
Final supernatant (25,000*g*)	20	0·014	18	0·016	5	0·026	8	0·092	7	0·101	2	0·119

Both liver homogenates (in sucrose–EDTA and sucrose–EDTA–sodium fluoride) were centrifuged for 10 min at 700*g*. Precipitate was discarded. The supernatant (first supernatant) was centrifuged at 5,000*g* for 10 min. The precipitate (mitochondrial fraction) was separated and the supernatant fluid recentrifuged at 25,000*g* for 10 min. After removal of the fatty layer, the upper part of the supernatant was sucked off (final supernatant), and the middle portion discarded. The suspension at the bottom was removed with the syringe (microsomal fraction) and the tube inverted to pour off the remaining microsomes. The pellet remaining at the bottom is referred to as particulate glycogen. The different precipitates were suspended in known volumes of sucrose–EDTA or sucrose–EDTA–sodium fluoride solutions for the assay of glycogen synthetase and phosphorylase activities respectively. The figures are representative of 3–6 assays each fraction.

* Microsomal fraction is usually contaminated with the final supernatant.

tumour cells. After 6–7 days, the animals were killed and the liver and ascitic tumour removed from each. The tumour cells were suspended in a wash medium described by Wu and Racker[6], sedimented by centrifugation at 80 *g* for 6 min and repeatedly washed and centrifuged at this speed to give tumour cells free of erythrocytes and most of the polymorphonuclear leucocytes. The washed tumour cells were finally centrifuged in a graduated tube at 1,200 *g* for 10 min. The tissues (liver and tumour cells, uncorrected for raffinose space) were homogenized in a Potter–Elvehjem homogenizer with a 'Teflon' pestle for 6 min at 600 r.p.m. to give a 10 per cent homogenate in 0·25 M sucrose solution containing 0·001 M ethylenediamine tetraacetic acid (EDTA) for the assay of glycogen synthetase activity. An equal volume of 10 per cent homogenate was prepared in sucrose–EDTA solution containing sodium fluoride (final concentration, 0·1 M), for the assay of phosphorylase activity. The homogenates were then subjected to the fractionation procedure as described by Leloir and Goldemberg[4].

All sedimented fractions were suspended in known volumes of sucrose–EDTA or sucrose–EDTA–sodium fluoride solutions. Normal rat livers served as control tissues, and were subjected to an identical fractionation procedure. Glycogen synthetase and phosphorylase activities were measured according to procedures described by Nigam *et al.*[2]. Protein determinations were made according to Lowry *et al.*[7], with human serum albumin (Connaught Laboratories, Toronto) serving as a reference standard. The results of the protein determinations and enzyme assays in the various fractions of normal liver, liver of tumour-bearing animals and tumour are shown in Table 1. It can be observed that in normal rat liver, glycogen synthetase and phosphorylase activities are present largely in the glycogen pellet. The synthetase activity is also present in the microsomes and mitochondria and to the least extent in the supernatant fraction. The phosphorylase activity is evenly distributed in the supernatant, mitochondrial and microsomal fractions. These results are in agreement with those reported by Leloir and Goldemberg[4]. The growth of hepatoma, however, provokes certain changes in the distribution pattern of the two enzymes. The liver of the tumour-bearing animal shows a marked loss of glycogen synthetase and phosphorylase activities from the mitochondrial and glycogen pellet fractions. This loss is not accompanied by any appreciable increase in the activity of these enzymes in the supernatant fraction. The microsomal fraction shows loss for glycogen synthetase but not for phosphorylase activity.

In the tumour cell glycogen synthetase activity is present in mitochondria, microsomes and particulate glycogen (the particulate glycogen fraction in tumour is different from liver particulate glycogen since it contains little glycogen) although it is highest in the supernatant fraction. Tumour phosphorylase is also mainly localized in the supernatant and is inactive in the particulate glycogen fraction. The tumour cell thereby resembles, to a certain extent, the liver of a tumour-bearing rat. In both these tissues the synthesizing and degrading enzymes are largely a manifestation of the soluble cytoplasmic fraction.

From these observations it is clear that, in normal liver, phosphorylase is either in an inactive form or unable to approach the glycogen molecules since it does not degrade glycogen in spite of being adsorbed on the glycogen granules. The growth of hepatoma is associated with certain changes that bring about loss of glucose and glucosyl-oligosaccharides[2]. Under such conditions it is likely that an impairment of glycolytic activity in the soluble cytoplasm occurs due to limiting amounts of substrate. This process would be followed by a release of phosphorylase and adenosine triphosphate (ATP) from the mitochondria in amounts sufficient to activate the phosphorylase, bridge its link to glycogen molecules and subsequently degrade the polymer. The absence of glucose would thus be a prerequisite for any appreciable glycogen degradation since in its presence adenine nucleotides formed during pyruvate oxidation are held inside the mitochondria and are not readily available for extra-mitochondrial phosphorylase activation or for glucose phosphorylation as observed in case of Pasteur effect[8].

The phenomenon of glycogen removal from liver during tumour growth can therefore be explained more appropriately on the assumption of an initial activation of phosphorylase due to the removal of cytoplasmic glucose, followed by degradation of the glycogen granules and loss of synthesizing capabilities of the degraded particles. The activation of phosphorylase can be ascribed to the availability of ATP, which not only phosphorylates dephosphophosphorylase but also provides 3′-5′ cyclic AMP (a co-factor for phosphorylase reaction) by the action of adenyl acylase on ATP. Such stimulation of phosphorylase activity has been shown during the administration of epinephrine and glucagon[9] and has been proposed for glycogen depletion during puromycin administration[10]. The case of tumour can be explained on lines parallel to those discussed for liver. It is proposed that in tumours the degradative apparatus is always in an active state due to a constant supply of glycolytic energy as evidenced by high rates of aerobic and anaerobic glycolysis. The formation of saccharides from UDPG in tumour homogenate supports the view that a certain amount of glycogen is synthesized in the tumour cell only to be degraded by an active phosphorylase, and perhaps a slow acting tumour or circulating blood amylase.

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Characterization of some Urinary Protein Fractions in Patients with Multiple Myeloma

THE urinary protein described in 1847 by Henry Bence Jones is a diagnostic tool in multiple myeloma and has been subjected to intense and detailed biochemical investigation. The characteristics of this protein (BJP) are well established[1]. But so far the supernatant urine after precipitation of the BJP by heating (65° C) has not been thoroughly studied. Therefore, in patients with proved multiple myeloma we investigated: (1) the whole urine; (2) the BJP; (3) the supernatant urine after removal of the BJP[2]. The work was then extended to other patients with evidence of a urinary paraprotein, and to patients with macroglobulinæmia Waldenström with or without BJP.

Samples of urine were lyophilized after exhausting dialysis and dried over phosphorus pentoxide; then they could be stored indefinitely. The aforementioned three urine fractions were examined by means of paper electrophoresis for proteins, glycoproteins and reducing hydroxyl groups, by paper chromatography for carbohydrates, amino-acids and glycopeptides, by diffusion measurements according to Northrup and Anson[3] for calculation of molecular weight, and by analytical ultra-centrifugation. In addition, protein, carbohydrate, sialic acid, hexosamine and hexose content were estimated chemically. Cholesterol and phosphatide contents were determined chemically and by thin-layer chromatography.

The following results were obtained:

(1) The known characteristics of the BJP were confirmed (Table 1*A*).

Table 1. PERCENTAGE COMPOSITION AND MOLECULAR WEIGHT OF URINARY FRACTIONS FROM PATIENTS WITH POSITIVE BENCE JONES PROTEIN

	A	*B*	*C*
Protein	86	50	31
Hexose	1·2	12	22
Sialic acid	0·3	2·0	2·0
Hexosamine	0·5	4·0	9·8
Molecular weight	32,000–40,000	13,000–17,500	9,000–12,000
Sedimentation coefficient ($S_{35,w}$)	3·5	—	1·8

A, BJP; *B*, supernatant urine after removal of BJP; *C*, water-soluble glycopeptide after lipid extraction of *B*.

(2) The supernatant urine, from which the BJP had been removed by heating twice to 70° C at *p*H 6–7, contained a well-defined glycolipoprotein which is quite different from the BJP and from the uromucoids described by Tamm and Horsfall and other authors[4–6]; this urinary protein has not yet been described. Its biochemical characteristics are presented in Table 1*B*.

(3) This glycolipoprotein from the supernatant urine was further prepared by lipid extraction with methanol and methanol-chloroform. After removal of the lipid extractable material an especially interesting water-soluble and non-dialysable fraction remained, consisting of 40 per cent carbohydrate and 40 per cent protein. For characteristics of this substance see Table 1*C*.

It should be emphasized that this fraction could be recovered several times from the same patients over a period of two years, thus proving the reproducibility of the methods used for urinary preparations. This glycoprotein is homogeneous by electrophoresis and ultra-centrifugation. Investigation for the presence of reducing hydroxyl groups with silver nitrate reveals a strong positive reaction. This low molecular weight glycopeptide seems to be important in the uroprotein constellation in paraproteinæmia.

(4) Comparison with urine from myelomatous patients without typical BJP made evident that the carbohydrate concentration and the relation of carbohydrate to protein are important in the precipitation of BJP and its heat reversibility. The BJP will be negative at a carbohydrate concentration above 30 per cent or a protein–carbohydrate ratio below 2 : 1. Urine with a higher protein concentration may reveal a BJP on electrophoresis but no evidence for BJP on heating. Reversible BJP will be found at a protein–carbohydrate ratio about 11 : 1 (Table 2).

These results indicate that in patients with multiple myeloma in addition to the BJP it is possible to

Table 2. RELATIONSHIP BETWEEN BENCE JONES PROTEIN AND PROTEIN–CARBOHYDRATE RATIO

	I	II	III	IV
Protein–carbohydrate ratio	40 : 1	11 : 1	9 : 1	2 : 1

I, Irreversible BJP; II, reversible BJP; III, negative BJP, but positive urine electrophoresis; IV, negative BJP, negative urine electrophoresis.

isolate other well-characterized non-dialysable urinary fractions which seem to be typical uroproteins in paraproteinæmia. These substances possibly play an important part in the physicochemical variability of the BJP as we presume that the BJP may be present in the native urine in the form of a glycolipoprotein complex which is broken down by a mild water hydrolysis when the urine is heated to 70° C.

A detailed study, including similar urinary protein findings in macroglobulinæmia Waldenström, will be published elsewhere[7].

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Inhibition of Spontaneous Splitting of γ-Globulin Preparations with ε-Amino-caproic Acid

In our previous work[1] in which changes of human γ-globulin preparations were examined during storage it was established that splitting occurs in them under the formation of two new fragments, gamma$_X$ and gamma$_Y$; this is especially marked in preparations prepared from placental extracts. Similar results were achieved by other authors[2]. Further, it has been established that a similar splitting occurs when the solution of human γ-globulin is treated with some proteolytic enzymes, for example, with plasmin, trypsin and papain[3]. It can be therefore presumed that a process of a proteolytic character occurs during storage.

The inhibition of proteolytic processes, especially of such connected with the action of plasmin by ε-amino-caproic acid, has already been described[4,5]. This is why we used this inhibitor for the stabilization of human γ-globulin preparations.

We prepared several sterile samples of 16 per cent solutions of human γ-globulin isolated from placental material in a 3 per cent ε-amino-caproic acid solution (ACA) as well as in a 2·25 per cent glycine solution, which is at present used as stabilizer of commercial γ-globulin preparations. The solutions were stored for 5 months at the temperature of 0° to + 4° C; samples were withdrawn at monthly intervals and analysed by immunoelectrophoresis, agar electrophoresis and by ultracentrifugation. Fig. 1 shows the results of immunoelectrophoresis of one of the preparations after a 5-months' storage in both kinds of solutions under precipitation with anti-human γ-globulin rabbit serum. In γ-globulin stored in glycine solution the usual products of splitting (gamma$_X$ and gamma$_Y$) were formed, while in the ACA solution the splitting was markedly inhibited. In this case only characteristic γ-globulin line precipitates, the splitting being detectable by a forking of its end at the cathode side.

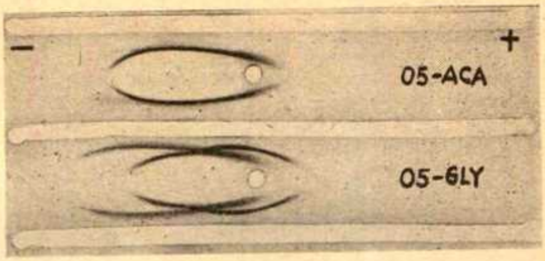

Fig. 1. Immunoelectrophoresis of placental γ-globulin preparations stored for five months in ε-aminocaproic acid and glycine

The inhibitory effect of ACA is still more definitely shown by the results of ultracentrifugation analysis (Fig. 2, Spinco model *E*, 52,000 r.p.m., time 56 min). In both samples three components appear: the lightest of S_{20} = 3·9, medium of S_{20} = 7 (unsplit γ-globulin) and the heaviest of S_{20} = 10·5 (dimer of γ-globulin, usually in preparations prepared by ethanol treatment). While in the inhibitor (ACA) containing preparation the 7*S* γ-globulin forms 92 per cent, the lighter fraction amounting to 4 per cent, in the control preparation (glycine solution) the 7*S* γ-globulin forms only 22 per cent, 78 per cent being formed by the 3·9 *S* fraction. Former analyses of isolated fractions of splitting demonstrated their very close sedimentation constants, and thus the lighter fraction can be considered a mixture of both products of splitting, gamma$_X$ and gamma$_Y$.

These results indicate that ε-amino-caproic acid is a suitable stabilizer of human γ-globulin preparations against spontaneous proteolysis. As the ε-amino-caproic acid shows no toxic or noxious effects on

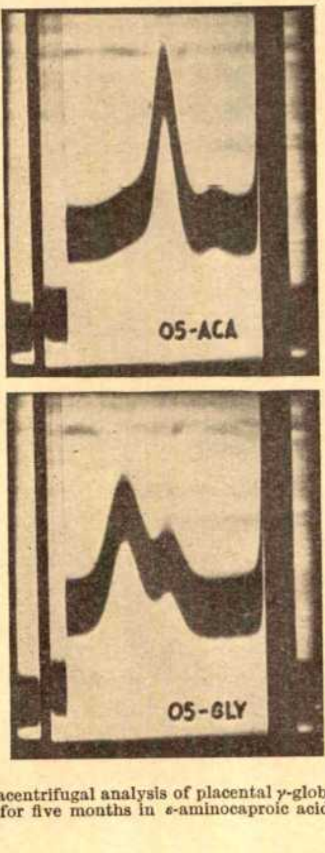

Fig. 2. Ultracentrifugal analysis of placental γ-globulin preparations stored for five months in ε-aminocaproic acid and glycine

parenteral administration[6] it appears suitable also for the production of injection γ-globulin solutions[7].

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Uricolysis by Human Leucocytes

ALTHOUGH uricase is absent from the tissues of the human being, there is nevertheless evidence that uric acid is destroyed in man. Benedict, Forsham and Stetten[1] first demonstrated by intravenous injection of isotopically labelled urate that only two-thirds to three-fourths of the daily urate turn-over of the body actually appears as urinary uric acid. Furthermore, the total recovery of the injected isotopically labelled uric acid in urinary uric acid over a 7-day period is similarly reduced[2]. The missing isotope has been demonstrated in a variety of metabolites in the urine, including ammonia, urea, allantoin, allantoic acid, respiratory carbon dioxide and fæcal bacterial bodies, after intravenous administration of uric acid labelled with nitrogen-15 and uric acid-2-^{14}C (refs. 3, 4).

We were led to re-examine this problem by observing that the inflammatory response to the intra-articular injection of sodium urate crystals involves a phagocytosis of the crystals by leucocytes[5]. A similar phagocytosis of urate crystals in synovial fluid is also seen in spontaneous attacks of acute gouty arthritis[6]. Our investigations show clearly that human leucocytes are capable of degrading sodium urate.

Human leucocytes essentially free of erythrocytes were prepared according to Fallon *et al.*[7] using fibrinogen sedimentation. The leucocytes were suspended in Krebs–Ringer bicarbonate buffer with added glucose (1·5 mg/ml.) to give a leucocyte concentration of about 15,000 per mm.^{3}. Erythrocytes from the initial sedimentation were washed twice with isotonic saline, and the packed cells, essentially free of leucocytes, were diluted with 10 volumes of Krebs–Ringer bicarbonate containing added glucose. Sodium urate-6-^{14}C with a specific activity of 2·8 μc./mg was crystallized from hot neutral solution of uric acid-6-^{14}C (Isotope Specialties).

Incubations were carried out aerobically at 38° C in modified Warburg flasks. Test flasks contained 0·5 ml. of the leucocyte suspension or erythrocyte suspension and 0·5 ml. of plasma containing 100 μg of sodium urate-6-^{14}C, either as microcrystals or in supersaturated solution. All incubations were performed in duplicate, and were terminated in 2 h by adding 0·3 ml. of 60 per cent perchloric acid to the incubation mixture. The carbon dioxide evolved was collected in 0·8 ml. hyamine and counted in a Packard 'Tri-Carb' liquid scintillation counter. Sonicated leucocytes were prepared with a Mullard probe sonicator using four interrupted 30-sec cycles while the sample was chilled in ice. Uricase activity[8] was determined spectrophotometrically in a Beckman model *DU* spectrophotometer. Leucocyte peroxidase was demonstrated spectrophotometrically according to Maehly[9]. Hydrogen peroxide was generated by adding glucose and glucose oxidase (Worthington) to each cuvette.

Table 1 shows the uricolytic ability of erythrocytes as compared to leucocytes as well as a comparison of the degradation of uric acid-6-^{14}C in solution as compared to microcrystalline suspension. As might be expected since leucocytes readily ingest crystals of sodium urate, sodium urate in crystalline form resulted in greater uricolytic activity in the leucocyte suspension while sodium urate-6-^{14}C in solution provided a better substrate for uricase action. Similar results were obtained in three other experiments. The calculated rate of uricolysis in this work was 0·25 μg sodium urate/h/10^6 leucocytes. Uricolysis by intact erythrocytes was negligible.

Table 1. COMPARISON OF URICOLYTIC ACTIVITY OF HUMAN ERYTHROCYTES AND LEUCOCYTES*

	$^{14}CO_2$ production in c.p.m. from Sodium urate-6-^{14}C	
	Solution	Microcrystalline
Plasma	136	147
Plasma + uricase	204,000	136,000
Plasma + erythrocytes	173	—
Plasma + leucocytes	960	2,081

* These are the average values of duplicate determinations.

No uricase activity was detectable in the sonically disrupted leucocyte with incubations up to 2 h at 25°. However, a peroxide-dependent uricolysis was demonstrable in the leucocyte peroxidase assay when a hydrogen peroxide generating system was used and accounted for the disappearance of 0·3 microgram of uric acid per 10^6 leucocytes/h.

That the gastrointestinal tract might be a primary site for uricolysis was suggested by the finding of Geren and associates[10] that after oral administration of uric acid labelled with nitrogen-15 only 27 per cent of the nitrogen-15 could be recovered in urinary uric acid over a 3-day period, while intravenous administration resulted in a recovery of 88 per cent of the isotope. Although sulphonamide bacteriostasis of the gut failed to alter uricolysis[3], Sorensen[4] reported that treatment with a combination of drugs, which effected much superior bacteriostasis than sulphonamides alone, reduced the recovery of carbon-14 in uricolytic fragments from 22·5 per cent before bacteriostasis to 3 per cent after bacteriostasis. By contrast, the recovery of carbon-14 in urinary allantoin was lowered only slightly from 2·1 to 1·8 per cent with bacteriostasis. Sorensen concluded that the primary site of uricolysis in the human is the intestinal tract and is the result of bacterial action on uric acid contained in intestinal secretions.

Although Bien and Zucker[11] have reported that overnight incubation of whole blood was accompanied by a decrease in uric acid content, *in vitro* studies by Sorensen[4] using uric acid-6-^{14}C and a variety of human tissues, including whole blood, led him to conclude that uricolysis did not occur in any human tissue.

Despite the absence of uricase in human tissues there are a variety of enzymatic reactions which could destroy uric acid. Canellakis, Tuttle and Cohen[12] have demonstrated that the products of peroxidative destruction of uric acid in phosphate buffer are allantoin, carbon dioxide, and urea, and that a variety of peroxidases can attack uric acid, including verdoperoxidase. Verdoperoxidase accounts for 1 per cent of the dry weight[13] of human leucocytes. Howell and Wyngaarden[14] have demonstrated the peroxidative destruction of uric acid catalysed by methæmoglobin and other hæm proteins *in vitro*.

Thus, leucocytes have been shown to contain the enzymatic equipment necessary for degrading uric acid, although such a destruction by intact human leucocytes has not heretofore been reported. Canellakis[12] states that in unpublished experiments leucocytes from a sterile abscess in the rat were found capable of degrading uric acid. Soberon and Cohen[15] have demonstrated a hydrogen peroxide requirement for uricolysis using the rat leucocyte.

Although most of the uricolysis in the normal human is accounted for by the action of the bacterial flora within the intestinal tract, uricolysis might very well account for the persistent appearance of essentially the same quantities of carbon-14 in urinary allantoin before and after intestinal bacteriostasis observed by Sorensen[4].

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Sialic Acid in the Bulbo-urethral Glands of the Boar

THE two bulbo-urethral glands (Cowper's glands) of the boar lie parallel to the pelvic urethra to which they are united by a heavy muscular attachment. They are normally about 15 cm long by 3–5 cm in diameter and contain a sticky, white mucin which is ejected into the pelvic urethra during ejaculation of semen. In semen freshly collected by means of an artificial vagina the mucin has lost its sticky character and appears as small gelatin-like lumps resembling tapioca. Each lump consists of a white opaque 'nucleus' surrounded by a water-clear gel. If the semen is left standing for several hours these lumps grow and coalesce into large masses of transparent elastic gel which may occupy more than 50 per cent of the semen volume. Many spermatozoa are trapped in the gel mass.

The change in the physical properties of the mucin that occurs when it mixes with the semen is induced by the secretion of the seminal vesicles[1]. Thus if a small quantity of the bulbo-urethral mucin is immersed in the fluid secretion of the seminal vesicles a transparent gel-layer forms rapidly on the surface of the mucin, and this increases in thickness until a lump of gel is obtained which closely resembles those found in freshly collected semen. Semen from which the small lumps have been removed can be used successfully for artificial insemination. Thus the role of the gel in reproduction is not understood although it is possible that it functions as a plug in the uterus of the sow and so prevents back-flow of semen.

Table 1. COMPOSITION OF THE BULBO-URETHRAL MUCIN

	Percentage of dry weight	Molar ratio
Sialic acid (N-acetylneuraminic acid)		
Before hydrolysis (Svennerholm)	26·6	(1·00)
After hydrolysis (Warren):		
With neuraminidase	24·6	
With acid	25·2	
Galactosamine		
Galactosamine standard	12·8	0·83
N-Acetylgalactosamine standard	13·3	0·87
Neutral sugars (as galactose)	4·0	0·26
Methylpentose (as fucose)	0·78	0·047
Uronic acid (as glucuronate)	$\ngtr$0·1	
Ester sulphate (SO_4^{2-})	0·52	0·054

Odin[2] found that 14·7 per cent of the dry weight of the semen gel consisted of a sialic acid which was identified as N-acetylneuraminic acid. This finding is unexpected since N-glycolylneuraminic acid is the predominant sialic acid in epithelial secretions of this species[3].

Analyses of the secretions of the reproductive tract of the boar showed that the bulbo-urethral mucin contains between 4·0 and 4·5 per cent (fresh weight) of sialic acid. The concentration of sialic acid in the seminal vesicles is less than 1 per cent of that in the bulbo-urethral mucin while the other glandular secretions contain negligible amounts. The analysis of carbohydrates in the mucin is given in Table 1. Sialic acid was determined, as N-acetylneuraminic acid, in the intact secretion by Svennerholm's resorcinol method[4]. It was also determined by Warren's method[5] after liberation of sialic acid either by incubation with neuraminidase[6] or by heating for 1 h at 80° in 0·1 N sulphuric acid. The sialic acid produced by acid hydrolysis was examined by paper chromatography[7] and shown to consist of N-acetylneuraminic acid plus a trace of the N-glycolyl derivative. Authentic samples of these acids were kindly supplied by Dr. G. Blix. Neutral sugars were determined by an orcinol method[8] and methylpentose by the method of Gibbons[9]. Hexosamine was analysed according to Rondle and Morgan[10] and has been expressed as galactosamine since the hexosamine liberated by acid hydrolysis[10] gave a single spot with R_F equal to that of galactosamine when it was examined by paper chromatography[11]. Both galactosamine and N-acetylgalactosamine have been used as standards in the Rondle and Morgan method. The standard solutions were subjected to the same hydrolysis procedure as the mucin. Uronic acid was determined according to Bitter and Ewins[12] and ester sulphate by a modification of the procedure of Heald and Robinson[13].

The predominance of sialic acid and, after acid hydrolysis, of galactosamine in approximately equimolar amounts suggests that the mucin is analogous to the submaxillary mucins of sheep and cattle. The carbohydrate in the submaxillary mucins consists mainly of disaccharide units with the structure 6-(N-acetyl-α-D-neuraminyl)-N-acetylgalactosamine[14,15]. The majority of these disaccharides are linked glycosidically to free carboxyl groups of aspartyl and glutamyl residues in a protein core[16]. For this reason N-acetylgalactosamine may be the more suitable standard for the hexosamine assay reported here[14].

Seminal plasma consists of the mixed secretions of several accessory glands in the male reproductive tract. Constituents of the plasma often originate mainly or entirely from a single gland and thus chemical analysis of seminal plasma can yield useful information on the secretory activity of the various glands[17]. In boar seminal plasma citric acid, ergothioneine and inositol are indicators for the activity of the seminal vesicles while the level of glycerylphosphophorylcholine is a measure of the contribution of the epididymal secretion to the seminal plasma. It is now clear that in boar semen the sialic acid, which is present almost entirely in the gel, can serve as a measure of the secretory activity of the bulbo-urethral glands.

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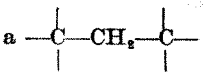
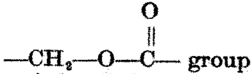
Chromatographic Isolation of Leucodrin

LEUCODRIN, the dilactone $C_{15}H_{16}O_8$ (refs. 1–3), which in the past was used as a remedy for malaria[4], has been isolated from the leaves of *Leucadendron adscendens* by chromatography on a column of finely powdered 'Perlon'.

Dried, powdered *Leucadendron* leaves were extracted continuously with boiling methanol until free from bitter principle, the solvent was evaporated from the extract, the residue dissolved in water and the cold solution passed through a column of powdered 'Perlon' ('Versuchsprodukt PP. 15/Sp.'). The column was first washed with cold water until the effluent was clear and then elution with boiling water afforded, after concentration, a clean, crystalline product in 15 per cent yield. This product was identical in all respects with the product obtained from *Leucadendron* leaves by Rapson[1], who used in its purification the rather more tedious method of precipitation of impurities with lead subacetate. The product from chromatography had m.p. 212·5°–213° (lit.[1] m.p. 212°–212·5°) (Found: C, 55·3; H, 5·0 per cent. Calc. for $C_{15}H_{16}O_9$. C, 55·5; H, 5·2 per cent), $[\alpha]_D^{20}-19{\cdot}5°$ (lit.[3] $[\alpha]_D^{17}-19{\cdot}2°$). Further elution of the column with warm, absolute alcohol and evaporation of the effluent afforded the yellow pigment, rutin, which melted indefinitely at 187°–192°. By following Rapson's procedure[1] on the same batch of leaves, leucodrin was obtained in 10 per cent yield.

The influence of powdered 'Perlon' in the separation of leucodrin is undoubtedly due to the formation of hydrogen bonds between the polyamide and this phenolic natural product. In fact, polyamides have been used previously as an adsorbent in chromatography for the separation of phenols[5], flavones and their glycosides[6], chalcones and flavones[7] and methyl gallates[8]. The high yields obtained, undoubtedly due to the fact that the phenolic material is not only held back on the surface but also inside of the polyamide[5], could make chromatography on powdered 'Perlon' a very efficient technique for the large-scale separation of naturally occurring phenols.

Preliminary investigations into the structure of leucodrin appear to point to the presence of two different lactone rings in the molecule rather than the spiro-γ-lactone type system which was put forward by Rapson[3] as a possibility—the infra-red spectrum shows two distinct carbonyl bands, ν_{max} (nujol) 1,805 and 1,778 cm^{-1}, λ_{max} (in ethanol) 205 mμ (ε 7,150), 230 mμ (ε 9,660) and 277 mμ (ε 1,430). The ^{1}H nuclear magnetic resonance spectrum at 60 Mc/s, for a solution of leucodrin in trifluoroacetic acid (reference compound, tetramethylsilane), shows two bands which cannot be ascribed to either of Rapson's structures: a moderately intense band at 9·09 τ which is probably associated with a  $-\overset{|}{\underset{|}{C}}-CH_2-\overset{|}{\underset{|}{C}}-$ group reasonably far removed from an aromatic nucleus, and a very intense band at 5·43 τ which might be associated with the protons of a  $-CH_2-O-\overset{\overset{O}{\|}}{C}-$ group.

A detailed account of degradative studies will occur in due course.

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PHYSIOLOGY

Phosphoproteins and Sodium Transport

A FAIR measure of agreement has been reached that active transport of ions and volume regulation of cells involves utilization of adenosine triphosphate (ATP). We have put forward the suggestion that the phosphorylation of phosphoprotein is the first step in this utilization of ATP.

We have shown[1,2] that phosphoprotein turn-over of liver slices is dependent on the sodium concentration of the external medium, and is sensitive to cardiac glycosides and other drugs inhibiting ion transport, such as 'Promethazine'. We now bring evidence that the phosphoprotein turn-over of liver slices is also markedly affected by external potassium ion concentration.

Liver slices can be severely depleted of potassium by first cooling and then incubating in potassium-free Ringer solution at 38° C. Intracellular sodium increases greatly in such slices. If 5–10 m.equiv. potassium is then added to the incubation medium, transport starts. There is no lag period before potassium accumulation begins; in this respect the system differs from liver slices that are recovering from cooling alone[3,4] as these have a delay of about 10 min before potassium accumulation starts. The rate of potassium uptake is between 8–12 m.equiv./min/kg dry weight, and is inhibited immediately by 'Ouabain' at 5×10^{-4} M. 'Ouabain' at 10^{-4} M has little effect until some 10 min have passed, whereupon inhibition begins and progresses. 'Promethazine' also inhibits at a concentration of 10^{-4} M.

If phosphorus-32 is included in the potassium-free medium, the slices incorporate the tracer into ATP and phosphoprotein. If potassium is added, after labelling the slices with phosphorus-32 in potassium-free medium, there is a rapid fall in the radioactivity of the phosphoprotein as transport commences. This is detectable within 15 sec and maximum at 30 sec. By 45 sec after potassium addition, the radioactivity has returned to initial levels. Table 1 illustrates such an experiment and shows that 'Ouabain' abolishes the effect of potassium. 'Promethazine' (not shown) also prevents the effect of potassium. There is little or no effect on ATP radioactivity caused either by potassium addition or by the drugs.

Table 1. EFFECT OF 'OUABAIN' AND POTASSIUM ON PHOSPHOPROTEINS OF LIVER SLICES

Exp. No.	K^+ 10 mM	'Ouabain' (5×10^{-4} M)	Specific activity of phosphoprotein c.p.m./μM P
1	—	—	16,000
	+	—	11,000
	—	+	15,500
	+	+	15,200
2*	—	—	27,000
	+	—	20,000

The slices were leached of potassium by cooling at 0° in 0·154 M sodium chloride for 30 min. They were then suspended in potassium-free Ringer, containing phosphorus-32, specific activity 3×10^6 c.p.m./μmole inorganic phosphate for Exp. 1 and 15×10^6 c.p.m./μmole inorganic phosphate for Exp. 2, for 30 min at 38°. Transport was initiated by addition of potassium chloride, 10 mM, and the experiment ended 30 sec later. 'Ouabain', when present, was added 3 min before potassium chloride. The rate of uptake of potassium was 8 m.equiv./kg dry wt./min in controls and about 1 m.equiv./kg dry wt./min in presence of ouabain.

* The figures in Exp. 2 represent the total radioactivity in phosphorylserine derived from the proteins by acid hydrolysis and subsequent chromatography.

Essentially similar results are observed in human red cells. If cells with high internal sodium are incubated with phosphorus-32 in a medium free of potassium, phosphoproteins become labelled. On addition of potassium, there is a fall in radioactivity which is blocked by 'Ouabain' (Table 2). In contrast to liver slices, the effect of potassium is to bring about a new steady state, so that phosphoprotein radioactivity in the presence of external potassium is about 67 per cent of that in its absence. Liver slices show an effect of potassium addition within a few seconds; red cells show the effect after half an hour. This slowness of response may be connected with the slow rate of ion transport found in red cells.

The fall in radioactivity is recorded as a fall in specific activity of the protein–phosphorus. The labile 10 per cent of the phosphoprotein–phosphorus contains nearly all the counts[5]; this fall in specific activity represents dephosphorylation of the labile fraction.

Table 2. EFFECT OF 'OUABAIN' AND POTASSIUM ON PHOSPHOPROTEINS OF HUMAN RED CELLS

K 25 mM	'Ouabain' 10^{-5} M	Specific activity, c.p.m./μM P in phosphorylserine
—	—	225
—	+	150
+	—	190
+	+	210

Cells were loaded with sodium by cold storage (sodium was 50 m.equiv./l. of cells). They were incubated in medium with and without external potassium or 'Ouabain' as shown in a system as described in ref. 8 containing phosphorus-32 of specific activity 16×10^6 c.p.m./μmole in organic phosphate. The phosphoprotein residue was hydrolysed in hydrochloric acid, with the prior addition of 10 μmoles of carrier phosphorylserine, which was isolated by chromatography on 'Dowex 50' as already described (ref. 1). The specific activities of ATP, not shown, were essentially the same in all systems.

Table 3. EFFECT OF 'PROMETHAZINE' ON CALCIUM

Preincubated in Ca^{2+}	Promethazine (10^{-4} M)	Specific activity, c.p.m./μM P after final incubation in 0·93 mM Ca, 10 mM Na: ATP	Specific activity, c.p.m./μM P after final incubation in 0·93 mM Ca, 10 mM Na: Phosphoprotein
2·8 mM	—	104,000	4,300
2·8 mM	+	123,000	7,200
0·93 mM	—	170,000	7,700
0·93 mM	+	200,000	9,000

Preincubation was in choline Ringer at 38° for 10 min, gas-phase 95 per cent oxygen–5 per cent carbon dioxide and 'Promethazine' as shown. For incubation, the slices were transferred to choline Ringer containing 10 mM sodium, 0·93 mM calcium and phosphorus-32, but no drug. Incubation time was 10 min. As a control, slices were preincubated in choline Ringer containing 0·93 mM calcium and transferred to the same medium as the experimental slices.

It is suggested that sodium and potassium take part in the following reactions of the phosphoproteins of liver cells and human erythrocytes:

$$\text{ATP} + \text{Protein} \xleftrightarrow{\text{Na}_i} \text{P} \sim \text{Protein} - \text{Na} + \text{ADP}$$

$$\text{P} \sim \text{Protein} - \text{Na} \xleftrightarrow{\text{K}_o} \text{P} \sim \text{Protein} - \text{K} + \text{Na}_o$$

$$\text{P} \sim \text{Protein} - \text{K} \longrightarrow \text{K}_i + \text{Protein} + \text{inorganic phosphate}$$

(Where Na_i and K_i are internal and Na_o and K_o are external.) 'Ouabain' (and also 'Promethazine') compete for the phosphoproteins with both sodium and potassium. 'Ouabain' and 'Promethazine' not only prevent the effects of addition of sodium and potassium on phosphoprotein turn-over, but also prevent certain calcium effects.

Liver slices respond to addition of sodium to the medium by increased phosphoprotein turn-over. This effect is competitively inhibited by calcium in media where osmolarity is regulated with choline chloride[2,9]. Thus 10 mM sodium is effective when calcium concentration is 0·93 mM but ineffective in the presence of 2·8 mM calcium. But slices pre-incubated with 2·8 mM calcium will not respond to a fresh medium containing 10 mM sodium and 0·93 mM calcium with stimulated rates of turn-over of phosphoprotein and ATP, whereas slices pre-incubated with 0·93 mM calcium will do so. If the prior exposure to 2·8 mM calcium was conducted in the presence of 'Ouabain' or 'Promethazine', and the slices were then removed to a fresh medium containing phosphorus-32 and 10 mM sodium these slices responded with greater activity in ATP and phosphoprotein than did untreated controls (Table 3). In other words, the drugs reduced the effect of the higher levels of calcium to that of the lower level. In liver slices, therefore, as in muscle[6,7], there is a balance between the activities of sodium, potassium and calcium, which all appear to centre around a common site or sites.

These results will be published in detail shortly[8,9].

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Activities of Various Concentrations of Triamcinolone Acetonide

Blank[1] has reported that 0·01 per cent triamcinolone acetonide, applied topically, was at least comparable in clinical efficacy with 1·0 per cent hydrocortisone, although somewhat less effective than 0·1 per cent triamcinolone acetonide, the concentration usually adopted clinically. In a larger group of cases Olansky[2] found that 0·1 per cent triamcinolone acetonide was significantly more active than the 0·01 per cent concentration, but he quoted earlier studies[3] which suggested that 0·025 per cent triamcinolone acetonide was difficult to distinguish from the 0·1 per cent concentration. Since there would be economic advantages in using a reduced concentration if this could exhibit the activity of 0·1 per cent triamcinolone acetonide in most dermatitides, we have conducted experiments to compare the effects of three concentrations of triamcinolone acetonide and 1·0 per cent hydrocortisone.

Chemical irritants such as cantharidin and volatile oil of mustard were found unsatisfactory for the production of uniform inflammation, while turpentine was considered to be possibly dangerous. It was therefore decided to use the 'Cellophane' tape method described by Wells[4] to produce a standardized trauma on the flexor aspect of the forearms of volunteers. 'Cellophane' tape, 0·5 in. wide, was smoothed on to a marked area and stripped off sharply in one direction. A fresh piece of tape was applied to the same area and stripped off in the opposite direction, this process being repeated until the surface became uniformly red and glistening. Erythema was produced in this way to a length of approximately 14 cm but only the central portion of 10 cm was used for division into control and treated areas.

Lotions containing 0·01, 0·025 and 0·1 per cent triamcinolone acetonide and 1·0 per cent hydrocortisone were prepared and packed in code-labelled containers. Measured quantities of these lotions were applied at random and under double-blind conditions to marked areas of the 'stripped' skin and were spread evenly, care being taken to avoid the control areas interposed between treated areas. By marking five areas, each 2 cm in length, it was found possible to compare three preparations simultaneously. After the lotions had been applied, the areas were examined at intervals and the responses were recorded after 2 and 6 h. The preferences at 2 h were analysed sequentially[5] with $\alpha = 0{\cdot}025$, $\beta = 0{\cdot}05$ and $\theta_1 = 0{\cdot}85$, although in some instances results continued to become available after a boundary had been crossed.

Table 1. Triamcinolone Acetonide (T.A.) 0·01, 0·025 and 0·1 per cent compared with Hydrocortisone 1·0 per cent (H)

T.A. concentration (per cent)	T.A. better		H better		No difference	
	2 h	6 h	2 h	6 h	2 h	6 h
0·01	13/32	14/32	9/32	8/32	10/32	10/32
0·025	15/32	21/32	6/32	3/32	11/32	8/32
0·1	17/32	23/32	5/32	0/32	10/32	9/32

Table 2. Triamcinolone Acetonide (T.A.) 0·01 and 0·1 per cent compared with Triamcinolone Acetonide 0·025 per cent

T.A. concentration (per cent)	T.A. 0·025 per cent better		T.A. 0·01 per cent or 0·1 per cent better		No difference	
	2 h	6 h	2 h	6 h	2 h	6 h
0·01	16/32	18/32	5/32	6/32	11/32	8/32
0·1	9/32	3/32	11/32	8/32	12/32	21/32

The total results are summarized in Tables 1 and 2.

The results read at 2 h indicate that 0·025 and 0·1 per cent triamcinolone acetonide have similar activities and that both concentrations are more active than 1·0 per cent hydrocortisone, while 0·01 per cent triamcinolone acetonide is similar in activity to 1·0 per cent hydrocortisone and less active than 0·025 per cent triamcinolone acetonide.

After 6 h the preferences for both 0·025 and 0·1 per cent triamcinolone acetonide over 1·0 per cent hydrocortisone are increased, but there are still no significant differences between these two concentrations of triamcinolone acetonide.

Wells[4] found that the effects of hydrocortisone were apparent on the 'stripped' area after about 4 h and became maximal after 12 h, but he applied the drug 3 times in 24 h if the experiment was prolonged. In our experiments, the activity of all the preparations appeared to be maximal after 2–3 h following a single application and the effects of hydrocortisone were often beginning to fade at the sixth hour.

The results obtained by the 'Cellophane' tape-stripping method support the clinical evidence that 0·025 per cent triamcinolone acetonide is indistinguishable from the 0·1 per cent concentration, although the latter may be preferred in refractory dermatoses. The experimental results also suggest that 0·025 and 0·1 per cent triamcinolone acetonide have longer, as well as greater, activity than 1·0 per cent hydrocortisone.

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Effect on the Plasma Citrate of Treatment of Primary Hypothyroidism with Triiodothyronine

During the course of metabolic studies on myxœdematous subjects it was noted that the plasma citrate-level was in some cases raised. In the hope that useful information might be obtained on the mode of action of thyroid hormones on tissue metabolism, the plasma citrate-level was followed during the treatment of four female patients suffering from primary myxœdema, using ordinary replacement doses of triiodothyronine. Triiodothyronine was also administered to four normal medical students, two male and two female, and their plasma citrate-levels were followed.

Plasma citrate was measured by the method of McArdle[1] on venous blood taken in the fasting state. The normal range of plasma citrate in our laboratory is 2·0–3·9 mg/100 ml. The initial basal metabolic rates in patients 1–4 were −29, −38, −35 and −13 per cent, respectively (standards of Robertson and Reid[2]), and rose to within normal limits in from 7–14 days. The total daily dose of triiodothyronine administered (orally) was as follows: case 1, 60 μg; case 2, 60 μg for 4 days, followed by 120 μg; case 3, 20 μg for 4 days, 40 μg for 6 days, followed by 60 μg; case 4, 20 μg for 8 days followed by 30 μg. The normal students received 80 μg per day.

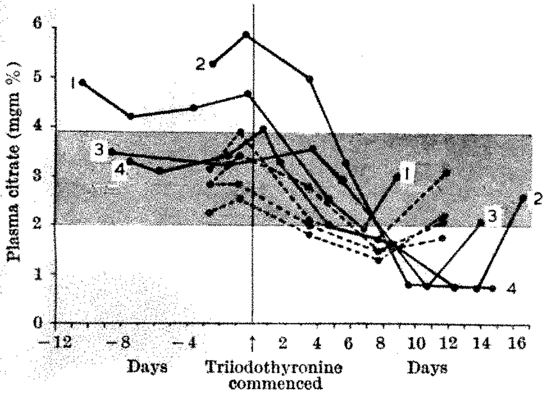

Fig. 1

The results are shown in Fig. 1; those obtained in patients are represented by solid lines, and in normals by dotted lines. Two of the hypothyroid subjects had high plasma citrate-levels initially. Triiodothyronine produced an abrupt fall in plasma citrate to subnormal levels in all subjects. The fall was on the whole greater in the hypothyroid subjects. A minimum value was reached in the controls at the eighth day; thereafter the levels began to rise again. In three out of four of the hypothyroid subjects the trough of the curve was delayed until the tenth to thirteenth day.

It seems reasonable to suppose that the initial fall in plasma citrate is in some way related to the effect of thyroid hormones in raising the metabolic rate particularly as only physiological doses have been used. It may be due either to a slowing in the rate of release of citrate into the circulation or to an increased rate of removal. It might be suggested that in the hypothyroid state the pathways involved in the oxidation of citrate are depressed, and that treatment corrects this. The subsequent return to normal in plasma citrate (Fig. 1) might be expected if the restoration of citrate synthesis took place more slowly than that of citrate catabolism. Examination of plasma-levels of other Krebs cycle intermediates might be of value in investigating the above possibilities. Relevant to these suggestions are the findings that succinoxidase activity in some mammalian tissues is depressed by thyroidectomy and increased by administration of thyroid hormone[3,4]. Pitt-Rivers and Tata[5] have, however, pointed out the difficulties in accepting that the thyroid hormones act by facilitating a rate-limiting enzyme reaction; the work of Tapley *et al.*[6–8] on the effect of thyroid hormones on mitochondrial swelling suggests that these hormones could regulate metabolism by controlling the permeability of mitochondrial membranes to enzyme substrates. The observations reported here could be equally well explained by such an effect.

I thank Prof. C. Wilson, Dr. J. M. Ledingham and Dr. A. Stuart Mason for their advice and permission to examine their patients.

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HÆMATOLOGY

Common Antigen between Human Red Cell and Glomerulus

The existence of a shared antigen between the kidney and the red cell has been demonstrated by Simonsen[1] for the dog and by me[2] for the dog and the rabbit.

During the course of isolation of a soluble fraction from human glomeruli, it was observed that rabbit antisera to this material contained a hæmagglutinin of considerable titre to human red cells. While initially only group O cells were evaluated, it soon became apparent that the hæmagglutinin was capable of also agglutinating group A and group B cells.

The procedure for the isolation of the soluble fraction from human glomeruli, to be reported in detail elsewhere, consists of isolating glomeruli from pooled human kidneys by Greenspon's method[3] and subjecting the glomeruli to trypsinization for 6 h at 37° C and storage in the cold overnight. The particulate material is removed by centrifugation and the supernatant subjected to treatment with trifluorotrichloroethane as described elsewhere[4]. The aqueous phase from this treatment is dialysed against water and then lyophilized.

Fig. 1 represents a double diffusion agar analysis of rabbit antisera directed against this extracted material. As can be seen, wells 1 and 2, containing two separate rabbit sera, indicate two reacting components present in the glomerular extract which is placed in the centre well. Wells 3 and 4, which show a single line, represent the same antisera as present in wells 1 and 2 respectively except that the antisera has been absorbed with human O, Rh negative cells to remove the hæmagglutinin.

Human O_h cells (suggested by Dr. Kurt Stern, University of Illinois Medical School, and kindly supplied by Dr. Philip Levine, Ortho Pharmaceutical Corp.) were evaluated against the rabbit anti-glomerular extract sera in an effort to determine the specificity of the hæmagglutinin. While the titres of the sera were not remarkably high, nevertheless agglutination occurred. Antisera developed against whole pooled human glomeruli exhibited much higher titres against all human red cells tested.

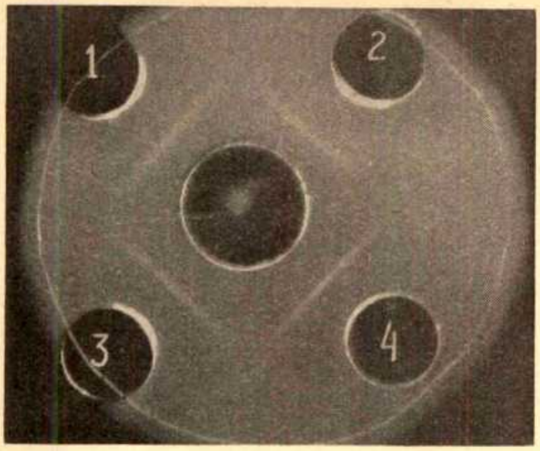

Fig. 1. No. 1. Rabbit anti-glomerular extract serum 45; No. 2, rabbit anti-glomerular extract serum 82; No. 3, rabbit anti-glomerular extract serum 45 absorbed with human O(H) Rh-negative cells; No. 4, rabbit antiglomerular extract serum 82 absorbed with human O(H) Rh-negative cells; centre well, glomerular extract 10 mg/ml.

Inasmuch as the starting material consisted of pooled kidneys, it is rather tenuous to conclude that the hæmagglutinin is directed against a component that is independent of the ABO system. Nevertheless, the hæmagglutinin can be removed by the use of any human A, B, O cell. That the hæmagglutinin may be due to contamination with red cell stroma cannot be absolutely ruled out except to point out that microscopic examination of the starting material is performed before trypsinization and also that a considerable amount of contamination would have to be present to contribute to the final, soluble extract.

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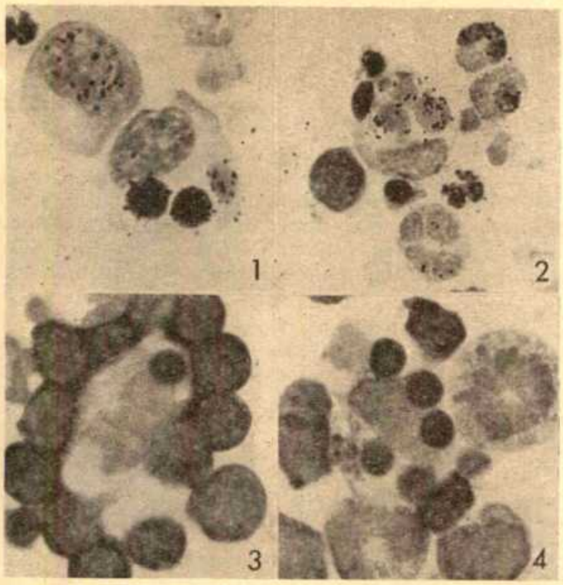
Nucleophagocytosis in Bone Marrow

INGESTED nuclear material has long been known within the reticular cells of normal lymphoid tissues and has led to the suggestion that the reticular cell may re-utilize lymphocytes and incorporate their deoxyribonucleic acid (DNA) into newly formed cells[1,2]. During autoradiographic studies of lymphocytes in the bone marrow, observations have been made on reticular cells including the ingestion of nuclear material labelled by the prior incorporation of tritium-labelled thymidine during DNA synthesis. Male Hartley guinea pigs weighing approximately 400 g were given a single, intraperitoneal dose of tritiated thymidine (1 μc./g body-weight: specific activity, 1·9 c./mM). After intervals of 25, 48 and 72 h humeral bone marrow was gently suspended in homologous serum and examined in smears. Autoradiographs of smears were prepared by coating with melted Eastman Kodak *NTB*[3] emulsion, exposing for 14 days and staining through the fixed emulsion with MacNeal's tetrachrome stain.

After each time-interval reticular cells could be found showing well-labelled bodies within their cytoplasm (Figs. 1 and 2). Even after the earliest interval some of these inclusions of labelled DNA appeared to be partially digested (Fig. 1) and, at later times, short strands of labelled material extended into the adjacent cytoplasm in some instances (Fig. 2). These appearances clearly indicate a true ingestion of nuclei which represent a population of cells with a notably short life span from DNA formation to phagocytosis. It is improbable that this phenomenon is merely a result of radiation damage as not all the nuclear inclusions were labelled. Furthermore, a special study of bone marrow preparations from healthy animals receiving no radioisotopes readily revealed a variety of reticular cells containing Feulgen-positive bodies within their cytoplasm (Figs. 2 and 3).

The precise identification of the inclusions rests, at present, on indirect morphological evidence. Some are small, round, dense bodies most readily interpreted as being the discarded pyknotic nuclei of late (orthochromatic) erythroblasts. Such nuclei, devoid of cytoplasm, may be seen in smears, both free and in the process of apparent extrusion from erythroblasts. Other inclusions stain less densely and are larger than the nuclei which normally appear to be lost by erythroblasts. These might represent the phagocytosis of earlier (polychromatic) erythroblasts *in toto*, possibly as a manifestation of 'ineffective erythropoiesis'[3], but attempts to show an association of the ingested bodies with hæmoglobin or its derivatives have been largely unsuccessful. Thus, Lepehne's reagent clearly demonstrates erythrocytes within the cytoplasm of reticular cells, but a stainable envelope around engulfed nuclei is seen only exceptionally. Moreover, no increased optical density is apparent when viewed by monochromatic light at wave-lengths approximating to that of Soret absorption of hæmoglobin (4150 Å), and the Prussian blue reaction reveals no satisfactory correlation with the presence of non-hæmoglobin iron. On the other hand, these nuclei resemble those of small lymphocytes in size and chromatin pattern where such features are not obscured by lysis. They also resemble the lymphocytic nuclei illustrated within phagocytic reticular cells in the lymphoid tissues[1,2] and it may be noted that the small lymphocyte forms a large population of cells in the bone marrow of this experimental animal[4]. If this identification is correct, it appears that certain small lymphocytes have a 'life-span' of only a few hours before being phagocytosed in the bone marrow. Labelled small lymphocytes do not appear in the thoracic duct lymph or the bone marrow until 2–4 h after tritiated thymidine, yet at 25 h they may be already partially digested within reticular cells (Fig. 1).

As individual reticular cells may contain as many as five or more ingested nuclei (Fig. 4), it is possible that the phagocytosis of lymphocytes occurs on a significant scale. The full extent of the phenomenon is difficult to determine because of the fragility of reticular cells and their characteristically uneven distribution in smears. In the single cell suspensions which are desirable for quantitative techniques[4] reticular phagocytes are infrequently seen. However, they are readily demonstrated in smears that have been carefully prepared after minimal agitation of the bone marrow suspension. Other fragile cell types, notably megakaryocytes, which are plentiful in histological sections of bone marrow but not



Figs. 1–4. Reticular cells containing ingested nuclei in bone marrow smears. (× *c.* 750.) Fig. 1. Autoradiograph, 25 h after tritiated thymidine, showing three labelled inclusions. Fig. 2, Autoradiograph, 72 h after tritiated thymidine, showing four ingested nuclei three of which are labelled and partially digested. Fig. 3. Rosette of basophilic erythroblasts. The central reticular cell contains one ingested nucleus. Fig. 4, Reticular cell containing five ingested nuclei and surrounded by erythroblasts and myelocytes

in routine smears, are also seen in greater numbers in those preparations.

The functional significance of the phagocytosis of small lymphocytes by bone marrow reticular cells is not known. It might merely represent the removal of effete or defective cells after a short and possibly functionless existence. Alternatively, the small lymphocyte may be making a specific contribution to hæmopoiesis in a manner analogous to the feed-back re-utilization theory developed for the lymphoid tissues. According to this view, lymphocytes are ingested by reticular cells which then become lymphocyte-forming cells[1,2], and, at least in antigenically stimulated tissues, the ingestion may occur after a very short life-span[5]. In the bone marrow, however, the immediate cellular relationships of the reticular cells make this less acceptable as a universal explanation. Although some phagocytic cells lie free in the bone marrow smears, many form the central cells of erythroblastic islets (Fig. 3), and others are closely related to a variety of myeloid and erythroid precursors (Fig. 4). Particularly in the case of erythroblastic islets, the relationships are so intimate that it may be difficult to discern a distinct line of junction between the cytoplasm of the reticular cell and that of the surrounding erythroblasts (Fig. 3). This no doubt contributes to the fragility of the reticular cell in smears and suggests the possibility that ingested lymphocytes may provide a source of DNA or other materials for the reticular cell, either for its own metabolism or to be passed out to the surrounding cells in a similar fashion to the ferritin transfer described by Bessis[6]. Such a mechanism might be increased in the event of a stimulus calling for an increased demand on reticular or stem cells. In recent long-term experiments Rieke[7] has noted an increased number and grain density of labelled bone marrow reticular cells in response to anæmia at a time when small lymphocytes appeared to be the only source of well labelled DNA. However, intermediate stages which might further support the hypothesis of lymphocyte incorporation have not yet been observed.

While the interpretation remains speculative, the present observations suggest that nuclear phagocytosis in the bone marrow includes a short-lived group of cells, possibly lymphocytic. If so, this would contribute to the turnover of the small lymphocyte population in normal and stimulated bone marrow.

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RADIOBIOLOGY

Cæsium-134 in Man in Scandinavia

Lidén and Andersson[1] have demonstrated the presence of cæsium-134 in Laplanders from north Sweden and Finland. They suggest that a possible source of the cæsium-134 was the Windscale accident of October 1957. The activity of cæsium-134 in the Laplanders in 1961 was found to be 1·1–1·6 per cent of the activity of cæsium-137, and to attribute this to the Windscale accident it must be assumed that: (*a*) the activity emitted from Windscale contained one-third as much cæsium-134 as cæsium-137; (*b*) one-tenth of the cumulative fall-out of cæsium-137 in Scandinavia to mid-1961 was due to emissions from Windscale.

After the Windscale accident a considerable number of samples of milk, grass and soil were taken from the surrounding district and analysed by γ-spectrometry for iodine-131. Some of these samples, and others taken later, were afterwards analysed for some of the longer-lived activities, especially cæsium-137. Results of the iodine-131 and cæsium-137 surveys[2-6] show that the patterns of deposition of these two isotopes in the Windscale district were very similar. The ratio of activities of iodine-131 to cæsium-137 worked back to the time of the accident was close to 50 : 1.

Fig. 1 shows (*A*) the cæsium-137/barium-137m peak at 0·662 MeV as it appeared in a milk sample from a farm 14 km south-south-east of Windscale, and (*B*) the same peak given by a soil sample taken from a point 2 km from Windscale in the same direction. The milk was collected on November 11, 1957, and measured on December 6, 1957, by which time the ratio of iodine-131/cæsium-137 was about 1/2. On the high-energy side of the peak a small peak occurs at about 0·8 MeV, the height of which is about 2 per cent of the height of the 0·662 MeV peak. It is possible that this peak is due to cæsium-134 (major γ-rays 0·60 and 0·80 MeV; half-life 2·1 years)

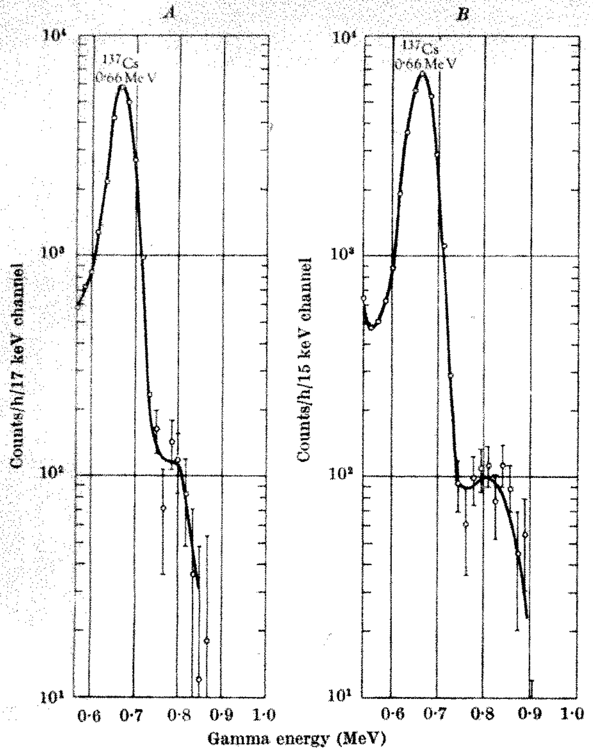

Fig. 1. *A*, Part of spectrum of 1 pint of milk collected on November 11, 1957, from a farm 14 km south-south-east of Windscale and measured on December 6, 1957. Cæsium-137 content of milk is 15 mμc/l.; *B*, part of spectrum of 3-kg sample of top soil collected in January 1961 from a site 2 km south-south-east of Windscale, and measured in June 1961. Level of cæsium-137 activity in soil is 240 mc/km^2

but this is not easy to confirm. The possible presence of the 0·60 MeV peak is masked by the 0·662 MeV peak of cæsium-137, together with a small contribution from the 0·64 MeV γ-ray of iodine-131.

The sample of top soil was collected in January 1961 and measured in June 1961; the naturally occurring activities in this sample have been subtracted from the curve using the spectrum of sub-soil from the same site, following the technique of Löw and Edvarson[7]. At the time of collection of this sample the zirconium-95/niobium-95 activity from weapon debris was very low, and did not contribute significantly to the counting-rate in the 0·76 MeV part of the spectrum. About 20 per cent of the observed cæsium-137 was due to weapon debris. Again a small peak appears in the spectrum at about 0·8 MeV, which is about 1·5 per cent of the height of the cæsium-137 peak; the accuracy of this 0·8 MeV peak is low because the statistics of the difference of these two curves are poor. Part of this peak may be due to manganese-54; but if it is all attributed to cæsium-134 deposited in 1957, back extrapolation suggests that the activity of cæsium-134 was about 4·5 per cent of that of cæsium-137.

From these two curves, which are typical of several obtained for milk and soil from the Windscale area, the activity of cæsium-134 released in the accident is known to be less than 5 per cent of the activity of cæsium-137, after correction for crystal efficiencies and γ-intensities from the recently published decay scheme[8]. It follows that the cæsium-134 found by Lidén and Andersson in Lapland cannot be attributed to Windscale, even if the assumption (*b*) above is correct.

The latter assumption is based on an interpretation by Hvinden and Lillegraven[9] of measurements of cæsium-137 deposition at Bergen; but it can be shown to be unlikely by consideration of the isopleths of cæsium-137 in soil around Windscale. The 20 mc/km^2 isopleth lay at an average distance of about 30 km from Windscale[6]. The cumulative fall-out of cæsium-137 at Bergen up to June 1961 was about 70 mc/km^2; if 7 mc/km^2 of this is attributed to Windscale it would imply that the deposition from the accident was reduced with distance by a factor of only 3 as the cloud travelled from about 30 km to 800 km.

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Mutagenic Effect of X-rays on Moths

Müller *et al.*[1] conclude from their experiments on the mutagenic effect of X-rays on moths that: "If very low doses have any effect at all, then it must be much smaller than should be expected from the results of irradiation with higher doses". This is contradicted by the results they present in their Fig. 1 and Table 1.

Their regression curve based on effect proportional to square of dose, which holds at high dose-rates (their Fig. 2), extrapolates to about 40 mutations per 10^6 scales at 0 dose, so that no significant increase is found even at 40 r. However, the actual number found was 19 at 0, 22 at 10 r., so that there is a net increase of 20 at 20 r., 25 at 40 r. Calculated increases at 20 and 40 r. are 2 and 7 on their result of 202 mutations at 200 r., an effect proportional to square of dose.

The increase in mutation frequency for low doses is not less, but very much more than would be expected from the results of irradiation with higher doses.

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[1] Müller, I., Löbbecke, E. A., and Oltmanns, O., *Nature*, **194**, 78 (1962).

We certainly agree with Dr. Kortschak that there is a larger increase in mutation-frequency from 0–20 and 0–40 r. than would be expected from the dose-relationship at higher doses. But in his considerations he leaves out of account the very low, and repeatably low, value at 10 r., and the sentence quoted by him refers to these very low doses between 0 and 10 r., where only an insignificant effect was found. Somewhere between 10 and 20 r. there must be a steep increase in mutation-frequency, which might be a rather sudden step. The hypothesis is that there might be a protective mechanism, which is destroyed by doses between 10 and 20 r. However, this needs to be proved by extensive experiments and because of their hypothetical character these considerations were not included in the short communication.

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BIOLOGY

Conversion in Plants of Benzoic Acid to Salicylic Acid and its βd-Glucoside

PREVIOUS investigations of the auxin metabolism in plant tissues led to the following findings of a ring hydroxylation resulting in 2-hydroxyindolyl-3-acetic acid[1,2], and a hydroxynaphthalene-1-acetic acid, which probably hydroxylated in the 8-position, and their glucosides[2]. Other known products of the auxin metabolism were the aspartic acid conjugates[3] and the βd-glucose esters[4,5] of the free auxinic acids. Analogues of the last two metabolites have also been described for benzoic acid[4,6]. In wheat coleoptiles no hydroxylation of benzoic acid could be demonstrated.

Using the method described earlier[4] etiolated *Helianthus* hypocotyls were incubated with 6×10^{-5} and 6×10^{-4} molar carboxyl-labelled benzoic acid. Hypocotyls were crushed directly on chromatographic paper '*S* and *S* 2043*b*'. The solvents used in chromatography were butanol / formic acid / water (10 : 1 : 2) for the first and isopropanol / ammonium hydroxide / water (80 : 15 : 5) for the second direction. The compounds labelled with carbon-14 were then detected by autoradiography of these paper chromatograms (Fig. 1). Besides the very large spot of the benzoyl-βd-glucose (*BGl*) and two hitherto unknown compounds (BX_1 and BX_4), it shows the free benzoic acid (*BA*), a very small spot of salicylic acid (*SA*), and a relative large spot of βd-glucosido-salicylic acid (*GlSA*). The glucoside was characterized by hydrolysis with emulsin. The hydrolysis products agreed with co-chromatographed authentic salicylic acid and glucose. The radioactivity and colour reaction with ferric chloride, and diazobenzene-sulphonic acid were in accordance with this phenolcarbonic acid.

Recently[7] gentisic and *o*-pyrocatechuic acid were found in leaves of various plants fed with salicylic acid. The same acids and *p*-hydroxybenzoic acid were formed in plants incubated with carboxyl-labelled benzoic acid[7]. Whether they did occur as free acids or as glycosides is questionable, because the investigations were carried out with acid-hydrolysed ethanol extracts of plant tissues. *Helianthus annuus* hypocotyls, *Solanum tuberosum* tubers, and *Pisum sativum* internodes and roots are able to form salicylic acid, and in much larger quantities βd-glucosidosalicylic acid from benzoic acid. No other phenolcarbonic acids or their glycosides in traceable amounts derived from benzoic acid.

In wheat coleoptiles no ortho hydroxylation of benzoic acid could be demonstrated. On the other hand, it was shown that wheat plants are capable of metabolizing salicylic acid to gentisic and *o*-pyrocatechuic acids[7]. Therefore, it seems that ortho hydroxylation of aromatic acids needs a specific reaction mechanism, while further hydroxylation appears a rather widespread and more unspecific reaction, presumably catalysed by polyphenoloxidases.

In the biosynthesis of coumarin[8] a high rate of conversion of cinnamic acid to βd-glucosido-*o*-coumaric acid was found. This glucoside has been discussed as a possible intermediate in the biosynthesis of coumarin. The same situation has been described for the synthesis of hydroxynaphthalene-1-acetic acid[2] and here of salicylic acid. But whether the glucosido-phenolcarbonic acids are intermediates or detoxification products is unknown. Due to its lactone ring, coumarin may be an exception; however, other phenolcarbonic acids are substrates for phenoloxidases. In the case of salicylic acid gentisic and pyrocatechuic acid, and possibly polyphenols, are formed. The glucosides of phenolcarbonic acids prevent this oxidation. Therefore, it seems that this property may be the general function of those phenol-glucosides. A balance favourable for this assumption is indicated by the fact that the quantitative relation between the free phenolcarbonic acids and their glucosides (about 1 : > 10) was always nearly the same, whether plants were fed with the free phenolcarbonic acids or their glucosides. The function of these glucosides is under investigation.

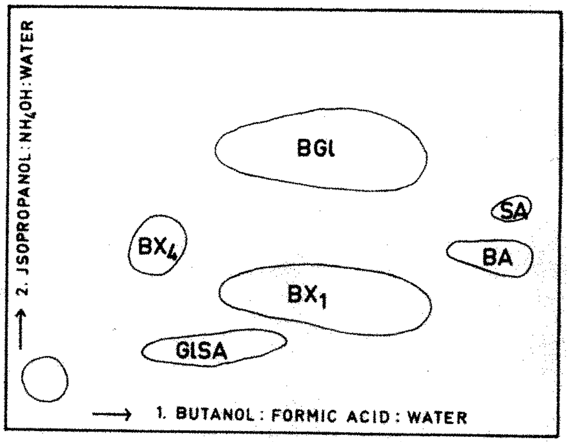
2. ISOPROPANOL : NH4OH : WATER
BGl
SA
BX4
BA
BX1
GlSA
1. BUTANOL : FORMIC ACID : WATER

Fig. 1

This work was supported by a grant from Deutschen Forschungsgemeinschaft.

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Antagonistic Effect of Urea and 2-Chloroethanol on the Resistance to High Salt Concentration in Wheat Seedlings

IT is considered that the ammonia produced by the hydrolysis of urea in the soil damages the roots of plants. Recently, Cooke[1] reported that at least one other factor may be operative in urea toxicity. Weber and Tanford[2] demonstrated that 2-chloroethanol strengthened the intramolecular hydrogen-bonding of ribonuclease and urea possessed the opposite character. The increase of resistance to high salt concentration by the seed treatment with dilute 2-chloroethanol solution was found by Miyamoto[3].

The present investigation deals with the antagonistic effect of urea and 2-chloroethanol on the resistance when their dilute solutions are applied to the wheat seed treatment.

Wheat seeds were soaked for 14 h at room temperature in the solutions which are indicated in Table 1. After the treatment, the seeds were spread on a filter paper and dried for 4 h. Control seeds were treated by water instead of the solution. A 200-g portion of sandy soil (*p*H 6·65) was placed in a Neubauer container and moistened by 25 ml. of water. Forty seeds were planted in the soil. At 6 days after the seeding, 3·0 or 4·0 g of ammonium nitrate in the form of 50 per cent solution was added on the surface of soil. The numbers of wilted plants were recorded and they were considered to be the criteria of the damage. Tests were replicated three times and the averages were calculated. The results are shown in Table 1.

Table 1. EFFECT OF SEED TREATMENT WITH UREA AND 2-CHLOROETHANOL ON THE RESISTANCE TO HIGH SALT CONCENTRATION IN WHEAT SEEDLINGS

Seed treatment (14 h)	Amount of applied NH_4NO_3 in g/200g soil	Wilted plants (%)		
		24*	48*	72*
0·06 % Urea	3	14·2	50·5	71·4
	4	32·9	69·3	88·6
0·08 % 2-Chloroethanol	3	0	12·9	20·4
	4	5·3	29·0	41·9
0·06 % Urea + 0·08 % 2-chloroethanol	3	7·3	28·4	38·9
	4	18·8	45·5	64·4
Control	3	6·3	27·6	40·4
	4	14·4	40·0	66·6

*Hours after the NH_4NO_3 application.

The results in Table 1 indicate that the seed treatment with 0·06 per cent urea reduces the resistance, and the effects of 0·06 per cent urea and 0·08 per cent 2-chloroethanol seed treatments are antagonistic. The seed treatment with 0·08 per cent ammonium nitrate solution did not influence the resistance, and no antagonistic effects between 0·08 per cent ammonium nitrate and 0·08 per cent 2-chloroethanol were observed. I consider that the weakening effect of urea on the intramolecular hydrogen-bonding of some macromolecular substance may be the reason for the decreasing of resistance, and it can be a factor other than ammonia in urea toxicity.

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Gibberellin-like Activity of Kudzu Vine Extract

PLANTS sensitive to the application of gibberellin A_3 (gibberellic acid) respond in a number of ways; but the most characteristic one is that of elongation of the stem. This response has been demonstrated and reported by many workers[1–5].

A number of investigators have reported gibberellin-like activity in extracts from a variety of plants and plant parts[6–12]. Generally, a positive growth response of the bio-assay plant stem to the test extract has been accepted as an indicator for the presence of a gibberellin-like substance[6–9,12].

Among the plants assayed in the Merck Chemical Division laboratory in July of 1958 for the purpose of determining the occurrence of gibberellin-like activity of extracts of both flowering and non-flowering species[13] was the kudzu vine (*Pueraria thunbergiana*). This perennial plant, a member of the Leguminosae, is a very long-running, somewhat woody plant which has become established in many parts of south-eastern United States as a soil-erosion control agent and as a minor forage crop. Its rapid growth (50–100 ft. per season) suggested the possibility of its extract possessing gibberellin-like activity. The initial assay gave a positive growth response of relatively great magnitude.

The sample of kudzu vine used in preparing the extract was collected in Georgia. Only the first twelve inches of terminal stem of each runner of the vine was collected, and several pounds were sent by air mail express so as to reach the laboratory in a still succulent condition. The parts collected consisted of the stem proper, leaves and leaf petioles. Three hundred grams were prepared for extraction by maceration in a Waring blender. Distilled water was added to facilitate blending, and the amount used was noted. The *p*H of the resulting slurry was adjusted to 3·5 with 5 N hydrochloric acid. The initial extraction was conducted with ethyl acetate, added in an amount to give a 1 : 1 ratio with the water already present in the slurry. Steeping, filtering and separation of the water layer from the solvent followed. Two more extractions of the residue solids were made with ethyl acetate. The individual ethyl acetate filtrates were combined, and concentrated to 30 ml. by evaporation at room temperature. The water layer from the first extraction was similarly reduced to 30 ml. A non-ionic surfactant was added as 1 per cent of the total volume to each concentrate.

Investigations of growth activity of the extracts were initially conducted with the pinto bean seedling bio-assay method. However, the technique utilized was slightly modified from that given by Gray[14]. A volume of 0·01 ml. of each material was placed at the terminal of the seedling stem. Measurement of stem height at the inception of the test and again six days after application yielded data for comparison with measurements of control plants and of plants treated with standard potassium gibberellate solutions.

The results obtained with the ethyl acetate extract of the kudzu vine are given in Table 1.

Table 1. RESPONSE OF PINTO BEAN SEEDLING TO THE EXTRACT OF KUDZU VINE AS INDICATED BY STEM HEIGHT INCREASE

Material	Age of pinto bean seedling (days)	Vol. of solution applied (ml.)	Average increase in stem height (in.) six days after application
Check	14	—	0·56
Standard solution of potassium gibberellate, 1 p.p.m.	14	0·01	0·75
Kudzu vine extract	14	0·01	6·00
Check	20	—	1·62
Standard solution of potassium gibberellate, 1 p.p.m.	20	0·01	3·93
Kudzu vine extract	20	0·01	17·00

Additional assays with Pinto bean seedlings, dwarf pea seedlings and *dwarf*-1 maize[4] confirmed the presence of gibberellin-like activity in the extract of the kudzu vine. The water filtrate has given comparable activity.

The magnitude of the responses obtained with the extract had been exceptionally high, and a log of the concentration-height increase study indicated a gibberellic acid activity equivalency falling within a 10–100 p.p.m. range for a volume of 0·01 ml. of the initial ethyl acetate extract. This activity has not been equalled by other plant extracts showing positive growth responses.

An initial examination of the extract by chromatography developed a number of fluorescent bands, one of which paralleled the band of the chromatograph of standard gibberellin A_3 control. An eluant of the former band was assayed by means of the pinto bean seedling technique, and the resultant height increase was again indicative of a plant growth activity of a high order.

Subsequent chromatographic and biogrowth work with kudzu vine extracts confirmed the presence of a gibberellic acid-like substance. Visual comparison of the fluorescence on the chromatograms to standard gibberellic acid indicated an approximate level of 80–500 parts per billion[15].

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Infection of Celery Seedlings by *Septoria* spp.

Celery seed often carries pycnidia of the fungi *Septoria apii* and *Septoria apii-graveolentis*. It is commonly assumed that spores from these pycnidia infect young seedlings, and that this is the most important way in which the diseases caused by these two fungi start each year. There are, however, the following reasons for doubting the validity of this assumption: (1) Seed treatment with fungicides does not satisfactorily prevent infection of celery seedlings, although similar treatments are effective in certain other diseases in which the fungi are seed-borne as pycnidia. (2) Infection of celery seedlings can, however, be prevented by treating infected seeds in hot water, a treatment known to be effective against certain fungi carried inside seed, for example, *Ustilago nuda* in wheat and barley.

These facts suggest that spores in pycnidia may be less important than has previously been supposed, and that seedlings become infected by parts of the fungi which are not carried superficially on the seed. We have obtained the following evidence in support of this hypothesis: (*a*) A considerable number of samples of heavily infected seed of different ages and from different sources were examined. In most of them no viable spores were found and only a few (*c*. 1 per cent) of the spores from the youngest seed, about 8 months old, germinated under optimum conditions. None of these samples had been treated in any way before it was examined. (*b*) Healthy seed were heavily contaminated with viable spores taken from young cultures of the two fungi. They were then sown in conditions favourable for infection of seedlings. None of the seedlings became infected. When seedlings of the same age, grown from uncontaminated seed, were sprayed with viable spores from cultures of the same type and age, almost all became infected. (*c*) Microscopic examination of seed carrying pycnidia showed that many contained hyphæ abundantly in the mesocarp; that in some the testa was invaded by hyphæ, and that occasionally the hyphæ had penetrated to infect the cotyledons. The hyphæ inside the seed have not yet been identified but microscopically they did not have any characteristics that would have precluded them from belonging to *S. apii* or *S. apii-graveolentis*.

Although the above facts do not preclude the possibility that seedlings become infected from the occasional spore that remains viable in infected seed, they do strongly suggest that more frequently seedlings become infected from mycelium carried within the seed.

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Differential Synthesis of Nucleic Acids in Sexual Differentiation of *Allomyces*

What could well be the final expression of a fundamental, sex-determining antagonism between deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) biosynthetic pathways has found a first chemical confirmation in our recent measurements of the percentage values of the DNA/RNA ratios in the gametes of the water mould *Allomyces*[1].

As expected from the cytological data (selective multiplication of the male nuclei[2]), the DNA/RNA ratio was found to be higher in the males (12·8 per cent) than in the females (6·9 per cent)[1]. Thus, it remained to follow the evolution of this nucleic acid ratio from the initial, undifferentiated hyphæ through the progressively differentiating male and female gametangia, in an attempt to determine, chemically, the crucial point of appearance of the diverging values of the ratio.

Male and female hybrid strains of *Allomyces macrogynus* × *A. arbuscula*[3] have been grown, at 25° C, in 50 ml. of either of the modified glucose–yeast extract (*GY*) solutions[4]: G_2Y, namely that containing twice the concentration of glucose (6 g/l.) and half that of yeast extract (Difco, 0·5 g/l.), which favours quick and relatively synchronized differentiation of the gametangia; glucose–casein–yeast extract (*GCY*) solution, with normal glucose (3 g/l.) but only 0·1 g/l. yeast extract and a supplement of 3 g/l. casein hydrolysate (Difco), favourable to vegetative growth and slowing down differentiation[5]. Through continuous agitation of the cultures, each small block of inoculum (12 per flask, cut from gametophytes grown on plates of 'YpSs' Difco medium[6]) gives rise to a mycelial ball of radially disposed hyphal tips which are starting to differentiate their gametangia earlier (second day on glucose–yeast extract, G_2Y solution) or later (third day on glucose–casein–yeast extract, *GCY* solution). Improved synchronization of the last stages of gametangial differentiation could be achieved by dipping young mycelial balls in the discharge, hypotonic saline solution[7]. Finally, the balls of each sex were pooled and their developmental stage[2] controlled under the microscope before drying them between pieces of blotting paper, measuring their fresh weight and keeping them in the freezer until analysis.

For the nucleic acid analyses, we have combined the principles of the classical methods[8], according to the following procedure: (1) initial grinding of the frozen balls in cold, 10 per cent trichloroacetic acid (TCA)[9], followed by successive washings of the residue with cold, 5 per cent TCA and twice with a chloroform/ethanol mixture (1 : 3) to remove lipids (and male carotenoids); (2) hydrolysis of the dried residue in 1 N potassium hydroxide at 37° C overnight[10]; exact neutralization of the hydrolysate with HCl has preceded the precipitation of DNA and protein with 2·5 per cent TCA (final) + hydrochloric acid 0·6 N, hydrolysed RNA remaining in the supernatant; RNA was determined on an aliquot of this TCA supernatant, using the orcinol, colorimetric (660 mμ) method[11,12], against a standard curve of pure RNA (Fluka AG., Buchs, Switzerland) and each time with a renewed standard of 10 μg/ml. (3) DNA was selectively extracted from the residue (2), with 0·5 N perchloric acid[13] for 20 min at 70° C, followed by washing with cold 2 N hydrochloric acid (puriss., Merck Co.). Finally, DNA was determined at 260 mμ with a model *DU* Beckman spectrophotometer, in the perchloric acid–hydrochloric acid solution and against standards of pure DNA (Fluka AG.), dissolved in the same solvents also used as blank.

Table 1

Developmental stage of *Allomyces* (statistically predominant)	Nucleic acid ratios* DNA/RNA (per cent)		RNA/DNA	
	♂	♀	♂	♀
Vegetative hyphæ + club-like progametangia (*GCY*, 3 days)	8·5	10·5	11·8	9·5
Young gametangia† in early maturation (G_2Y, 3 days)	8·9	9·2	11·2	10·9
Mature gametangia‡ + free gametes (G_2Y, 4 days and 2 h in hypotonic saline solution)	11·9	6·1	8·4	16·4

* Average values from four experiments.
† Traces of carotenoids in the males.
‡ γ-Carotene-rich males.

As shown by the results summarized in Table 1, the final, sex differential values of the DNA/RNA ratios (per cent) in the mature gametangia (gametes liberation stage[2]) correspond closely to those previously measured in the free gametes of *Allomyces*[1], male values averaging 1·5–2 times higher than the female ones. Moreover, the origin of this final, higher value in the male gametes can now be traced back to the first maturation stage of the gametangia, characterized by the differential multiplication of the male nuclei[2,5,14].

In the female gametangia, the DNA-level can be considered as stationary, or, at the most, as slightly increasing as no, or only few, mitoses are elevating the given number of nuclei initially incorporated in the gametangial cytoplasm at the septation stage[2,14]. Consequently, the sharp drop in the DNA/RNA ratio in the female gametangia must result from an increase in the RNA-level as more clearly shown by the developmental evolution of the RNA/DNA ratios (Table 1, 2nd col.).

The sharp increase in the RNA/DNA values in the mature female gametangia provides direct, chemical evidence in favour of my previous assumption, mainly drawn from cytochemical observations[15,16], that differential, active RNA synthesis coincides with the formation of the basophilic nuclear caps in the mature gametangia. This has found additional support in my recent measurements of the increasing RNA/protein ratios in the maturing female gametangia[17].

By contrast, the RNA synthesis must be much less important in the male gametangia as shown by their final low RNA/DNA ratios and confirmed cytologically by the thinness of their gametic nuclear caps[18].

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Occurrence of the Genus *Archigetes* in Britain

DURING the course of investigations into the taxonomy and ecology of aquatic Oligochaeta we have observed two species of *Archigetes*, a genus not to our knowledge previously recorded from Britain. *Archigetes appendiculatus* Mrazek, 1897 (non Ratzel, 1868) was first observed in a specimen of *Limnodrilus hoffmeisteri* Claparède, 1862 obtained from a Liverpool pet shop along with a large number of uninfected 'Tubifex' worms. The same species

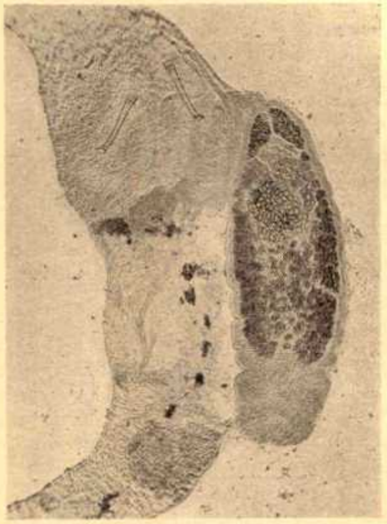

Fig. 1. *Archigetes appendiculatus* in the genital segments of *Limnodrilus hoffmeisteri*. Both host and parasite are sexually mature, eggs being clearly visible in the uterus of the parasite. Note the separation of the vitellaria into four distinct lobes. (× *c*. 17)

of parasite was later found in a pond near Fornalls Green Lane, Meols, Cheshire, on May 28, 1959. A second species *A. sieboldi* Leuckart, 1869, also from *L. hoffmeisteri*, was found from the Shropshire Union Canal near Chester on March 20, 1962. The cestodes were readily identified from recent descriptions of the genus[1,2]. In each instance the parasites were located in the genital segments of the tubificids. Both specimens of *A. appendiculatus* had eggs in the uterus (Fig. 1), while two specimens of *A. sieboldi*, from one oligochaete, contained no eggs.

At present we are collecting further data on the incidence and occurrence of *Archigetes* and other cestode parasites of Oligochaeta. We should appreciate correspondence with other workers who may find such material during the course of their studies on related fields of freshwater biology.

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Presence of Istiophoridae along the South Atlantic and Mediterranean Coasts of Spain

As a result of a co-operative undertaking of the Laboratorio del Instituto de Investigaciones Pesqueras of Cadiz with the Marine Laboratory of the University of Miami, carried out by us during the years 1960 and 1961, twenty-one specimens of Istiophoridae were obtained, of which nineteen were *Tetrapturus albidus*. Two of the twenty-one specimens were not identified and are not dealt with here. Some of these fish were captured on lines operated by fishermen for the capture of the swordfish (*Xiphias gladius* L.) off the south Atlantic coast of Spain and Portugal, and in the Straits of Gibraltar. The balance of these fish were caught in the tuna-trap (Almadraba). 'Nueva Umbria', situated off the town of Isla Cristinal not far from Huelva, on the south Atlantic coast of Spain, and in the trap 'La Atunara', situated off the town of La Linea, in the Mediterranean Sea, just east of the Straits of Gibraltar. Table 1 shows the catches of the 19 specimens identified as *T. albidus*.

In October 1961 Dr. C. Richard Robins, of the Marine Laboratory of the University of Miami, confirmed, at Cadiz, the identification of nineteen of the specimens as *T. albidus* Poey (= *Makaira albida* (Poey) of some authors). Dr. Robins is investigating the other two specimens still further before identifying them.

In the Spanish literature *Tetrapturus belone* Rafinesque and *Makaira nigricans* Lacepede have been recorded from the coast of northern Spain and the Balearic Isles for the former and off the southern coast for the latter. Fernando de Buen[1] reported *M. nigricans* taken at Isla Cristina in August 1923, giving little descriptive data. Again[2] this author recorded *M. nigricans* on the south Atlantic coast of Spain and *T. belone* in the north of Spain and the Balearic Isles. Luis Lozano y Rey[3] identified the fish recorded by de Buen in the north of Spain and from Isla Cristina with *T. belone* Rafinesque.

We doubt the validity of the identification of *T. belone* Rafinesque in the above papers, with the possible exception of the listing of this species for the Balearic Isles by de Buen[2]. In fact, we agree with Robins and de Sylva[4] that a survey of all literature referring to the Istiophoridae reveals no certain record of *T. belone* from the eastern Atlantic exclusive of the Mediterranean Sea.

We believe that this is the first report in the literature of *T. albidus* from the southern Atlantic and Mediterranean coasts of Spain, with the exception of a statement in Tortonese[5] which in part reports a statement by the junior author referring to some of the above 19 specimens.

The common or vernacular names of these fish on the south Atlantic coast of Spain vary from west to east as follows: Isla Cristina, 'pez aguja'; Huelva, 'alfiler'; Barbate, 'cometa'; Algeciras, 'salton'.

We thank Mr. Antonio Solano, of the Consorcio Nacional Almadrabero, for his co-operation and the

Table 1. Catches of the 19 Specimens Identified as *Tetrapturus albidus*

Original Field No. Marine Laboratory, University of Miami (EATL)	Date	Location	Gear	Wt. (kg)	Body-length (mm)
39	Aug. 16, 1961	Between Tangier and Larache	Line	25·25	1,662
40	Aug. 16, 1961	Between Tangier and Larache	Line	40	1,850
41	Sept. 13, 1961	La Linea (Almadraba La Atunara)	Trap	42	1,825
42	Aug. 22, 1960	La Linea (Almadraba La Atunara)	Trap	31·5	1,670
43	Aug. 16, 1961	West of Tangier	Line	48	1,900
44	Aug. 1, 1961	Off Cabo Santa Maria	Line	63·5	2,030
45	Aug. 22, 1961	Middle Straits of Gibraltar off Punta Almina	Line	46	1,905
46	Aug. 1, 1961	Off Isla Cristina	Line	26·5	1,700
47	Aug. 1, 1961	Off Cabo Santa Maria	Line	39·5	1,810
48	Aug. 7, 1961	Off Cabo Santa Maria	Line	46·25	1,895
49	Aug. 1, 1961	Off Cabo Santa Maria	Line	35·5	1,800
50	Aug. 7, 1961	Off Cabo Santa Maria	Line	28	1,675
51	Aug. 7, 1961	Off Cabo Santa Maria	Line	29	1,685
52	Aug. 8, 1961	Isla Cristina (Almadraba Nueva Umbria)	Trap	24·5	1,600
53	Aug. 15, 1961	Straits of Gibraltar off Tangier	Line	41	*
54	Aug. 7, 1961	Off Cabo Santa Maria	Line	45	1,942
55	Aug. 8, 1961	Isla Cristina (Almadraba Nueva Umbria)	Trap	27	1,928
56	Oct. 5, 1961	La Linea (Almadraba La Atunara)	Trap	42	1,860
57	Oct. 5, 1961	La Linea (Almadraba La Atunara)	Trap	25·5	1,640

* Head off, no measurement.

donation of the specimens from the tuna-traps; Messrs. Jaime Perezy and Jose Troyano, who helped in the purchase of specimens; and Mr. Magin Vinielles, director of Industrias Gaditanas del Frio, S.A., who permitted the use of the freezer for the preservation of the specimens. We also thank Dr. C. Richard Robins for assistance in the identification of the specimens.

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MICROBIOLOGY

Bacterial Growth at Low Population Densities

ACCEPTING Monod's[1] formulation of the relationship between growth-rate (μ) and the concentration of the limiting substrate (s), Herbert *et al.*[2] calculated the latter for steady-state conditions in the chemostat as:

$$s = K_s \frac{D}{\mu_{\max} - D} \qquad (1)$$

where D is the dilution-rate (equal to the growth-rate), and K_s and $\mu_{\max}$ are growth constants. The concentration of cells produced (x) must be nearly proportional to the consumption of the substrate ($s_r - s$, s_r being the concentration of the growth-limiting substrate fed into the chemostat), when s is very small as compared with s_r:

$$x = y\,(s_r - s) \qquad (2)$$

or

$$x = y\left[s_r - \left(K_s \frac{D}{\mu_{\max} - D}\right)\right] \qquad (3)$$

where y is the yield coefficient which is constant at a given growth-rate. Since s is also constant at a given growth-rate and independent of s_r (ref. 3), x theoretically follows the broken curve in Fig. 1 at low values of s_r. When washout of the population occurs ($x = 0$), s_r and s become identical and can be taken as the relative threshold concentration of the limiting substrate for growth of a particular organism depending upon the growth-rate given. This was checked experimentally.

Approaching the washout of a population in the chemostat by lowering s_r at a fixed dilution-rate met with the difficulty of determining low population densities. *Spirillum serpens* did not tend to wall growth and exhibited considerable constancy of cell width and amplitude at a fixed growth-rate. With this particular organism, the calculation of cell mass after counting cell units of equal size on membrane filters has been found to yield results of sufficient accuracy down to concentrations of 10^7 cells or 0·1 mg dry weight per litre. The organism was grown in a basic mineral medium containing ammonia as the nitrogen source and lactate as the growth-limiting factor. The constants were at 20°: $\mu_{\max} = 0·34$ h^{-1}, $K_s = 2·2$ mg lactate/l., and $y = 0·42$.

When s_r was lowered stepwise at a fixed dilution-rate of $D = 0·5\ \mu_{\max}$ (where s is equal to K_s), cell concentration x adjusted to constant values according to equation (3). Below approximately 1 mM of lactate in the inflowing medium, however, the drop of x began to deviate from theoretical values as indicated by the solid curve in Fig. 1. From each point on this curve, a different value for s has been calculated by rearranging equation 2:

$$s = s_r - \frac{x}{y}$$

The lowest population that could be maintained at a constant level indicated a final washout at a threshold concentration of 12·8 mg lactate/l. (s_e). That is, about six times as high as the theoretical value.

Since the inconstancy of K_s is unlikely, it must be assumed that the growth limitation has changed. From several observations it can be concluded that growth-stimulating interrelationships between the cells occur as a consequence of the decreasing population density. If this is true, any external factor displaying the corresponding beneficial effect pertaining to growth of the population can be expected to lower the experimental threshold concentration of the growth-limiting substrate regardless of its nature. This has been demonstrated by decreasing the concentration of dissolved oxygen in a steady state culture of a microaerophilic *Spirillum*, where cells acted as reducing agents. This treatment not only

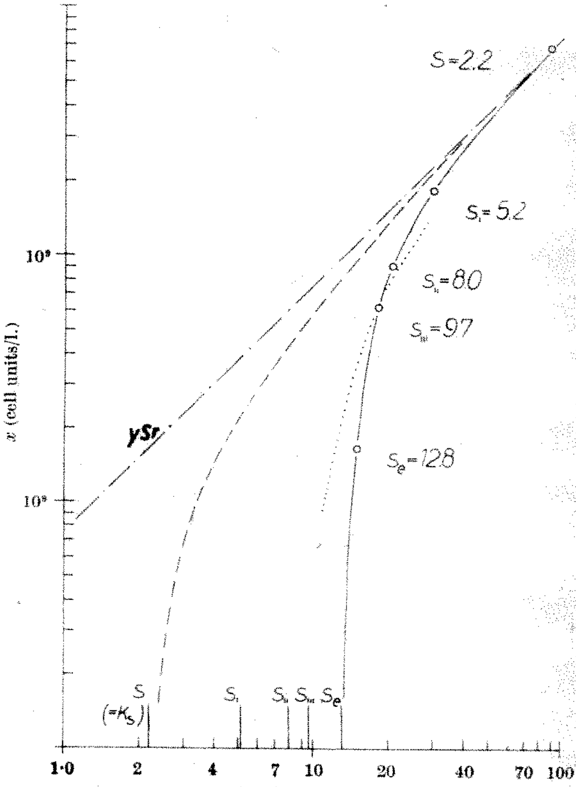

Fig. 1. Decrease of population density in the chemostat with decreasing concentration of the growth-limiting substrate in the inflowing medium. The experimental values (solid curve) deviate from the theoretical values (broken curve). From each point a specific value between s and s_e is calculated

lowered the experimental threshold concentration of the limiting substrate, but also the amount of the inoculum necessary to start growth of the population in batch culture. There are similar observations reported in the literature; for example (ref. 4), a reduced carbon dioxide production by a culture at low population densities may decrease the effectiveness of an inducer of β-galactosidase.

The determination of the experimental threshold concentration has been repeated at various dilution-rates. The deviation between theoretical and experimental values was found to increase with a decreasing growth-rate. This fact supports the concept of growth-stimulating interrelationships between cells of a bacterial population in a medium which does not meet all the requirements for growth of the single cell. This internal factor becomes growth limiting when the population density drops below a certain value. From this point of view, the existence of an *absolute* threshold concentration of the growth-limiting substrate is conceivable, below which an organism is unable to grow at any dilution-rate.

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Fimbriæ and Hæmagglutinating Properties in Strains of *Proteus*

FIMBRIATION and the hæmagglutinating pattern of 21 strains of *Proteus mirabilis*, 10 of *P. vulgaris* and 20 strains each of *P. morgani* and *P. rettgeri* have been investigated. The strains of *P. hauseri* were those previously used[1] and were either in the *Y*, *W* or *R* phases[2]. The *morgani* strains comprised 12 recently isolated local strains and 8 (Nos. 232, 235, 1707, 2815, 2818, 5845, 7381 and 10041) obtained from the National Collection of Type Cultures, Colindale, London. The *P. rettgeri* consisted of N.C.T.C. strains Nos. 7475, 7476, 7477, 7478, 7479, 7480, 7481 and 8893 and 12 local strains. Methods used were those of Duguid and Gillies[3]. Broth cultures were started from stock agar slopes and incubated at 37° C and at room temperature. Erythrocytes of fowl, sheep, horse, guinea pig, rabbit, man and ox were used and the hæmagglutination pattern of 20 serial 48 h broth cultures of each strain was determined.

All strains of *P. hauseri* became hæmagglutinating after 1–3 sub-cultures in broth at both temperatures. At this stage fowl and sheep cells were strongly agglutinated. Horse and guinea pig cells weakly and rabbit, man and ox cells were scarcely ever agglutinated and then only weakly. Our results, though showing the same tendency, differed from those of Duguid and Gillies[4] and Shedden[5] in not showing such a diffuse spectrum. The former workers did, however, encounter a few strains which were inactive for rabbit, human and ox cells. With further sub-cultures the spectrum tended to become narrower, and by the 10th and subsequent examinations all strains agglutinated only fowl, sheep and horse cells. Tests with the latter cells were often only weakly positive. *Y*, *W* and *R* variants had the same hæmagglutinating properties. Shedden[5] found his *B* phase cultures (which correspond very closely to our *W* variants) to be non-hæmagglutinating. All strains of *P. morgani* became hæmagglutinating after 2 or 3 sub-cultures at both temperatures. The pattern at this stage corresponded closely to those presented for *Proteus* strains by Duguid and Gillies[4] and for *P. hauseri* by Shedden[5]. Tests with ox cells were, however, only weakly positive or negative. At about the 10th sub-culture the spectrum characteristically narrowed and tests were afterwards only positive with fowl, sheep, horse and guinea pig cells. It would appear that under the conditions of growth used, further variants of *P. hauseri* and *P. morgani* are selected which determine the final agglutinating pattern of the culture. The hæmagglutinating property of strains of *P. rettgeri* differed from those of other *Proteus* groups in that all strains developed hæmagglutinating ability earlier (2nd or 3rd sub-culture) in broth at room temperature than at 37° C (6th–10th sub-culture). Secondly, the hæmagglutinating pattern differed from the other *Proteus* groups in that erythrocytes of all 7 vertebrate species used were agglutinated to the same degree—usually fairly strongly positive—and the pattern did not vary with further sub-cultures in broth.

The hæmagglutinating activity of *P. rettgeri* and *P. morgani* was the same when tested at 4°, 22°, 37° and 55° C. The hæmagglutinins of these two groups were cell-bound and non-diffusible and were not affected by acetone or 95 per cent alcohol but were completely destroyed by boiling at 97° C for 2 min. Agglutination of red cells by *P. morgani* and *P. rettgeri* is not inhibited by D-mannose. In all these respects the hæmagglutinins of these groups resemble those of *P. hauseri*[5]. Non-hæmagglutinating variants of *P. rettgeri* could be selected by serial platings on agar incubated aerobically at 37° C. This variation has been demonstrated for strains of *P. hauseri* and *P. morgani* by Duguid and Gillies[4]. Electron microscopy showed organisms of hæmagglutinating strains of *P. morgani* and *P. rettgeri* to be perifimbriate with denser aggregations at the ends. The fimbriæ are about 0·01μ in width and 0·5–2μ in length (Fig. 1). These fimbriæ have similar distributions and dimen-

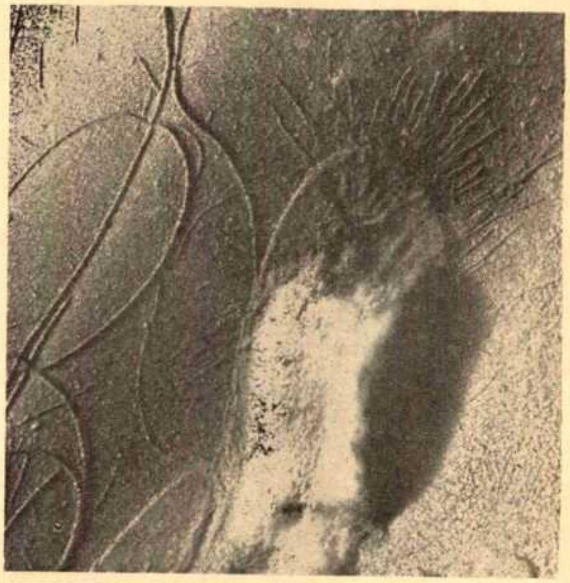

Fig. 1. Electron micrograph of *P. rettgeri* N.C.T.C. No. 7481. Numerous fimbriæ and a few flagella are present. (× *c*. 41,250)

sions to those of *P. hauseri*[5]. Non-hæmagglutinating variants of these organisms showed no fimbriæ.

P. morgani and especially *P. rettgeri* differ in many respects from *P. hauseri*. This investigation was an attempt to discover more differences and has been only partly successful. Although differences in the hæmagglutinating pattern between *P. hauseri* and especially *P. rettgeri* were demonstrated, the 3 groups of organisms were found to have hæmagglutinins with many similar physical properties.

This work was aided by grants to one of us (J. N. C.) from the South African Council of Scientific and Industrial Research.

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GENETICS

Demonstration of Antigenic Difference between the American Bison (*Bison bison*) and Domestic Cattle (*Bos taurus*)

THERE are reports[1-4] demonstrating that antiserum produced against the erythrocytes of one species which react with the cells of a closely related species may be made specifically reactive with the homologous cells by absorption with cells of the related species. Hence, one might expect that this method should differentiate the cells of all, or nearly all, related species.

Stormont *et al.*[5] have demonstrated the homology of nine genetic systems of cattle blood groups with those of bison. Owen *et al.*[6] were unable to demonstrate clearly definite differences between cattle and bison blood groups by means of cross-absorptions of hetero-immune (rabbit) antisera. This communication describes a reagent which reacts specifically with bison cells and which shows no cross-reactivity for cattle cells. A brief description of this reagent has appeared elsewhere[7].

Rabbits were immunized with bison bloods obtained from Wichita Mountains Wild Life Refuge, Cache, Oklahoma, through the courtesy of Mr. E. J. Greenwalt. After appropriate absorption with cattle cells, the resulting pooled antisera were tested against the erythrocytes of cattle, bison, cattle-bison F_1 hybrids and back-cross hybrids (B_1) in which the F_1 hybrids were back-crossed to cattle. All tests indicated that this reagent was specific for bison cells. Additional evidence obtained by two other groups of workers using this reagent confirmed these data, indicating that this reagent was bison-specific in its hæmolytic reactions. The number of animals tested by these two groups and by us exceeds 2,000 cattle, all negative (including animals of both dairy and beef breeds); some 400 bison, all positive (from 2 different herds). Table 1 shows the reactions of this reagent with the cells of each of these two species as well as with those of 13 F_1, 46 B_1, a number of second-generation back-cross hybrids (B_2) and a variety of hybrids from *inter se* matings of back-cross hybrids. These bloods were made available to us by Dr. S. L. Scheinberg, who obtained them through the courtesy of Drs. Peters and McArthur, Dominion Agricultural Experimental Station, Manyberries, Alberta. The data were consistent with the supposition that this reagent detected an erythrocytic antigenic specificity, or, more probably, at least two and possibly three specificities present in bison but absent in cattle. As shown in Table 1, all F_1 hybrids reacted. Furthermore, the segregation pattern of the B_1 hybrids suggested that at least three factors were present. Of the 31 B_1 individuals tested with both dilutions (¼ and 1/16), 27 were positive with one or both dilutions and only 4 were negative with both dilutions. This is a highly significant deviation ($P = 0{\cdot}01$) from 1 : 1 segregation and is consistent with the 7 : 1 ratio expected for a three-factor segregation. Unfortunately, at the time these investigations were made, sufficient volumes of the cells of various B_1 hybrids were not available for the necessary cross-absorptions of this reagent to establish whether the antiserum could be fractionated into two or more components.

Table 1. REACTIONS OF BISON-SPECIFIC REAGENT WITH THE ERYTHROCYTES OF CATTLE, BISON AND VARIOUS HYBRIDS

Groups tested	No. per reaction type	Reaction with bison-specific reagent, diluted 1/4	Reaction with bison-specific reagent, diluted 1/16
Cattle	300	0	0
Bison	200	+	+
F_1 hybrids	6	+	+
	7	nt	+
	13		
B_1 hybrids	14	+	+
	6	+	±
	7	+	0
	4	0	0
	3	nt	+
	5	nt	±
	7	nt	0
	46		
B_2 hybrids	4	+	+
	5	+	±
	3	+	0
	7	0	0
	19		
Inter se hybrids	2	+	+
	2	+	±
	5	0	0
	9		

Degree of hæmolytic activity: strongly reactive (+); weak but definitely reactive (±); no detectable reaction (0); not tested (nt).

The results clearly show a serological difference between cattle and bison blood group factors. Scheinberg[8], using bison-cattle hybrids, detected at least one serum protein specificity which also distinguished bison from cattle, although previous results by others were negative[9,10]. The differences in both cells and serum, however, should not affect the decision to classify these two species in the same genus as recently proposed by Stormont, Miller and Suzuki[5]. There are numerous cases reported of blood group systems which distinguish between individual species of the same genus[11].

We thank Drs. W. H. Stone and M. R. Irwin for the extensive test on the specificity of this reagent carried out at the Department of Genetics, University of Wisconsin. We also thank Drs. W. J. Miller and C. Stormont, School of Veterinary Medicine, University of California, Davis, for their analysis of an aliquot of this bison-specific reagent.

This project was supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and in part by a research grant *E* 1643 to M. R. Irwin and W. H. Stone from the Department of Health, Education and Welfare of the National Institutes of Health, Public Health Service.

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Mutagenic Effectiveness of Ethyl Methanesulphonate and Methyl Methanesulphonate in Barley

THE monofunctional alkylating agent ethyl methanesulphonate (EMS) has been shown to be capable of producing mutations in more than one-half of the spikes of treated barley plants[1,2]. Smaller mutation frequencies (7·8 per cent, 16 per cent) have been reported for populations treated with methyl methanesulphonate (MMS). We wished to compare, under similar conditions, the mutagenic efficiency of EMS ($CH_3SO_3C_2H_5$) with that of MMS ($CH_3SO_3CH_3$). Since MMS is rather highly toxic to plants we were forced to use, in this comparative study, low concentrations of the chemicals and short exposure periods.

Dry seeds of barley (*Hordeum vulgare* L.) variety Montcalm were treated with freshly prepared non-buffered aqueous solutions of EMS, *p*H 3·8, and MMS, *p*H 3·7, at 20° C. After the treatment with the chemical the seeds were washed with tap water, surface dried and sown immediately in well-watered greenhouse beds. Chlorophyll mutations appearing in the M_2 (offspring of the treated plants) were classified according to the method of Gustafsson[3].

Table 1. SURVIVAL IN M_1 AND CHLOROPHYLL MUTATIONS IN M_2 AFTER TREATMENTS WITH EMS AND MMS

Treatment	No. of seeds	No. of plants maturing	No. of M_1 spikes	No. of chlorophyll mutations	Mut. per 100 M_1 spikes	Mut. per 1,000 M_2 seedlings
EMS 0·2% by vol., 2 h, 20°C.	300	161	321	12	3·7	7·5
MMS 0·2% by vol., 2 h, 20°C.	300	102	212	69	32·5	57·0

With the similar concentrations and treatment times MMS was more efficient than EMS in inducing mutations (Table 1). The mutation-rate reported here with MMS is about twice as high as the maximum reported previously. Differences in the treatment methods are probably responsible for this difference in effects. A considerably larger dose of EMS than was used here is required to induce mutations at a rate similar to that obtained with MMS in this experiment.

This investigation was supported by a grant from the National Research Council of Canada.

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MISCELLANEOUS

Holey Films for Electron Microscopy

VARIOUS methods have been described[1] for the production of holey films required either for specimen support or astigmatism-correction purposes. Some of these methods are rather involved, and all can apparently produce pseudo-holes which are often difficult to detect and therefore very misleading.

A simple method in which films are formed from an emulsion of glycerol in 0·25 per cent 'Formvar' solution has been found reliably to produce holes ranging from about 0·05 to 25μ diameter. This size-range adequately covers all known electron microscopy requirements from astigmatism correction to the support of thin sections. The films are cast on microscope slides and after being allowed to dry for 10 min are exposed to a jet of steam for 1 min. They are then handled and mounted on grids in the usual

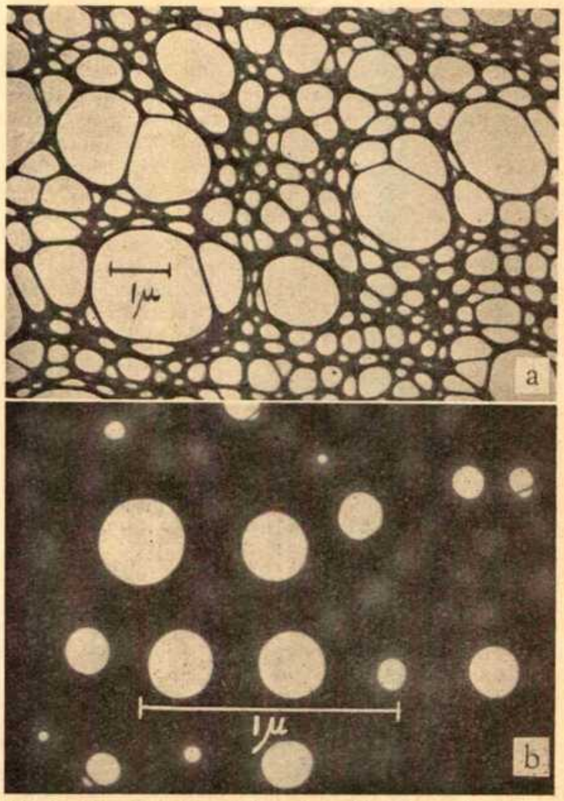

Fig. 1. Holey films formed from glycerol in 'Formvar' solution. *a*, 32 parts 'Formvar', 1 part glycerol; *b*, 120 parts 'Formvar', 1 part glycerol

Table 1

'Formvar' solution	Glycerol	Maximum hole diameter (μ)
8 parts	1 part	25
16 ,,	1 ,,	14
32 ,,	1 ,,	7
120 ,,	1 ,,	4

manner for films cast on glass and finally coated with carbon. Steaming of the cast film before it is dry will result in the formation of a multilayer meshwork which will contain many pseudo-holes.

The glycerol content controls the number of holes and also their size (Table 1), and therefore permits films to be made best suited to the particular requirement. Films made by this method are stable in the electron beam and sufficiently robust to permit such operations as the staining and washing of biological specimens.

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Storage of Standardized Radioactive Solutions

A recent communication, Hamilton[1], directed attention to changes in concentration of standardized solutions stored in polythene bottles caused by transpiration. The conclusion was drawn that 'Pyrex' or quartz containers were necessary for the storage of such solutions to prevent loss of water.

Radioactive solutions were originally stored here in glass vials, but these have been replaced by polythene ampoules of 6 ml. or 40 ml. capacity[2]. Less adsorption of inorganic material from solution occurs in polythene and the ampoules, being unbreakable, are safer to handle. If the neck of the ampoule is drawn into a fine capillary, solutions may be easily drawn into or expelled from the container. The ampoule is perfectly sealed by melting gently the tip of the capillary and closing it by pressing with a pair of tweezers. Permanent labelling of polythene is easily accomplished with a hot wire.

The results of a series of international intercomparisons on radioactive counting techniques indicated that specific activity of solutions was increasing with time. This increase was attributed to transpiration from the polythene ampoules in which the solutions were transported. To test this theory several ampoules containing various quantities of water or dilute nitric acid were sealed and stored open to the atmosphere, but shielded from draughts. Monthly weighings over a period of one year showed that a fairly regular loss in weight occurred in the ampoules (Fig. 1). As one would expect, the loss varied with the wall thickness and surface area of the ampoules, but was independent of the volume of solution. This means that the concentration of the solution increases faster in ampoules containing smaller amounts of solution. Rates of change of up to 8 per cent per annum have been observed.

Rather than lose the advantages of polythene by reverting to glass vials the transpiration was reduced to acceptable levels by storing the ampoules in an enclosed space over water. A desiccator in which the desiccant is replaced by water makes an ideal store. Results already obtained (dashed lines in Fig. 1) show that no appreciable loss of weight occurs. As it might be argued that constancy of weight does not necessarily imply constancy of concentration, a direct experiment is also being carried out on cobalt-60 solutions.

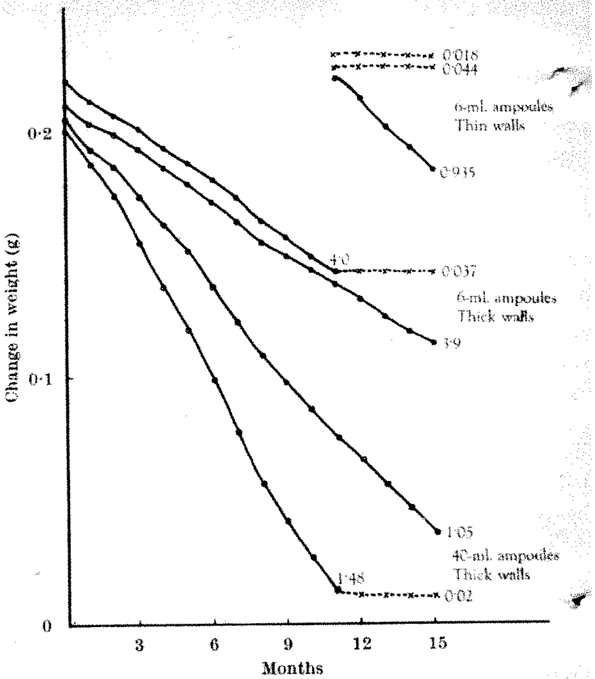

Fig. 1. Change in weight of polythene ampoules during storage. Total percentage change is given at the end of each storage period. ○, stored open to atmosphere; ×, stored over water

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The volume of standard solutions used in isotopic geological age investigations range from 100 to 1,000 ml., the latter being the most common. Keith's conclusions seem admirable for small volumes of solution. In my previous work similar experiments were carried out, but were discontinued for the following reasons:

(*a*) Preliminary experiments using radioactive traces indicated that over long periods of time the polythene walls of the bottles acted as a semi-permeable membrane. As the standards were stored in acid solution the use of an enclosed volume of water-vapour to reduce transpiration seemed inadvisable.

(*b*) The use of water in an enclosed space may require temperature control to prevent condensation of water vapour on the polythene and its adsorption into the outer walls of the polythene bottles, thus forming conditions suitable for osmotic transfer.

If the migration of acid out through the polythene walls is impossible then it is possible to flame spray the polythene with an impervious layer of zinc metal (private communication from Imperial Chemical Industries, Ltd.), or some other material. In this laboratory quartz bottles with ground stoppers are now used and variations of standard concentrates are being observed.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 5

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. D. E. Broadbent. "Theories of Short-Term Memory"

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. C. de Jager (University of Utrecht). "The Emission of Gas by the Sun".*

Institution of Mechanical Engineers, Steam Plant Group (at Nevill Hall, Westgate Road, Newcastle upon Tyne), at 6 p.m.—Meeting on "Some Design Problems arising in the Development of Very Large High Speed Turbines".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—Mr. H. Gibb· "Rituals of Rice" (Colour films with commentary).

Royal Institute of Chemistry, London Section (joint meeting with the Woolwich Polytechnic Chemical Society, at the Woolwich Polytechnic, Thomas Street, London, S.E 18), at 7.30 p.m.—Prof. R. N. Haszeldine· "Double Bonds that are Different".

Tuesday, November 6

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C 1), at 1.15 p.m.—Mr H. W Thorp: "Magnesia from Sea-Water—a New British Industry".*

University of London (at the Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m —Dr. F. A. Vick. "Science and Its Standards".*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Dorothy Hodgkin, F.R.S.· "The X-ray Analysis of Large Molecules". (Seventh of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C 1), at 5.30 p.m.—Prof L. Dudley Stamp: "Some Aspects of Medical Geography. 1· Climate and Disease".* (First of four Heath Clark Lectures.)

Institution of Electrical Engineers and the Royal Aeronautical Society London Joint Group (at Savoy Place, London, W.C.2), at 6 p.m.—Lecture on "Electrical and Electronic Problems in Service Aircraft".

Plastics Institute, London Section (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. R. N. Lewis· "Progress in Epoxide Resins".

Wednesday, November 7

Colour Group (Great Britain) (at the Royal College of Art, Kensington Gore, S.W.7), at 3 p.m.—Visit to the Stained Glass Department; 3.50 p.m.—(in the New Physics Department, Imperial College, S.W.7) —Meeting on "Stained Glass, Its Early Glory, Decline and Revival".

Royal Geographical Society (at 1 Kensington Gore, London, S W.7), at 5 p.m.—Mr. David Oates: "New Discoveries at Nimrud".

Institute of Fuel (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p m.—Dr G. Milner "Some Aspects of Present-Day Town-Gas Supply".*

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. C. Bruinzeel (Amsterdam). "Electric Discharges during Simulated Aircraft Fuelling".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5 30 p.m.—Mr. G. Millington· "V H.F. Diffraction Problems introduced by the Rough Earth".

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5.30 p.m.—Mr E. Knighting: "Numerical Forecasting" (Evening Discourse)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C 1), at 5.30 p.m.—Prof. L. Dudley Stamp: "Some Aspects of Medical Geography. 2: Climate and Health".* (Second of four Heath Clark Lectures.)

British Institution of Radio Engineers, Education Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C 1), at 6 p.m.—Meeting on "The Teaching of Communications in a Modern Context".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Prof. R. G. Bolten. "The Mechanics of Instrumentation".

Society for Analytical Chemistry (at the Wellcome Building, Euston Road, London, N.W.1), at 6 p.m.—Meeting on "Fluoride, Teeth and the Analyst".

Society of Chemical Industry, London Section and Heavy Organic Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. B. G. Ayling and Mr. J. O. Hughes· "Pipelines for Chemicals" (two papers).

Society of Chemical Industry, Plastics and Polymer Group (joint meeting with the Newcastle Section of the S.C.I., and the N.E. Section of the Plastics Institute, at King's College, Newcastle upon Tyne), at 6.30 p.m.—Prof. C. E. H. Bawn, F.R.S.· "New Developments in Vinyl Polymerization".

Thursday, November 8

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 10 a m.—Symposium on "Some Textile Processing Mechanisms".

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. W. A. H Rushton, F.R.S.· "Visual Adaptation" (Ferrier Lecture).

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. Sydney Smith: "W. S. MacLeay, Charles Darwin, and Cirripedes"; Dr. F. B. Hora "Observations and Experiments on Toadstools in a Natural Habitat".

Mineralogical Society (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—General Meeting.

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 5.30 p.m. Mr. W. B. Gosney, Miss M. V. Griffith and Mr. K. C Hales. A Review of the Washington Meetings of the International Institute of Refrigeration.

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5 30 p.m.—Mr I. L. Davies: "Optical Masers".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. A. C. Stevenson "Radiation-Induced Genetic Changes".* (Eighth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Oil and Colour Chemists' Association, London Section (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. E. J. W. Vogelzang (Delft). "Some Industrial Uses of New Non-drying Alkyd Resins".

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, Adelphi, London, W C.2), at 7 p.m.—Mr. W. J. Bray: "Satellite Communication Systems".

Royal Institute of Chemistry, London Section (at Croydon Technical College, Fairfield, Croydon, Surrey), at 7.30 p.m.—Dr. E C. Barton-Wright· "Judge, Jury and Expert Chemical Witness".

Friday, November 9

North East Coast Institution of Engineers and Shipbuilders (at Bolbec Hall, Newcastle upon Tyne)—Mr. N. Carter: "Calculations for the Sub-division of Passenger Ships".

Clay Minerals Group of the Mineralogical Society (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 11.25 a.m.—Autumn Meeting. 4.30 p.m —Annual General Meeting.

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Prof. J S Mitchell· "Some Problems in the Study of Radiosensitizers and Radioactive Drugs".*

Bone and Tooth Society (at the Institute of Orthopaedics, 234 Great Portland Street, London, W.1), at 4.30 p.m.– Symposium on "Citrate Metabolism"

Arthur Stanley Eddington Memorial Trust (at the Arts' School Lecture Theatre, Bene't Street, Cambridge), at 5 p.m —Prof. W. C. Kneale· "On Having a Mind".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. Audrey I. Richards: "The Pragmatic Value of Magic in Primitive Societies".

Saturday, November 10

Biochemical Society (at the Glaxo Laboratories, Greenford, Middlesex), at 10.55 a.m.—Scientific Papers.

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Dr. John F. Potter "Journey to the Centre of the Earth".*

Monday, November 12

University of London (at Friends' House, Euston Road, London, N.W.1), at 5.30 p.m —Sir Vivian Fuchs. "The Human Element in Exploration" (Rickman Godlee Lecture).*

Plastics Institute (at the Royal Institution, Albemarle Street, London, W.1), at 6.30 p.m.—Dr. J. C. Swallow. Second Swinburne Award Address.

Society for Visiting Scientists (at the English-Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Matching Men and Machines". Chairman· Dr. W. F. Floyd; Speakers: Dr D. J. K. Stuart, Dr. H. P. Ruffell and Mr. D. E. Broadbent.

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p m —Dr. I. G. Gass· "Return to Tristan da Cunha".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or *before the dates mentioned*:

Lecturer or Assistant Lecturer (with qualifications in pure mathematics) in the Department of Mathematics—The Registrar, The University, Manchester 13 (November 10).

Assistant Lecturer (with an honours degree in archaeology, or its equivalent, and a good general knowledge of European and British prehistory, with some emphasis on the periods before the Neolithic) in the Department of Archæology—The Secretary, The University, Edinburgh (November 12).

Lecturer in the Chemistry of High Polymers in the Department of Textile Industries—The Registrar, The University, Leeds 2 (November 14).

Lecturer (Grade II) in Philosophy—The Registrar, The University, Bristol 8 (November 15)

Lecturer or Senior Lecturer in Livestock Husbandry at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 15)

Lecturer (*with special qualifications in experimental psychology*) in Psychology—The Secretary, The Queen's University, Belfast, Northern Ireland (November 15).

Lecturer or Assistant Lecturer in Philosophy—The Registrar, The University, Nottingham (November 16).

Assistant Lecturer in Dairying; and a Demonstrator in Dairying—The Registrar, University College of Wales, Aberystwyth (November 17).

Assistant Lecturer in Physiology—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (November 17).

Chair of Physics—The Registrar, University of York, The King's Manor, York (November 17).

Lecturer in Organic Chemistry—The Secretary of University Court, The University, Glasgow (November 17).

Temporary Lecturer (with qualifications and experience in the *general field of physical or inorganic chemistry*) in Chemistry, for one calendar year from January 1, 1963—The Registrar, University College, Singleton Park, Swansea (November 17).

Professor of Chemistry in the Faculty of Science, University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 18).

Lecturer (preferably interested in inorganic or physical chemistry) in Chemistry—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (November 20).

Lecturer and an Assistant Lecturer in the general field of *Applied Mathematics and Statistics—The Registrar*, The Manchester College of Science and Technology, Sackville Street, Manchester 1 (November 24).

Lecturer and/or Assistant Lecturer in the Department of Pure Mathematics—The Registrar, University College of North Wales, Bangor, North Wales (November 24).

Lecturer or Assistant Lecturer in the Department of Applied Mathematics—The Registrar, University of North Wales, *Bangor, North* Wales (November 24).

Librarian (graduate)—The Vice-Chancellor, University of Essex, c/o Divisional Education Office, Park Road, Colchester, Essex (November 24)

Tutorial Research Student in Mathematics (Pure or Applied)—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (November 26).

Chair of Forestry—The Secretary, The University, Aberdeen (*November* 30)

Lecturer (preferably with a special interest in economic geography) in Geography at the University of Melbourne—The Secretary, Association of Universities of the *British Commonwealth (Branch* Office), Marlborough House, Pall Mall, London, S W.1 (Australia and London, November 30).

Lecturer in Sociology at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, November 30).

Lecturer or Assistant Lecturer (with a good honours degree *in geology* and research and teaching experience in geochemistry and petrology) in the Department of Geology—The Registrar, The University, Keele, Staffs (November 30).

Assistant Lecturer in Biochemistry in the *Department of* Physiological Chemistry—The Registrar (Room 22, O.R.B), The University, Reading (December 1).

Lecturer (preferably with research experience in drug metabolism and the application of radio-isotopes in biological chemistry) in Pharmaceutical Chemistry at the University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (Australia and London, December 7)

Senior Demonstrator in Botany (Plant Physiology) at the University of Melbourne, Australia—The Secretary, Association of *Universities of the British Commonwealth (Branch* Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 30).

Head (with considerable experience in any suitable field of research work, preferably in applied plant physiology or in plant pathology) of the Department of Hop Research—The Secretary, Wye College (University of London), Near Ashford, Kent (December 31).

Lecturer in Botany (Genetics or Cytogenetics) at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

REPORTS and other PUBLICATION

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Forestry Commission. Booklet No. 7: *The Plan of Operations:* Guide to the Preparation of the Plan of Operations for Dedicate and Approved Woodlands. Pp. 43. 3*s*. net. Forest Record No. 3 Tariff Tables. *By Dr. F. C. Hummel, G. M. L. Locke and J. P. Vere* Revised edition. Pp. 32. 2*s*. 6*d*. net. Leaflet No. 3: Pine Shoot Beetle By D. Bevan. Revised edition. Pp. 8. 8*d*. net. (London: H. Stationery Office, 1962.) [12

National Union of Teachers. Current Grammar School Problem By Mervyn Pritchard. (An address to the meeting of Grammar Scho Teachers at the Annual Conference, Scarborough, 1962). Pp. 1 The Winds of Change. By Sir Ronald Gould. (Address of the Gener Secretary of the National Union of Teachers to the Annual Conferenc Scarborough, 1962.) Pp. 18. (London: National Union of Teacher 1962.) [12

Government of Northern Ireland: Ministry of Agriculture. Leafl No. 140· Fowl Typhoid Pp. 2. (Belfast: Ministry of Agricultur 1962.) [12

Time Units in the Solar System—Geometrisation. By S. Ryzewsk Pp. 32. (Southampton S. Ryzewski, 15 Landguard Road, 1962.) [12

Building Research Station Digest, (Second Series). No. 26· Ligh weight *Aggregate* Concretes—2. Uses. Pp 4. (*London: H.M. Statio* ery Office, 1962.) 4*d*. [12

Biorheology, Vol. 1, No 1, (July, 1962). Edited by Prof. A. Copley and Dr. G. W. Scott Blair. Pp. 1–82. Annual Subscriptio rates. (A) For libraries, government establishments, research labor tories, etc. £10 (30 dollars); (B) For individuals who place their orde directly with the publisher and certify that the journal is for the personal use £5 (15 dollars). (Oxford and New York· Pergamo Press, 1962.) [12

Bulletin of Mechanical Engineering Education, Vol 1, No. 1, (Ne Series), January—June, 1962. Edited by Dr. J. H. Lamble, Dr. Parker, and J. L. Duncan, in collaboration with Prof. W. Johnso Pp. 1–88. Annual subscription rates: (A) For all institutional, industri and government establishments, and educational establishments outsid the British Commonwealth £3 10s. (10 dollars), (B) For individua and educational establishments within the British Commonwealt £1 (3 dollars). (Oxford and New York. Pergamon Press, 1962) [12

The University of Sussex. 1962–1963 Prospectus. Pp. 60 + 3 plate (Stanmer, Brighton: University of Sussex, 1962.) [12

Ciba (A.R.L.), Ltd. Technical Notes, No. 236, (August, 1962 Encapsulated Motors. Pp. 8. (*Duxford*: Ciba (A.R.L.), Ltd , 1962.) [12

British Medical Bulletin, Vol. 18, No. 3, (September, 1962): Syn posium on Electron Microscopy. Pp. 179–254 + 32 plates. (Londo British Council, 1962.) 35*s*. [12

Other Countries

United States Department of the Interior: Geological Surve Water-supply Paper 1475-K: Ground-Water Reconnaissance at Pi nacles National Monument, California. By R. E. Evenson. Pp. iii 375–382 + plate 22. Water-Supply Paper 1536-E: Theory of Aquif Tests. By J. G. Ferris, D. B. Knowles, R. H. Brown and R. W Stallman. Pp. vii + 174. Water-supply Paper 1598 : Geology an Ground-Water Resources of the Ahtanum Valley, Yakima Count Washington. By Bruce L. Foxworthy. Pp. v + 100 + 3 plates. (Was ington, D.C.: Government Printing Office, 1962.) [12

Bulletin of the American Museum of Natural History. Vol. 12 Article 3: A Study of Normal and Malignant Thymus Tissue of t Teleost *Astyanax mexicanus* in Tissue Culture. By Libby Rosenbloo Friedman. Pp. 69–100 + plates 39–51. (New York: American Museu *of Natural* History, 1962.) 1.50 dollars. [1

Unesco. Termites in the Humid Tropics· Proceedings of the Ne Delhi Symposium, jointly organized by Unesco and the Zoologic Survey of India, 4–12 *October* 1960. Pp. 259 + 42 plates. (Par United Nations Educational, Scientific and Cultural Organizatio London: H.M. Stationery Office, 1962.) 24·50 NF.; 35*s*.; 7 dollars. [1

Fiskeridirektoratets Skrifter: *Serie Havundersøkelser. (Report* Norwegian Fishery and Marine Investigations.) Vol. 13, No. Measurements of the Reflection of Sound by Fish. By L. Midttun a I. Hiff. Pp. 18. (*Bergen*: A. s. John Greigs Boktrykkeri, 1962.) [1

Conseil Permanent International pour l'Exploration de la Me Charlottenlund Slot, Danemark. Bulletin Statistique des Pêch Maritimes, Vol. 45 pour l'Année 1960. Redige par Arni Fridrikss Pp. xi + 61. (Copenhagen. Andr. Fred. Høst et Fils, 1962.) K 15.00. [1

Companhia de Diamantes de Angola (DIAMANG). Servicos Cu turais. Dundo—Lunda—Angola. Museu do Dundo. Publicaco Culturais No. 58: Subsidios para 1 Estudo da Biologia na Lunda-Estudios Diversos, (XXIII). Pp. 154. (Lisboa: Companhia de Di mantes de Angola, 1962.) [1

United States Department of Commerce: Weather Bureau. Technic Paper No. 44: A Catalog of 100 FCO-Positioned Transosonde Flight By J. K. Angell. Pp. ii + 201. (Washington, D.C.: Governme Printing Office, 1962.) 2 dollars. [1

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PROBLEMS OF COMMUNICATING SCIENTIFIC IDEAS

ONE of the most fundamental discussions at the meeting of the British Association for the Advancement of Science in Manchester was that arranged by Section X on September 4 on problems of communication. It could be said that the subject was a dominant one in the proceedings of the Section. Prof. Bruce Pattison's presidential address on "English as an International Language" discussed a particular means of communication, and afterwards the special linguistic problems of foreign students in the United Kingdom were described to the Section by Mr. G. Perren of the British Council's staff. The general theme of Section L, "The Unity of Knowledge", also touches closely on problems of communication, and apart from Mr. P. Wilson's excellent presidential address on the unity of knowledge as a new dynamic for the schools, that Section heard a meritorious address from Dr. S. Toulmin on science and the British intellectual tradition, while Prof. D. Daiches was concerned with university education and the unity of knowledge, and Dr. C. S. R. Kitson-Clark on the use of history.

All these addresses bear on some aspect or other of the communication of knowledge or of ideas, and the whole meeting was remarkable for a painstaking effort to assist joint discussion between Sections and the cross-fertilization of ideas. The discussion on problems of communication went fairly to the root of the problem, particularly in the paper by Mr. B. C. Brookes, which dealt with the communication between scientists, and in that of Mr. J. Maddox, which discussed communication between scientists and the educated layman. Those by Prof. P. Sargant Florence, which discussed basic English for the social sciences, and by Dr. Elsie C. Graham, which considered basic English as an international language and its value for science, dealt with a more limited or specific aspect, and even though Dr. Graham recognized the range and wealth of meaning in ordinary English words, her paper scarcely met Prof. Bruce Pattison's fundamental objection to the artificial languages that they have no roots.

To the extent to which basic English denies itself that wealth of association and range of meaning, and to which Prof. Pattison referred, it is open to that general objection to an artificial language and cannot claim the advantages which English already holds as a language of science and learning—not simply as a channel for European and American experience, particularly in science and technology, but also for communication inside many of the new developing countries. Prof. Pattison's survey, in fact, points rather to the high importance of teaching English in these countries, to the value of spoken English and the provision of better trained teachers, especially with better command of spoken English. Rather than the exploitation of a partly artificial language, he urges the use and deliberate use of the opportunities which English already affords, and that we should be concerned to keep clear those old channels of communication and to open new ones from them.

Mr. Brookes's stimulating paper follows naturally from this point of view. He began by stressing the fact that the scientific method is essentially social and utterly dependent on the co-operation of scientists. The history of science is still written so largely in terms of the contributions of individuals that he suggested what was needed was a Trevelyan to restore the balance by displaying the importance of its internal social aspects. Mr. Brookes himself brings out emphatically some of those neglected human aspects of the whole scientific system, its dependence on the whole social corpus of scientists who operate the system and through them on the scientific societies and academies. Only with their founding could a scientific community of thought and attitude and a coherent approach to scientific problems become possible.

The importance of scientific societies is not always appreciated, though Sir Cyril Hinshelwood has directed attention to their functions on several occasions, and their place in the technological society of to-day merits close and critical assessment, particularly in the light of the limits of communicability, to which also Mr. Brookes wisely directs attention. It is not entirely on the ability of the scientist or technologist to express himself clearly that effective communication depends. The limit of communicability is also set by the inability of those addressed to discard or change the mode and structure of thought which they have established for themselves. Here the scientific or technical society may be an adverse or a favourable factor, and it has to be remembered that it is an essential part of the critical method that defence of old theories should be as tough as the criticism which overthrows them. The issue is decided by the uncommitted young generation of scientists and because of this the structure of the scientific society or institution merits some consideration in respect of age groups.

While Mr. Brookes accepts that it is largely true that a new scientific truth triumphs through the senescence of its opponents and the growing up of a new generation familiar with that truth, he looks critically and constructively at factors which impede freedom of scientific discussion and criticism, and his review is far more realistic than many contributions to this field. He recognizes, for example, that the overwhelming spate of scientific papers that are now published has both made inevitable the appearance of specialists in the handling of information who act as intermediates between the scientist and his literature, and fostered a disinclination on the part of the scientist to depend on published sources. More important, he examines the implications of both developments and his comments are penetrating and constructive.

For example, he points out that the 'information' scientist or specialist may easily become an obstacle to the primary transaction in communication. Moreover, encouraging the writing or presentation of scientific papers in a form more suitable for mechanical abstracting and indexing is bad in principle if it reduces the scientist's direct responsibility to keep himself informed. Here, as elsewhere, spoon-feeding is bad and could be disastrous. Again, he lays a welcome stress on the importance of personal contacts, and it is not merely that modern conditions have facilitated personal contacts overseas and encourage the scientist to resort to informed channels even if they mean by-passing formal channels of communication. Personal contacts, to-day as always, represent a factor of which more account should be taken before deciding whether or not the establishment of costly and elaborate mechanical systems is justified. They could sometimes represent a more decisive factor than the application of the law of scatter in relation to indexing or abstracting of scientific papers would suggest or allow.

That is a matter which concerns the scientist and the information officer or librarian alike, but the second factor on which Mr. Brookes makes some pertinent comments is of the scientists' own making. This is the practice of early or even premature publication, and, in view of what has previously been said in these columns, it seems unnecessary to add more than that Mr. Brookes expresses the hope that scientists will eventually recognize the soundness of Gauss's publishing motto: *Pauca sed matura*. Further, he firmly deprecates alike the pressure to publish which develops from the dependence of promotion at universities and like institutions on published papers and from the tendency to duplication of publishing. More serious, however, is the way the pressure set up by the demand on publishing space, especially in the leading periodicals, discourages or hinders the publication of unorthodox views. Mr. Brookes remarks succinctly that the progress of science demands that heretics be heard. Much more could be said, but the scientific societies should be well aware of a problem that is by no means easy to resolve and which bears vitally on the existence of science itself.

Of secrecy, whether imposed by real or imaginary demands of security or of industry, Mr. Brookes points out that it is always expensive and detrimental to science, and suggests that the shedding of scientific secrecy by industry might be a most rewarding policy in its effect on the quality of its scientific staff. He is, however, just as much disturbed at the indirect effects of the commercial exploitation of the development of scientific information, and the misgivings he expresses about the consequences of some of the centralization and mechanization in this field deserve attention. The risk of vested interests developing which are concerned with the means and not with the scientist or the science to be served is not to be ignored.

These problems undoubtedly merit more careful examination from the point of view of the scientist, not the information officer or the librarian, than they have received, and Mr. Brookes's suggestions for assisting the scientist, and especially the young scientist, to play a part in the system of communication on which science depends could contribute materially to such an examination. At present, as he rightly observes, the young scientist does not appear to be trained to that end at all, and while Mr. Brookes neither accepts at its face value much of the present-day criticism of the quality of scientific writing or lecturing, nor advocates seriously distracting the young scientist from laboratory work or theoretical studies, he believes that the young scientist could be greatly helped by the removal of uncertainty of purpose and provision of an appropriate model or standard for his written work. The scientific author is not normally a professional writer and needs assistance in planning the framework and in getting started; but if he is to be helped he must be treated as Rutherford treated him—as a responsible person, with something worthwhile to communicate and something that is essentially communicable.

Those responsibilities as a communicator are part of the scientist's task as a creative or critical worker, and to help the young scientist to become a better communicator is to help him become a more effective creative scientist. This, at least for the young scientist, is the right approach to the problem of communication: it is an element in his intellectual development. There are other aspects of communication even between scientists, but this may well be the most important, and if it is wisely handled could do much to relieve difficulties elsewhere.

They could also stimulate attention by scientists to the communication of science to the public and assist to overcome that indifference or neglect which Mr. J. Maddox noted in his following address. This indifference was quite inconsistent with the fact that science was an integral part of the intellectual life of civilization, and the communication of scientific ideas should be fostered for the same reason as was the cultivation or appreciation of great paintings, music or literature. It enlarged men's minds, and without proper appreciation of the intellectual significance of science in society even the administration of public affairs suffered. This was equally true of industrial activity, and scientists could not escape their responsibility for reducing the inevitable time-lag in the adoption of new ideas.

All this is, of course, implied in the debate instigated by Sir Charles Snow's thesis of the old idea of the two cultures, but on the positive side of improving communications Mr. Maddox had little new to contribute. He saw ample room for improvement in the part played by the schools and by the Press, but the weight of his criticism fell on the scientists. The British Association was an example of what could be done, but there were definite limits to its activities, and Mr. Maddox suggested that one of the outstanding needs was some organization which would permit the free expression of scientific opinion.

That suggestion calls for some examination, for although scientists might well do more to assist the formation of informed opinion it is open for discussion how far 'scientific opinion' can exist except in the sense of considered opinion on specific scientific or professional opinion on specific issues. Such opinion could largely be formulated by existing professional institutions if they recognized their responsibility and used their opportunities, particularly in association with, for example, the Parliamentary and Scientific Committee. Meanwhile, Mr. Brookes, in any event, has indicated some opportunities for constructive work by the professional institutions, and the British Association could well make sure that this general problem of communication is adequately discussed at future meetings as well as kept under continuous review as an aspect of the Association's function in serving the community.

HUMAN ENDOCRINOLOGY

Hormones in Human Plasma

Nature and Transport. Edited by Dr. Harry N. Antoniades. Pp. xvii + 667. (Boston, Mass.: Little, Brown and Company, Inc.; London: J. and A. Churchill, Ltd., 1960.) 25 dollars; 180*s*.

THIS is a most useful book. There has long been emphasis on hormonal excretion products, too long in the view of many, simply because they are more accessible. It is good to see the increasing importance attached in the past few years to hormones in the circulating blood. Previously there had been available the book of the Ciba Foundation Symposium on *Hormones in Blood*, but this dealt in a less-comprehensive way with mammals generally. In the book edited by Dr. Antoniades, we have a good account by thirty-three authors of the whole subject of hormones in human plasma. This book is fully comprehensive in its treatment of hormones and the history of their discovery, and even the zoological limitation to man is only nominal. It is the first of its kind, and as Dr. Thorn says in his preface, it may well be the last, because the growth of information in this field will make it impracticable in future to compress the subject in full detail into a single volume.

The book is divided into five parts: methods of blood collection and plasma fractionation; hormones of pancreatic origin; hormones of pituitary origin; steroid hormones; and hormones produced by thyroid gland, the adrenal medulla and the placenta. This subdivision is not quite logical in that there is a potential overlap between steroid and placental hormones; discussion under the latter heading, however, is restricted to gonadotrophins. An attractive feature of the book is the integration of new knowledge with the pioneer observations. In particular, it is pleasant to see recognition given to the work of R. T. Frank, who as early as the 1930's recognized that the place to look for functionally effective œstrogens was in the circulating blood, not in the urine, but whose work, owing to technical imperfection, was largely ignored at the time. Many books published at the present time seek only to scoop off a thin layer of the cream of current knowledge without acknowledgment that it has evolved from the work of many years. The book edited by Dr. Antoniades is a welcomed exception.

In a book containing so much good material it is perhaps invidious to pick out particular parts for mention, but I myself was naturally attracted by the chapters dealing with steroid sex hormones in human plasma. That on androgens in the testis, the ovary, the adrenal cortex or as metabolic products of the corticosteroids puts the subject in proper perspective in a satisfactory way. Much the same applies to W. H. Pearlman's chapter on progesterone, which is a very complete review of the whole subject of progestagenic substances in blood and deals not only with the sources of the substances but also with their occurrence in lower mammals and even in birds, frogs and reptiles. A further chapter on steroids deals with their binding by plasma proteins. Two other chapters deal respectively with gonadotrophins. That on hypophysial gonadotrophins reflects by its brevity the relative lack of information in this field. That on extra-hypophysial substances, although it deals with pregnant mare's serum gonadotrophin, in addition to human chorionic gonadotrophin, is also somewhat sketchy. As to the rest of the book, it need scarcely be said that the chapters on insulin and related substances, on adrenocorticotrophic hormone and on thyroid hormone are very substantial. This is a reference book which should be accessible to every endocrinologist.

A. S. PARKES

EVOLUTION OF MAN

Mankind Evolving

The Evolution of the Human Species. By Prof. Theodosius Dobzhansky. Pp. xiii + 381. (New Haven, Conn. and London: Yale University Press, 1962.) 7.50 dollars; 55*s*.

NO branch of science is more difficult to write about without bias than is the application of genetics to human history and evolution. The difficulties arise, not so much because of the possible conflict between science and theology, but because most statements about human genetics have far-reaching political implications. On one hand, reformers who have wished to believe that the application of some specified social policy would cure all human ills have also found it necessary either to deny that any of the existing differences between human beings are genetic, or to assert the Lamarckian view that appropriate environmental changes will, without the need for the cruelties of natural selection, produce desirable genetic changes. On the other hand, those, from Plato onwards, who have felt that the Golden Age is in the past, and that the preservation of the *status quo* is the best that can be hoped for, have usually found it necessary to justify the existing differences between the 'haves' and the 'have-nots'—whether they be individuals, classes or races—by arguing that all important differences between human beings are hereditary.

Faced with these twin dangers, a few geneticists have incautiously attempted to explain the whole of human history, politics and sociology in genetic terms. A commoner, if more cowardly, attitude has been to admit the relevance of genetics in such cases as blood groups or certain diseases, but tacitly to ignore its possible relevance to differences of personality or mental capacity, or to differences between classes or races. Relatively few have been able to steer a successful course between the Scylla of hereditarianism and the Charybdis of environmentalism. We should therefore be particularly grateful to Prof. Dobzhansky for writing *Mankind Evolving*. He has the breadth of knowledge and the wisdom to give a balanced picture of the importance of genetics in human affairs, and he writes so beautifully that his book will be read with pleasure both by professional geneticists and by those with no biological training.

The first half of the book deals with genetics and the theory of evolution, with the environmentalist thesis and hereditarian antithesis, and with the problems of nature and nurture and of natural selection in man. Later chapters deal with the fossil history of man, the origin of human mental faculties, with class, caste and race, and with eugenics. These are important subjects, and some of them have been discussed with passion for three thousand years. During the past hundred years they have gradually ceased to be matters on which a man can permit his views to be dictated by his religious and political prejudices, and are becoming matters on which opinion must be influenced by ascertained facts and accepted theories. This is a desirable change, and Prof. Dobzhansky's book will do much to accelerate it.

J. MAYNARD SMITH

MEANING OF MEASUREMENT IN THE SCIENCES

Quantification

A History of the Meaning of Measurement in the Natural and Social Sciences. Edited by Harry Woolf. Pp. 224. (Indianapolis and New York: The Bobbs-Merrill Company, Inc., 1961.) N.p.

THESE nine essays are accounts of papers read at a conference held under the auspices of the U.S. Social Science Research Council. Physics, chemistry, psychology, sociology, medicine and economics are among the fields covered, so it is no surprise that there are differences in approach—one almost has the impression of reading essays in different languages. In spite of this, there is a unanimous determination to put measurement in its place. Measurement for its own sake is useless without a theory to give it direction, and all the writers insist that qualitative work must precede quantification in any science. T. S. Kuhn, rejecting the over-simplified text-book picture of a physicist measuring and then finding a theory to fit his measurements, goes so far as to write (p. 43): "Knowing what results they should expect from chemical analyses, chemists were able to devise techniques that got them".

The fields of psychology, economics and sociology differ from the others in the need to use mathematical statistics and probability as an instrument of measurement. This has made the quantification of these subjects more difficult, leading to controversies of various kinds. The book contains plenty of examples of prolonged conflict between schools of thought, both in the social sciences, as "political arithmetic" versus "university statistics", and in the natural sciences, as the wave and corpuscular theory of light. It is, of course, impossible to give a history of measurement in any field without giving a good deal of background history of the field itself.

Among individual essays, I was most interested by T. S. Kuhn with his provocative view of the rolé of measurement and "crisis states" in the development of physics, and P. F. Lazarfeld, with his rich human story of the personalities who developed measurement in sociology. The repetition of key names, such as Descartes, Leibniz, Lavoisier, Laplace, shows how thinkers of the past applied their talents to many fields. Nowadays, intense specialization sets up barriers between different disciplines, and a book like this, written by experts in different lines, may help to break these barriers down

A. M. MACBEATH

SPACE TECHNOLOGY

Proceedings of the 6th Symposium on Ballistic Missile and Aerospace Technology

Held in Los Angeles, August, 1961. Edited by C. T. Morrow, L. D. Ely and M. R. Smith. Vol. 1: Design and Reliability, and Invited Addresses. Pp. x+403. 64*s*. Vol. 2: Ballistic Missile and Space Electronics. Pp. x+453. 72*s*. Vol. 3: Propulsion, Space Science and Space Exploration. Pp. x+445. 72*s*. Vol. 4: Re-entry. Pp. x+240. 40*s*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.)

THESE four volumes contain about half the papers presented—namely, those which escaped military classification—at the symposium on "Ballistic Missile and Aerospace Technology" held at Los Angeles in August 1961. The first volume includes 14 papers on missile design, structure and reliability. The second volume has 18 contributions under four headings: ballistic missile and space guidance, navigation and control; computers and digital techniques; information processing; communications. Volume 3, also with 18 papers, is devoted to rocket propulsion, space science and the exploration of the Moon and planets. The nine papers in Volume 4 deal with the design of space vehicles for re-entry into the Earth's atmosphere. The contributors are all from the United States, most of them belonging to industrial organizations. The papers are generally of a high standard and impressively thorough, being especially valuable for their wealth of detail and illustration. The volumes, though apparently printed photographically from typescripts not always uniform in layout, are nevertheless not unpleasing in appearance. Non-American readers would, however, be well advised to skip the "Addresses" occupying the first 14 pages of Volume 1, which are embarrassingly nationalistic.

In a short review only a few of the individual contributions can be mentioned, to illustrate their scope. There are two notable papers on the shielding of manned space-vehicles from high-energy particles in space: R. H. Levy describes shielding by magnetic means, with superconducting coils, and concludes that the weights required may be comparable with conventional methods; while A. J. Beck, E. Divita and S. L. Russak give a long and detailed account of the shielding of spacecraft of particular designs during a lunar journey. Volume 3 contains an interesting paper by V. Ilsen on rocket exit-nozzles of unconventional shape, designed for increased efficiency. R. Hermann reports on investigations into the problems of evaporative film-cooling at hypersonic speeds. R. H. Battin and J. S. Miller discuss the guidance requirements for a novel low-thrust approach trajectory to the Moon, spiralling out from the Earth and in towards the Moon, while J. W. Salisbury reviews the geological problems in establishing a base after arriving at the Moon. Together the volumes constitute a most useful and wide-ranging survey of space technology.

D. G. King-Hele

THE RUTHERFORD JUBILEE AND NUCLEAR PHYSICS

Proceedings of the Rutherford Conference

Edited by J. B. Birks. Pp. 856. (London: Heywood and Co., Ltd., 1961.) 189*s*.

The scientific proceedings of the Rutherford Jubilee International Conference on Nuclear Physics which was held in the University of Manchester during September 4–8, 1961, to commemorate the discoveries of Rutherford at Manchester, and in particular, the fiftieth anniversary of the Rutherford scattering law and the discovery of the atomic nucleus, are recorded in the present volume. The addresses delivered at the special commemorative session by four of Rutherford's former colleagues are to be published separately in a special volume which will contain also other biographical, historical and bibliographical articles and a selection of the important papers published from Manchester during 1907–19.

The contents consist of the brief welcoming address by the vice-chancellor of the University of Manchester (Prof. W. Mansfield Cooper) and the reply by the president of the Conference (Sir Ernest Marsden); contributed papers; invited papers; discussion; open session; and conference summary by D. R. Inglis. All the manuscripts, whether issued as preprints in time for the conference or obtained by tape-recording during the conference, have been corrected by their respective authors. Abstracts of the 180 contributed papers are included, together with a list of delegates and an index of authors.

There were seven sessions in addition to the commemorative session (Session 4) and the open session. In the first session, on high-energy investigations of nuclei, there were invited papers by H. Pierre Noyes on nuclear forces; H. McManus on the interaction of fast nucleons with nuclei and R. H. Dalitz on hypernuclei and hyperon-nucleon interaction. In the second session, on collective motion in nuclei, B. H. Flowers spoke on pairing correlations in nuclei; B. L. Cohen on experimental evidence for collective oscillations and G. E. Brown on the theory of collective oscillations. The third session, on the nuclear ground state, included papers by D. H. Wilkinson and P. E. Hodgson on the nuclear surface and J. S. Bell on the many-body problem. Session 5 dealt with direct interactions with three invited papers; session 6 consisted of two invited papers, by S. Devons on limitations and needs in instrumentation for nuclear physics and by W. A. Fowler on "Rutherford and Nuclear Cosmochronology" respectively; and session 7 was concerned with weak interactions, with invited papers by R. J. Blin-Stoyle; S. A. Moszkowski; C. Rubbia on muon capture in nuclei; and V. L. Telegdi on the spin dependence of the interaction responsible for muon capture. In the open session (Session 8) there were four invited speakers, including R. E. Peierls, who took as his subject "Velocity Dependent Nuclear Forces", and Bohr, who discussed selected topics concerning nuclear spectra.

The volume is excellently produced and will remain both of scientific and historical interest for a very long time. The discussions are valuable, but because they are given verbatim they sometimes reflect the heat of the moment and quite often descend to ridiculous comments like "Oh, no ! no !". It is a pity that some editorial deletions or alterations were not made before publication. The price is high, but not too high for the material the volume contains.

S. Weintroub

SALT RELATIONS OF PLANTS

Mineral Salts Absorption in Plants

By Dr. J. F. Sutcliffe. (International Series of Monographs on Pure and Applied Biology. Division: Plant Physiology, Vol. 1.) Pp. x+194. (London and New York: Pergamon Press, 1962.) 35*s*. net.

This is a straightforward account of the principles involved in the absorption of mineral constituents of plants. After a brief review of the methods and plant materials now generally used in investigations on salt absorption, the problems presented by the subject are dealt with in seven chapters. Under the heading "Mechanisms of Ion Transport" the various processes which might be concerned in the movement of ions into and in the plant are briefly considered; they are diffusion, mass flow, ion exchange, Donnan equilibria, adsorption, chemical combination and active transport. How various external and internal factors can affect salt absorption forms the subject of the next chapter, while the relation of salt absorption to metabolism rightly claims a chapter to itself.

Much of a chapter on the location of absorption mechanisms is naturally devoted to a discussion of the concept of free space, but brief consideration is also given to the salt relations of cell walls, cytoplasm, mitochondria and chloroplasts. The plasmatic membranes are indeed described but these important components of the cell might well have received a more detailed treatment. In a chapter entitled "Salt Relations of Vascular Plants" there are considered the zones of ion absorption in roots, salt absorption and translocation, transport across the root cortex, upward transport of salts, distribution of salts in the shoot, foliar absorption and excretion of salts. These altogether constitute a vast subject on which much work has been done and much written in the past thirty or forty years, and in a book of the size of the one under notice the brief outline which is given is all that can be expected. Much the same can be said of the subject-matter of the next chapter dealing with the soil as a source of mineral salts. The final chapter, apart from an epilogue, deals with salt tolerance, particularly of halophytes. The term diffusion pressure deficit, that is, suction pressure, is here introduced without definition. While many readers will no doubt be acquainted with the term this should probably not be assumed.

The book is clearly and pleasantly written and should be found useful to students who want a general account of the aspects of plant nutrition with which the book sets out to deal.

W. Stiles

NEW ZEALAND OBSERVATIONS OF THE HIGH-ALTITUDE EXPLOSION OF JULY 9 AT JOHNSTON ISLAND

GEOMAGNETIC and atmospheric effects of the explosion of July 9 observed in New Zealand and the Pacific were discussed at an informal conference held at the University of Canterbury, Christchurch, on August 16. This article presents a summary of the main observations, which are expected to be published by individual authors in a special issue of *The New Zealand Journal of Geology and Geophysics*. The titles of organizations supplying information are quoted in brackets throughout this summary. Outstanding features of the explosion were the intensity of the effects, which caused 'off scale' readings on many instruments, and evidence for the precipitation of particles over an area extending about 25° farther south than the magnetic conjugate point.

The time of detonation (hereafter referred to as *H*) was determined from the reception at Lower Hutt of a strong click type sferic, occurring at 0900 h 09·0238 sec ± 0·0005 sec U.T.; and the height of the fireball was deduced, from a newspaper photograph, as between 355 and 395 km (Geophysics Division, Department of Scientific and Industrial Research).

Auroral displays were observed from locations between Tarawa (1° N.) and Campbell Island (53° S.) (New Zealand Meteorological Service). At *H*, a white or greenish-white flash was observed in two areas, one between Fiji and Samoa, and the other, just east of the South Island of New Zealand, approximately between magnetic latitudes 45° and 50° S. At Apia, the flash was seen in the direction of the conjugate point, which lies to the south-south-west. A bright glowing area suddenly appeared, extending high in the north-west sky, with distinct ray structure through it. Colours were mainly light yellow, tinged with violet, and with a suggestion of green at the edges. At Tarawa, the observing point closest to the explosion, the flash was of greenish tinge. Rays were seen to emerge from the upper portions of the flash, and to take zig-zag paths which eventually became straighter as they extended southwards. Observations of the flash off the east coast of New Zealand were hindered by cloud; but its occurrence is established. White glows appeared in many areas. A red glow appeared over wider areas within a few seconds of *H* and dominated the white glows after about 1 min. Also within a few seconds, a blue-white ray was seen from Apia to rise above the horizon, at the azimuth of Johnston Island, and extend along the magnetic field direction upwards and southwards. Numerous reports exist of white glow and rays, and of red glow, and those available so far are summarized in Figs. 1 and 2 respectively (Dominion Physical Laboratory Auroral Station; New Zealand Meteorological Service). Comparison of these figures show that while quiet white and red displays coincide in many instances, especially near the conjugate point, the white rays were located independently of the red glow. At about *H* + 4 min, the red glow was at maximum intensity; there is little doubt that it covered the region from 0° to 50° geomagnetic latitude, with longitude boundaries within ±5° approximately of the magnetic meridian through the fireball. The absence of reports of white ray structure in the area between New Zealand and the conjugate point is noticeable. While this cannot be dissociated completely from an absence of observers in the same area, observations of radio propagation and of magnetic disturbances, discussed here, support the concept that the white ray structure occurred in the two regions shown in Fig. 1, and not significantly between them.

The sustained white ray observed from Apia in the direction of Johnston Island resembled a searchlight. One theodolite measurement of it gave an apparent angular width near the horizon of about 0·2°, from which it is deduced that the main luminosity was confined within 10 km diameter at a height of 700 km near Johnston Island. No measurements of the luminous intensity were made. At high elevations, other rays appeared successively to separate from this in upward and westward directions during the 12

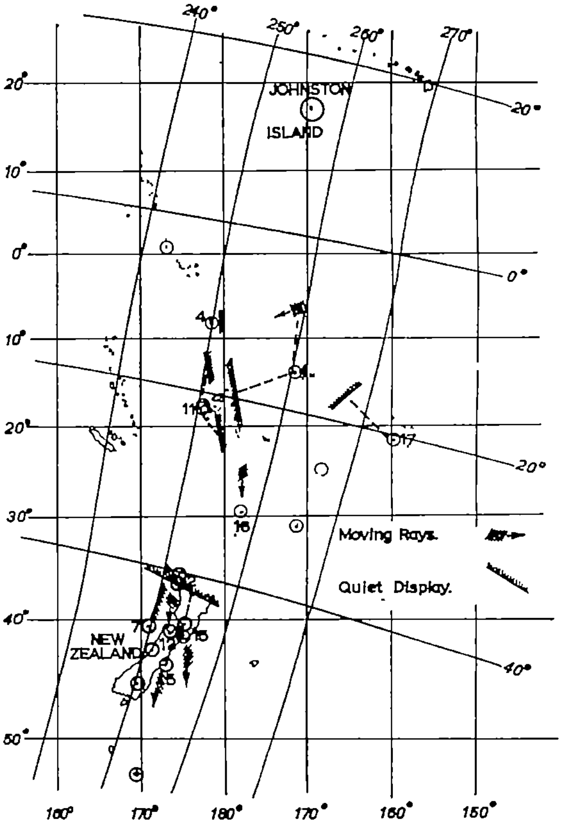

Fig. 1. The location of white auroral glows and rays due to the Johnston Island explosion. (Key to observing positions: 1, Apia; 2, Auckland; 3, Campbell Island; 4, Funafuti (Ellice Is.); 5, Christchurch; 6, Hokitika; 7, ship, 41° S. 171° E. 8, Lauder, Central Otago; 9, Lauthala Bay, Fiji; 10, Hauraki Gulf; 11, Nandi, Fiji; 12, Nelson; 13, Ohakea; 14, ship, 24° S. 167° W.; 15, Paraparaumu; 16, Raoul Is.; 17, Rarotonga; 18, ship, 31° S 172° W.; 19, Tarawa; 20, Wellington) (see also Fig. 2)

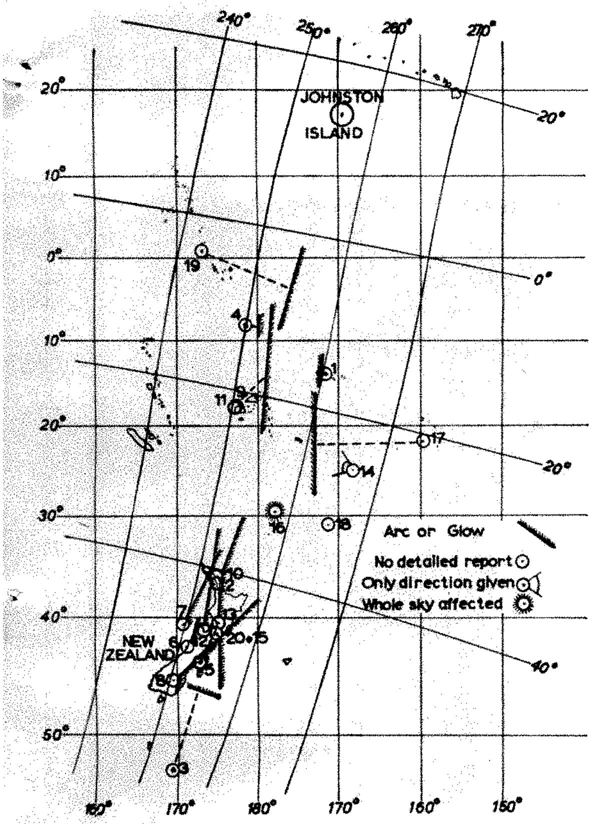

Fig. 2. As for Fig. 1, but showing location of red auroral glow

min of its existence (New Zealand Meteorological Service). The white ray structure clearly marked out the geomagnetic field directions. Luminosity was low at large elevations, giving the appearance of a gap in the rays near their zenith. The height of the rays was provisionally estimated as 700 km over Apia. The appearance of ray structure near the conjugate point observed at an azimuth of 331° and elevation of 54° from Apia is shown in Fig. 3 (University of Canterbury, Physics Department). Reports of the form of the display continue to be received from Pacific Islands. A colour sketch from Funafuti Island (New Zealand Meteorological Service) shows that, as seen from the west, the red glow extended in a north–south direction and filled the sky from horizon to an upper boundary obviously determined by the geomagnetic field direction. (The red area took the form of an archery bow, with string held horizontal, and colour between the bow and string.) South of the zenith of this glow, white rays appeared, starting at higher elevations than the red glow and approaching the horizon much more steeply. These rays are probably part of the structure shown in Fig. 3. The Funafuti Island observations clearly show differences in structure of the red glow and white rays, and establish a geomagnetic control of the upper limit of the red glow. The auroral displays at all locations faded between 0915 and 0920 U.T.

Spectrophotometry of the displays was carried out near Christchurch (University of Canterbury), and at Lauder, Central Otago (Dominion Physical Laboratory, Auroral Station). Near Christchurch, measurements were made of the variations of intensities of the *OI* emissions, λλ5577 and 6300 Å, and the N_2 + emission, λ4278 Å, during the display. At λλ5577 Å and 4278 Å, peak intensities occurred at $H + 2\frac{1}{2}$ min. The maximum intensity of λ5577 Å in the vertical direction was $2{\cdot}1 \times 10^4$ rayleighs, about 160 times the average intensity for the preceding month (University of Canterbury). At Lauder, an interrupted measurement suggested that the same radiation was increased only 20 times; and also that the rate of decay was faster than near Christchurch. Near Christchurch, the intensity of λ6300 Å showed a different time variation. Peak intensity was not reached until $H + 4$ min; and the maximum value was $1{\cdot}8 \times 10^5$ rayleighs, about 2,400 times the average intensity for the preceding month. This red glow corresponded to a Class III aurora. At Apia, a spectrogram taken in the first 11 sec showed that the display then corresponded essentially to a red type *B* aurora, with only a little hydrogen emission. The strong *OI*λ5577 Å line, and the much weaker *OI*λ6300 Å lines, indicated a height of the order of 100 km as the region of maximum intensity (University of Canterbury).

Magnetic effects of the aurora were observed at Lower Hutt (Geophysics Division, Department of Scientific and Industrial Research), at Amberley (near Christchurch) and at Apia (Magnetic Survey, Department of Scientific and Industrial Research). The scalar magnitude of the disturbance in total force at Lower Hutt was 300γ. At Amberley, the maximum disturbance in horizontal component was 220γ; and at Apia, the maximum disturbance was 55γ. Fig. 4 shows a tracing of the magnetic records from Amberley. The initial impulse was estimated as occurring at 0900 h 12 secs ± 1·5 secs U.T. From these records, the total force F is found to decrease steadily until $\Delta F = -45\gamma$ at $H + 45$ sec; then to increase, with ΔF reaching $+120\gamma$ at $H + 80$ sec, remaining near that value until $H + 105$ sec, followed by a rapid decay until $H + 150$ sec. A slow recovery continued for approximately 1 h. At Apia, the disturbance took a much simpler form. The large changes at the New Zealand stations, as compared with that near the conjugate point, are noteworthy.

Earth-potential gradients were measured near Christchurch (Magnetic Survey, Department of Scientific and Industrial Research), near Invercargill (University of Auckland, Physics Department; New Zealand Post Office), and at Lauder (Dominion Physical Laboratory, Auroral Station). Disturbances were noted within 0·2–0·5 sec after H. Off-scale deflexions occurred, but at Invercargill, the maximum range was (south) positive, 450 mV/km, negative 120 mV/km. At Lauder, fluctuations of quasi-period 3 sec and amplitude 7 mV/km occurred, but the main 'period' was in the region 40–100 sec. Comparison of the records from these latter two sites, 190 km apart, shows less occurrence at Invercargill of fast variations than at Lauder. The effect is believed not to be instrumental, and is ascribed to the existence of spatial irregularities in the exciting regions which affected Lauder but not Invercargill. If so, these regions were probably not more distant northwards from Lauder than a few hundred kilometres—a conclusion in general agreement with visual observations of the southerly limit of the auroral display.

A number of radio-wave experiments provided information on electron densities at various locations. These will be considered in order of increasing altitude. Recordings of transmissions from *NPM*, Hawaii (19·8 kc/s), showed a reduction in signal strength of 20 db. by $H + 0{\cdot}1$ sec, with recovery, starting at $H + 0{\cdot}3$ sec, to within 3 db. of earlier value at

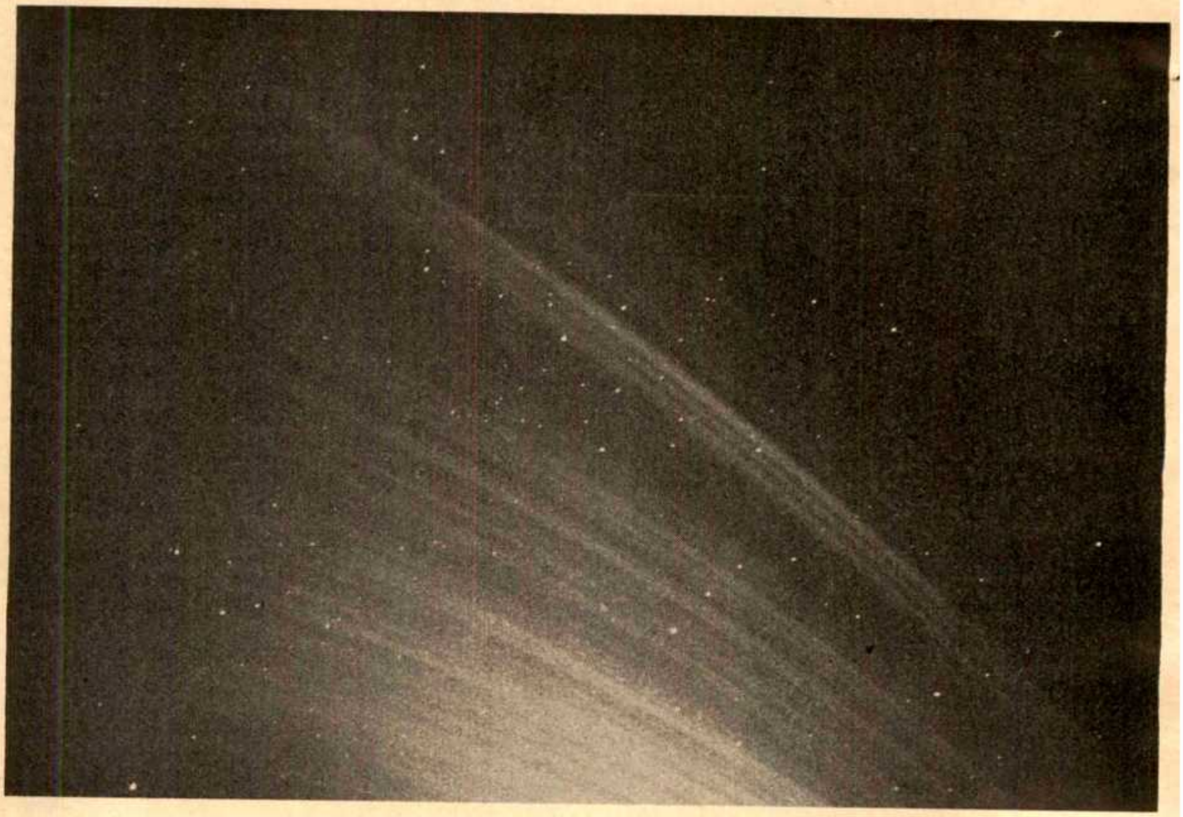

Fig. 3. Photograph of ray structure in aurora due to explosion, showing white rays from north merging into upper part of fading red glow. (Exposure from $H+10$ min 39 sec to $H + 11$ min 00 sec, with centre of photograph at 331·3° azimuth, 53·8° elevation, and field of view 40° × 28°, at Apia)

$H + 0{\cdot}6$ sec. This demonstration of momentary production of ionization, occurring a short time after H, and having a rapid recovery, suggests that the effects were located in a region relatively close to Johnston Island and deep in the atmosphere (Dominion Physical Laboratory). Comparisons of the time-scales of the variation of electron densities in the lower ionosphere, as a function of distance from the explosion, are possible through observations of cosmic noise absorption at 42 Mc/s, made with similar instruments located at Apia and at Lauder, New Zealand. At Apia, more than 10 db. reduction was noted for a few seconds after H, decreasing to zero in about 1 min. Assuming that the ionization was due to electrons of 300 KeV average energy, an electron density of approximately $10^4/\text{cm}^3$ occurred in a region centred around 70 km altitude (University of Canterbury). The rate of decay of the absorption appeared to be that of the ionizing agency, rather than determined by atmospheric processes. At Lauder, similar cosmic noise observations showed a slower rise to a maximum of 0·8 db. at $H + 2$ min, with rapid recovery starting at $H + 4$ min. The ionization involved was estimated to be located around 60 km, at a density of $4 \times 10^3/\text{cm}^3$. This could be produced by a proton flux of $10^3/\text{cm}^2$ sec and energy 15 MeV, or electrons of 1 MeV (Dominion Physical Laboratory, Auroral Station).

Information on low-height ionization away from the magnetic meridian was provided by observations of very low frequency transmissions from *GBR*, Rugby. At Lower Hutt, these are received predominantly by the shorter great circle path which passes within 3,000 km of Johnston Island, to the west. Immediately after H, a phase-change of 720° occurred, recovering in 3 h. This is interpreted, on the assumption of TM_{01} wave-guide mode propagation, as a decrease in effective guide boundary height from 85 to 68 km due to the explosion. A similar result was deduced from observations of station *NBA*, for which the phase-change was 630° (Dominion Physical Laboratory). To the east, at Rarotonga, ionosonde records showed moderate D-region

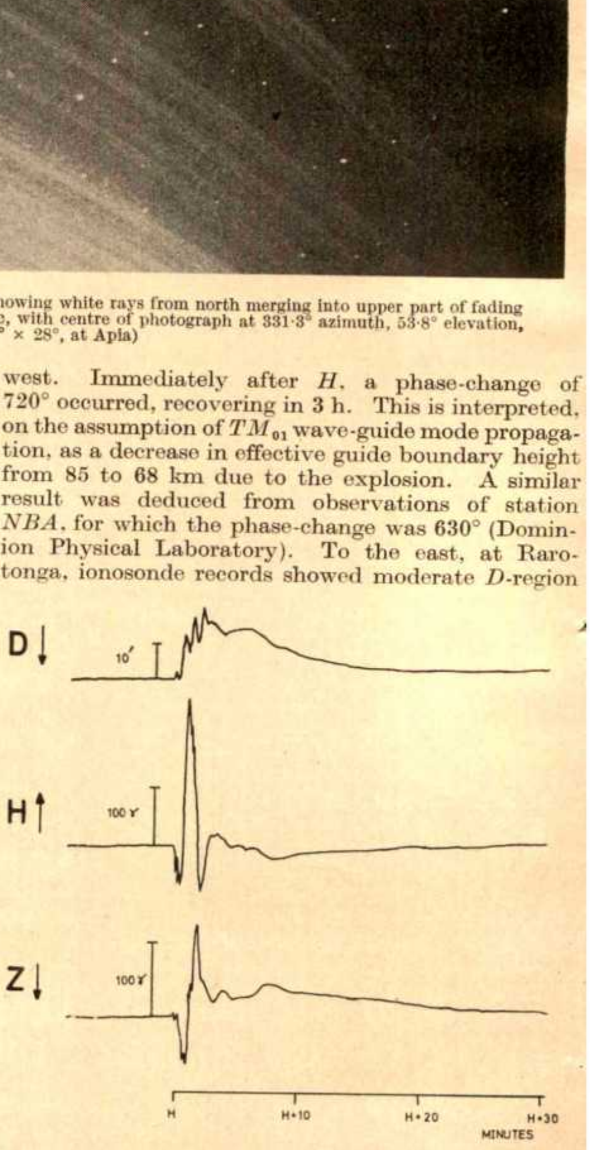

Fig. 4. Tracing of magnetic records from Amberley, New Zealand, showing disturbance due to explosion

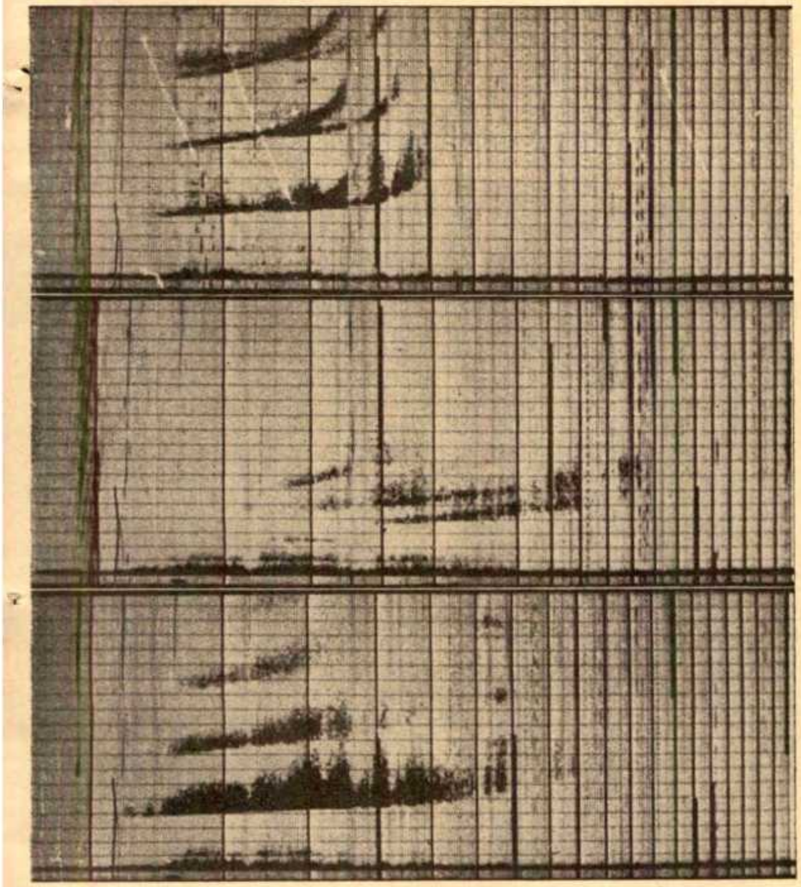

Fig. 5. Records from sweep frequency ionosonde at Christchurch showing effect of explosion. Top, at $H - 2$ sec; middle, at $H + 2$ min; bottom, at $H + 1$ h. Height markers are at 50 km intervals, and first frequency marker is at 1 Mc/s

absorption having essentially the same time variations as those noted at Apia. In the southerly direction, slight absorption was noticed at Christchurch and at Campbell Island, and there were negligible effects at Scott Base (Geophysical Observatory, Department of Scientific and Industrial Research).

The behaviour of electron densities at E-region heights was not measured directly, but upper limits could be set from inspection of ionosonde records. No strong sporadic or other E-region reflexions appeared at Christchurch or Campbell Island; and hence the corresponding electron densities probably did not rise above 5×10^4/cm^3. At Rarotonga, the appearance of the F-region trace suggested stratification, or a thick layer in E-region below. Information is available from a very high frequency pulsed forward scattered transmission at 52·5 Mc/s, for which the mid-point of the path is near Christchurch, as to variations in signal strength resulting from lower ionosphere electron density changes (Dominion Physical Laboratory). The transmission path, of length 910 km, extends from Invercargill to a receiver in the North Island, and makes an angle of 15° approximately with the magnetic meridian. A smooth increase in scatter strength started about $H + 2$ sec rising by 15 db. in 25 sec. Slight increases in greatest and least delays of the pulsed signals were recorded over the same period. At $H + 40$ sec, a rapid increase in strength by a further 20 db. was recorded. After a further interval of constant strength, a further rapid increase of strength at $H + 70$ sec caused 'off-scale' indications. Decay commenced about $H + 100$ sec and continued with an initial time constant of 13 min for a period of more than 2 h. The lowest height involved in the scattering was estimated from delay times to be between 80 and 85 km, and the greatest to be about 120 km. Signals with greatest delays showed 'filamentary' structure.

A second effect at E-region heights was detected by an auroral radar, operating at 55 Mc/s and located near Invercargill. The multi-lobed antenna radiation pattern was directed so as to intersect at right angles the magnetic field lines at E-region heights. This occurred over a distance of nearly 800 km, in a direction 175° true from the transmitter, over an area which lies across the magnetic meridian from Johnston Island. At $H + 40$ sec ± 6 sec, strong echoes appeared between ranges of 280–1,240 km. Using the known polar diagram of the array, it was deduced that the ionization responsible was field aligned, and located in a horizontal sheet, less than 6 km thick, at an average height of 103 km ± 1 km, some 8 km lower than the average height of thin layers giving rise to auroral echoes. The echoes showed an apparent motion to the south, with equivalent velocities, for horizontal motion at 103 km, lying between 25 km/sec and 1 km/sec. These values are greater than the velocity of sound in E-region. The echoes persisted for 17 min (Dominion Physical Laboratory, Auroral Station).

The main ionospheric effects in F-region were detected by the three ionosondes at Rarotonga Christchurch and Campbell Island, with greatest effects appearing on the Christchurch instrument (Geophysical Observatory). Fig. 5 shows a series of records from this location starting at $H - 2$ sec, $H + 2$ min and $H + 1$ h. The record at $H + 2$ min shows the maximum electron density noted in F-region after the explosion. At $H + 1$ min, an oblique echo appeared, due apparently to a high-density electron cloud. The density of the cloud increased up to $H + 2$ min and then decreased by $H + 6$ min. At $H + 7$ min, no reflexions were detectable from F-region, which was assumed to be too diffuse to give an echo. The region started reforming after $H + 8$ min. For $H + 3\frac{1}{2}$ h, 'spread-F' condition, much in excess of normal, persisted, as shown in the record for $H + 1$ h. Similar effects occurred at Campbell Island. The maximum density of the cloud was about ten times normal, and its approximate semi-thickness, assuming a parabolic layer, was estimated at 225 km. The probable total extent of the cloud is $5 \cdot 4 \times 10^{13}$ el/cm^2; and the minimum possible content $3 \cdot 6 \times 10^{13}$ el/cm^2.

At Rarotonga, the F-region effect took the form of an atmospheric wave which started about $H + 30$

min, reached a maximum about $H + 70$ min, and passed by $H + 120$ min. The disturbed condition of F-region precluded observations of the wave at Christchurch. The mean velocities of the onset, maximum and recovery of the wave were 147, 63 and 37 km/min respectively. This latter velocity, when corrected for electron recombination effects, is about the velocity of sound (40 km/min) in the region.

Effects on radio wave reception as a consequence of the ionization changes described here were widespread, and noticeable at frequencies from a few kc/s to 50 Mc/s. The click-type sferic used for determining the instant of detonation was followed by a clear multiple path whistler, of characteristics such that it can reasonably be attributed to the explosion (Dominion Physical Laboratory).

As expected, the interruption to communications circuits was greatest for those nearest the conjugate point. At Apia, complete and abrupt fade-out, commencing at H, of frequencies between 500 kc/s and 2·2 Mc/s, lasted until $H + 20$ min, for circuits to Fiji. Aeradio communications from Fiji were disrupted for periods of 5–25 min. Reports from ships indicate a complete interruption to transmissions between 4 and 9 Mc/s in the area 167°–176° W. and 24°–32° S. for 27 min (New Zealand Post Office).

In New Zealand, a complete fade-out occurred at Invercargill for frequencies about 2 Mc/s. A comparison of absorption of frequencies near 500 kc/s showed that paths over the South Island were affected more than those over the North Island, for north–south directions. High absorption affected all reception at Invercargill until $H + 80$ min. Trans-Tasman circuits suffered up to 60 db. absorption until $H + 5$ min. The recovery period extended throughout the night. Transmission from the Pacific coast of North America and from Hawaii to New Zealand, at frequencies near 10 Mc/s, faded out completely for 3 min, followed by 15–20 min of weak signals with rapid fading (New Zealand Post Office; New Zealand Broadcasting Corporation). Continued observations of broadcasting stations showed that high absorption persisted through the night of July 9, and that it was also present on July 10 and 11 around the New Zealand area.

The conference on August 16 was held essentially for comparison of observations, and a number of discrepancies was noted. The summary of observations permitted recognition of the three main visual effects, corresponding to initial flash, presumably due to decay electrons from fusion neutrons; the white ray structure due to material escaping from the explosion along the easy direction of the magnetic field, and the red glow, constrained within the magnetic field, and apparently due to excitation of atomic oxygen by low-energy electrons. The existence of much larger effects near New Zealand than near the conjugate point and the unexpected southward extension of the region affected were well established. Individual speakers were able to deduce hydromagnetic wave propagation as best explaining delay times of about 40 sec for E-region effects, and the atmospheric wave at higher altitudes was compared with those from previous explosions. Detailed accounts of these deductions will be presented by the workers concerned.

The conference acknowledged the indebtedness of various groups to the Royal New Zealand Air Force for transportation to locations in the Pacific.

J. B. GREGORY

CATASTROPHIC STORM EFFECTS ON THE BRITISH HONDURAS REEFS AND CAYS

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INTEREST in the effects of catastrophic storms on reefs and reef islands has been stimulated by investigations at Jaluit Atoll, Marshall Islands, following typhoon *Ophelia*[1,2], and at Ulithi Atoll, Caroline Islands, following another storm of the same name. Hurricane *Hattie*, which passed across the British Honduras reefs on October 30–31, 1961, presented an unusual opportunity to add to these Pacific investigations, since, during two expeditions in 1959–60[3] and 1961[4] I had completed field mapping of most of the sand cays of this coast, photographed all the cays from the air, and also observed living reefs, both on the three outer atolls (Turneffe, Lighthouse and Glover's Reefs) and the 150-mile long barrier reef. Accordingly, the hurricane-damaged areas were re-surveyed during February–June 1962. This article presents some of the main conclusions from the re-survey; the detailed reports on individual cays will be presented elsewhere.

Hurricane *Hattie*[5] was first identified as a tropical storm near San Andres Island, Nicaraguan coast, on October 27, 1961; it moved steadily northwards and intensified until the early hours of October 30, when it turned westwards in the neighbourhood of Grand Cayman Island and made for British Honduras. Heavy swells began to break on the outer reefs of British Honduras during October 29–30, and the first high winds reached Lighthouse Reef about 1600 h (Belize time) on October 30. The storm centre crossed Sandbore Cay, at the north end of Lighthouse Reef, at about midnight, October 30–31, when the minimum pressure recorded was 27·4 in. At this time the cay was covered to a depth of 7–8 ft. by a surge associated with the storm. The M.V. *Tactician*, 45 miles to the south-west in the barrier reef lagoon, also recorded 27·4 in. as the eye passed over at 0500 h, October 31. A minimum pressure of 28·5 in. was recorded at Belize, 20 miles north-west of the storm track, at 0600 h. The storm surge reached Belize at about this time, and by 0800 h sea-level at Belize had risen 10–15 ft. above normal, subsiding by 1030 h. No wind-speed records are available at Stanley Field, Belize, after 0500 h, when the anemometer ceased recording after gusts up to 115 m.p.h., but sustained speeds of 150 m.p.h. within 15 miles of the storm centre, with gusts up to 200 m.p.h., are considered likely. Hurricane *Hattie* was thus one of the most severe Atlantic hurricanes of 1961, and th

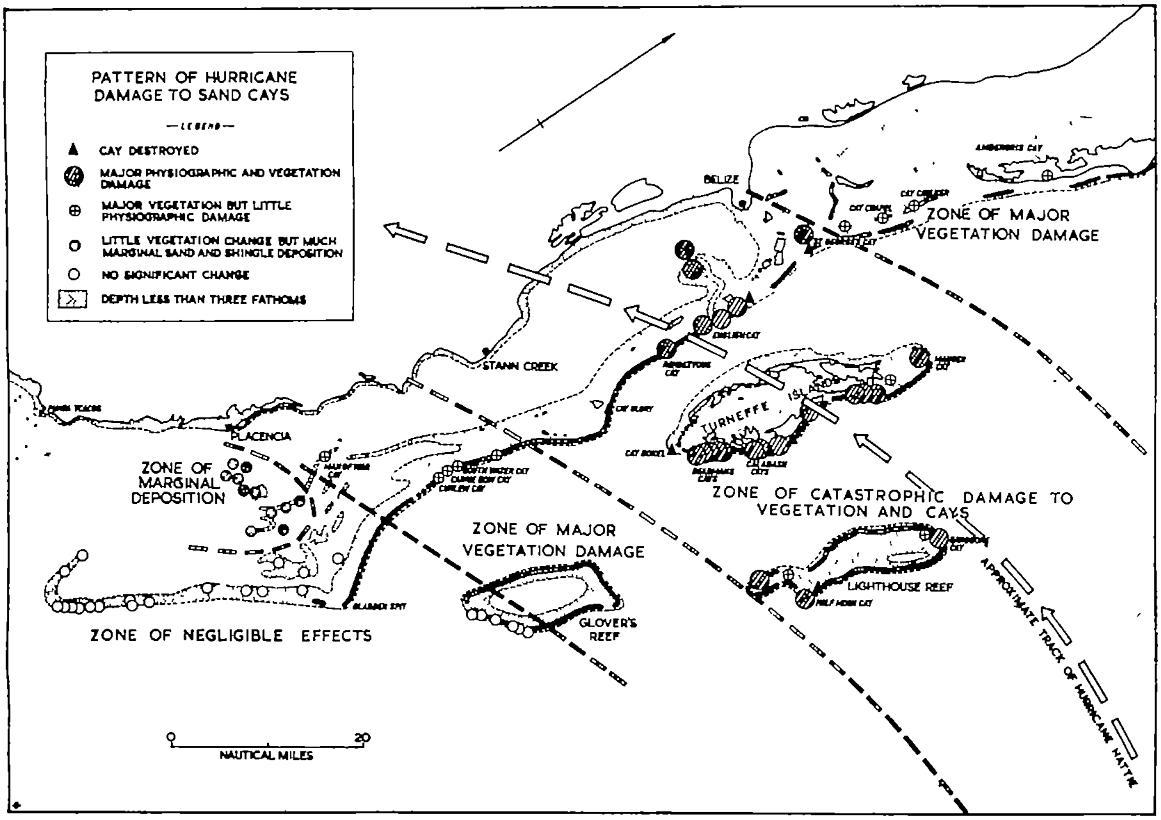
PATTERN OF HURRICANE DAMAGE TO SAND CAYS
—LEGEND—
CAY DESTROYED
MAJOR PHYSIOGRAPHIC AND VEGETATION DAMAGE
MAJOR VEGETATION BUT LITTLE PHYSIOGRAPHIC DAMAGE
LITTLE VEGETATION CHANGE BUT MUCH MARGINAL SAND AND SHINGLE DEPOSITION
NO SIGNIFICANT CHANGE
DEPTH LESS THAN THREE FATHOMS
BELIZE
AMBERGRIS CAY
ZONE OF MAJOR VEGETATION DAMAGE
STANN CREEK
ENGLISH CAY
TURNEFFE ISL
PLACENCIA
ZONE OF MARGINAL DEPOSITION
ZONE OF CATASTROPHIC DAMAGE TO VEGETATION AND CAYS
ZONE OF MAJOR VEGETATION DAMAGE
LIGHTHOUSE REEF
HALF MOON CAY
ZONE OF NEGLIGIBLE EFFECTS
GLOVER'S REEF
APPROXIMATE TRACK OF HURRICANE HATTIE
0 20
NAUTICAL MILES

Fig. 1

most severe in the British Honduras area since Hurricane *Janet* in 1955. Intense wind and wind-driven wave conditions combined with the high storm surge to the immedate north of the storm track to inflict severe damage to reefs and reef islands over a limited segment of the British Honduras coast.

Effect on Reefs. Damage to living coral reefs was variable and in places catastrophic. The windward reefs of Lighthouse Reef, immediately south of the storm track, suffered little damage, and the groove-buttress system remained intact. Damage to the windward reef of Turneffe was greatest near the north end, where fresh reef debris now forms a submerged shingle carpet on the reef crest, but the groove-buttress system is still largely intact. On the barrier reef, however, between English Cay and Rendezvous Cay, over a zone extending for 5 miles north of the storm centre, 80 per cent of the reef corals have disappeared. Patch-reefs which previously were coated with surface-breaking brown and orange *Acropora* now rise, greenish white, devoid of corals, to near sea-level. Along the reef-front all trace of the groove-buttress system has disappeared, and cannot be traced until one reaches Cay Glory, 10 miles south of the storm centre, where reef remnants indicate a pre-existing lineation. Reef species most affected were *Acropora cervicornis*, not seen living along the whole of the central barrier reef north of Curlew Cay; *Acropora palmata; Porites porites;* and numerous more fragile species, including *Porites furcata*, *Cladocora arbuscula*, *Manicina aerolata*, *Siderastrea radians*, *Eusmilia fastigiata*, *Favia fragum*, *Isophyllastrea rigida*, *Mycetophyllia lamarckana*, and even *Agaricia agaricites*. Consistently, the only species to survive in any quantity was *Montastrea annularis*, in massive hemispherical colonies. Other corals of similar habit, such as *Diploria labyrinthiformis*, *D. strigosa*, *D. clivosa*, *Siderastrea siderea*, *Solenastrea bournoni* and *Porites astreoides*, also survived in lesser numbers. The survival of the more massive, slower-growing species and destruction of fragile rapidly growing ones has also been observed at Low Isles, Great Barrier Reef of Australia, by Stephenson and others following the minor 1950 cyclone[6]. Many corals have been inverted or rolled across the reef-flat, but remain alive. Most of the reef constituents were washed into deeper water round the reef and are no longer visible, though in many places submerged areas of *A. cervicornis* debris indicates disintegration of this species *in situ*. Very little material larger than shingle size has been thrown above high-water-level, except on the east side of Turneffe, where mangrove hinders transportation into deeper water across the reef flat. South of Cay Glory, damage to the reef decreases rapidly; and no change was seen at Glover's Reef or along the southern barrier reef.

Effects on Reef Islands. The degree of damage to any particular cay depends: (*a*) on its location relative to the storm track; (*b*) on the type of cay, whether sand or mangrove; (*c*) on the size of the island; (*d*) on the nature of its vegetation and the extent of human interference. Four zones of cay damage are distinguished in Fig. 1, and will be discussed in turn.

Damage is greatest in a zone extending 15–20 miles north and south of the storm centre, especially on small cays where the natural vegetation has been replaced by coconuts with little undergrowth. In such cases, the cay has either disappeared or suffered severe marginal erosion; surface sand has been stripped to a depth of 1–3 ft., exposing coconut roots; fresh gravel has in some cases been patchily deposited on the cay surface; scour holes and channels have been cut on the cay surface and on the lee shore; and vegetation has been wholly or partly removed. Very large cays in comparable situations suffer only limited marginal erosion; surface erosion and deposition is restricted to a relatively narrow peripheral zone; and while coconut and other trees are thrown down in large numbers, much of the original ground vegetation survives. It is very noticeable that where cays retain much original vegetation, such as thickets of *Cordia sebestena* and *Bursera simaruba*, the effects are different: thus, along the south shores of Half Moon Cay, Lighthouse Reef, the section cleared for coconuts, formerly 7–10 ft. in height, suffered 3–5-ft. vertical erosion, whereas along the section covered with *Cordia* bush, much sediment was heaped against the vegetation barrier, with resultant increases in height of 3–5 ft. Similarly, scour channels were only cut across cay surfaces where the original dense vegetation had been cleared for coconuts or houses, as at St. George's Cay, Mauger Cay and Sandbore Cay.

Cays 20–40 miles from the storm track suffered less severe damage; they were unaffected by the storm surge and were subject to less extreme wave conditions. Physiographic changes were limited to minor shore retreat, exposing fresh beach-rock (South Water Cay, Carrie Bow Cay) or cay sandstone (Cay Chapel); the disappearance or modification of small spits; minor near-shore sand-stripping and root-exposure; and deposition of narrow carpets of sand and shingle inland from this erosion zone. Dominant storm effects were vegetational: on cays planted to coconuts 50–80 per cent of the trees were knocked down, often leaving small hollows filled with ground-water. Damage to other trees was generally restricted to defoliation and snapping off of branches, though in exposed areas large specimens of *Coccoloba uvifera* were seen overturned. Damage was greatest where natural vegetation had suffered most human interference, especially where this allowed unrestricted passage to waves across narrow portions of cays, as at Cay Caulker. Here, scour holes, channels and sand-stripping were well developed. Again, the larger the island the less was the damage sustained inland.

A third zone includes the cays between Placencia and Gladden Spit, barrier reef lagoon, where the main hurricane winds blew from the south across water 15–25 fathoms deep. In most of these cays the vegetational effects were insignificant, but all had sand or shingle deposited on their south and east shores. These deposits take the form of narrow carpets 1–5 ft. thick overlying the old cay surface, broad shingle flats adjoining the old shoreline, and offshore shingle ridges. Some of the offshore deposits are already being destroyed by wave action, the finer materials being flushed out leaving a coarse lag gravel. This zone is not duplicated to the north of the hurricane track, and its existence seems to depend on local conditions.

Finally, the cays of Glover's Reef and of the southern barrier reef and lagoon suffered no vegetational and little or no physiographic change, apart from insignificant shoreline readjustments which may or may not result from the hurricane itself. The Glover's Reef cays lie only 40 miles south of the storm track; while at Punta Gorda sustained gale force winds, perhaps reaching hurricane force, were insufficient to defoliate mangrove or lead to physiographic changes to the cays off Punta Ycacos. This zone is not represented in British Honduras to the north of the storm track, partly because of the absence of cays in that area, partly because of the greater wind velocities and storm surge, and hence greater extent of damage in the northern sector of the storm.

Beach-rock. Old relict beach-rock was remarkably successful in resisting damage, even where cays were completely destroyed, as at Cay Glory. Intertidal beach-rock within 20 miles of the storm centre was swept bare of larger algæ by wave action. Shore re-adjustments on many cays led to the exposure of new beach-rock, including low 'promenades' (Deadman's Cays) with pitted upper surfaces[7], similar to features previously interpreted as due to elevation of reef-rock. Much of this new beach-rock is poorly cemented; but in some cases areas where incipient cementation was previously noted now exhibit one or two lines of hard well-indurated beach-rock. Exposure of fresh areas of lithified sands above present sea-level at Harry Jones, Grand Bogue Point and Big Cay Bokel, Turneffe, and Cay Chapel, barrier reef, indicate that the rock is a cay sandstone rather than an elevated intertidal beach-rock.

Vegetation change. The most conspicuous vegetation changes were: (*a*) defoliation of mangrove within 40 miles of the storm centre; (*b*) the widespread destruction of coconut palms. Mangrove defoliation gradually decreased away from the storm centre, until at 20 miles distance on the south side, mangrove in the centre of large cays escaped defoliation. In early 1962, six months after the storm, some of the *Rhizophora* was again beginning to bear leaves, chiefly on the side of the cays in the lee of hurricane winds and waves, but it is not yet clear how much will ultimately recover. Large-scale fall of coconuts (in excess of 50 per cent) was found within 20–40 miles north and south of the storm track, and gives an indication of direction of extreme hurricane winds. Tree-fall direction at any point is generally constant within 30°–40°. Trees were uprooted, snapped off a few feet above the ground, or lost their crowns. Other trees which suffered heavy damage include *Cordia sebestena*, *Coccoloba uvifera*, *Bursera simaruba* and *Terminalia catappa*. All except *Cordia* tended to remain upright, losing leaves or branches; *Cordia* was generally uprooted and the debris piled by waves against obstructions. In the shallow northern barrier reef lagoon, uprooted trees, generally palms, have been carried several hundred yards from the nearest land, deposited on the lagoon floor, and are now visible above sea-level. *Rhizophora* showed great tenacity: not only are the details of mangrove areas almost unchanged by the storm, as shown by air photographs, but also in one case, Big Calabash East Cay II, the island was completely washed away, leaving only a ring of dead *Rhizophora* still in place. Greatest proportionate damage was caused to those species most abundant in exposed situations. *Tournefortia gnaphalodes* and *Suriana maritima*, formerly widespread near-shore plants, are not now to be found in the central zone of cay damage. On devastated cays and areas of freshly deposited sand, the chief colonists during the 4–7 months since the hurricane were, during the re-survey, *Portulaca oleracea*, species of *Euphorbia*, *Sesuvium portulacastrum* and *Ipomoea pes-caprae*.

Human settlement. The cays were previously habited by lighthouse keepers, coconut workers and hermen. The lighthouses at Sandbore Cay (Lightuse Reef) and Cay Bokel (Turneffe) were destroyed the storm, and that at Mauger Cay (Turneffe) was t out of action. Only the Half Moon Cay and nglish Cay lights remained in operation. Of the ur cays previously inhabited at Lighthouse Reef, ree have been abandoned; of the six settlements at urneffe, all are abandoned; and several cays on e barrier reef are no longer inhabited. The main urce of livelihood, apart from the lighthouses—the coconut industry—suffered very heavy damage, and all the loading installations at Calabash Cays were destroyed. It will be at least seven years before coconuts planted now begin to bear; but it is unlikely that private or public capital will be available for coconut investment following the succession of recent hurricanes. The larger islands near the storm centre, which were inundated by the storm surge, suffered contamination of their fresh-water lenses. Ground-water on these cays is now brackish and almost undrinkable. Since previous inhabitants, however, relied almost exclusively on rain-water vats for water supply, this is of less immediate practical importance than might be expected.

Birds. Two bird colonies in the devastated area were not markedly affected by the storm. The Half Moon Cay red-footed boobies, *Sula sula sula*, were not diminished in numbers, in spite of great damage to their nesting area, but the nesting period was delayed. Normally the young hatch in January–February, but in 1962 hatching began in mid-April and continued into May. *Fregata magnificens* was nesting in considerable numbers on Man of War Cay, barrier reef lagoon, in April.

Prospect. Investigations at Jaluit[8] indicate that, over a period of years, cays tend to revert to the position obtaining before the hurricane struck, shown, for example, by landward migration and erosion of shingle ridges, and by vegetation recovery. This is already beginning on some British Honduras ays. On the other hand, as at Jaluit, certain prosses are irreversible: the stripping of fine surface aterial and exposure of roots, erosion of scour holes nd scour channels in cay surfaces, deposition of shingle and sand carpets above high-tide-level, all are likely to be permanent. The large-scale destruction of living reef along the central barrier reef has both provided much new debris for cay accumulation and permitted larger waves access to cay shores. As a result sandbores have appeared in areas where none was previously seen, and many cay beaches are steeper. This, too, is likely to be a short-term effect, for studies in Australia indicate a period of 10–20 years for reef recovery following hurricane damage[6]. Future vegetation changes are difficult to assess. With the decline of economic interest in coconut plantations, and hence of the incentive to clear, many areas may tend to revert to bush, consequently decreasing the danger of catastrophic damage during subsequent hurricanes. Recovery will be most delayed in areas where the cay was completely destroyed, and has re-appeared or is likely to reappear, as a shifting unvegetated sandbore. A second re-survey in 3–4 years, as at Jaluit, could be instructive in assessing the long-term effects of hurricanes on reefs, reef islands and their human utilization. At present the most important practical conclusion, within the general zonation of damage, is the severity of change where human interference with island vegetation has been most intense.

I thank the Royal Society for supporting the re-survey expedition, and Miss E. L. Pruitt, Geography Branch, Office of Naval Research, Washington, for transatlantic transportation. Previous expeditions were supported by the Cambridge Expedition to British Honduras 1959–60, the Office of Naval Research, and the Coastal Studies Institute, Louisiana State University. I also thank Prof. J. A. Steers, Dr. F. R. Fosberg and Dr. Adrian Richards for help with the present investigation.

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CANCER RESEARCH IN CHILE

By Dr. R. S. d. ZALDÍVAR*

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CANCER research, along many different lines, is being successfully pursued in Santiago. These vestigations are concerned with experimental, nical and epidemiological problems.

Profs. G. Gasic and O. Badínez, Dr. F. Loebel and rs. Tatiana Baydak (Departments of Oncology and mbryology, University of Chile School of Medicine) ve for several years been studying the process of etastasis. At first, these investigations dealt with e capacity of cortisone in increased metastatic read in mice bearing mammary adenocarcinoma[1]. urther work dealt with the invasiveness of transanted neoplasms in mouse brain. It has been shown t in four different combinations of host and breast mour there is a close relationship between the ability of neoplasm for metastatic spread and its potency for invading the Virchow–Robin perivascular spaces when transplanted into the brain. However, sarcoma 180 and leukæmia *H* apparently show a different behaviour[1,2]. Recently, investigation has been focused on intercellular cementing substances and metastasis. The ground-substance of eight transplantable sarcomas of mice was examined by means of the Hale reaction. Hale-positive material is considered to be a non-sulphated acid mucopolysaccharide. A large amount of Hale-positive material was present in the ground-substance of non-metastasizing sarcomas. On the contrary, in tumours with moderate or high metastatic capacity the Hale reaction of the ground-substance was negative. Examination of the intercellular cement in five

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carcinomas with the same histochemical reaction confirmed this relationship[4]. Then the Golgi zone was examined using the Mowry variant of the Hale reaction. Several ascitic and solid tumours as well as normal tissues were examined; in almost all tissues, cells showed a positive-reacting Golgi zone[5]. Experiments to examine the chemical nature and biological meaning of this reacting Golgi material are in progress.

Dr. R. Iglesias (Institute of Experimental Medicine, National Health Service) has continued, through transplantation, his investigations of the biological behaviour of some spontaneous neoplasms in *A* × *C* rats and the influence of steroid hormones on such tumours. He found in the right ear of an animal a melanoma which did not show metastasis. Two groups of animals received inoculations, subcutaneously and intramuscularly. After 288–558 days, the neoplasm grew in four of the animals injected subcutaneously, and in only one of the rats with intramuscular inoculation. This tumour is being transplanted, and the probable influence of the gonads and of steroid hormones on tumour growth investigated[6]. Precocious metastasis of a transplantable sarcoma has been reported[7]. Lately it was found that the responsiveness to androgen of the Iglesias functional ovarian tumour, which was previously reported[8,9], has been changed. One tumour (*A*) maintains its original responsiveness, growing faster in intact males than in castrates and testosterone propionate stimulates its growth. But the other (*B*) is now growing faster in castrated males than in intact males and than in castrated males receiving the androgen[10]. Investigations dealing with the activity of large and sub-physiological doses of testosterone propionate on growth of two ovarian neoplasms were performed. The author concludes that masculinizing and sub-physiological doses of this hormone stimulate the growth of both tumours[11].

Dr. R. S. d. Zaldívar (Department of Biology, University of Chile) has been engaged in research with guinea pigs on gastric lesions induced by 3-methylcholanthrene and on the pathological effects of heated fat in the gastric mucosa. After intraperitoneal administration of 3-methylcholanthrene crystals, depositing the hydrocarbon on the gastric serosa of 66 animals, 9 pre-cancerous lesions (5 diverticula and 4 infiltrating epithelial lesions) and a true adenocarcinoma were induced (15·1 per cent). Also the liver developed proliferative changes as cholangiofibrosis, bile-duct hyperplasia, and cholangio-cellular tumour[12]. When the same carcinogen was implanted into the wall of the gastric antrum, 14 out of 41 animals (34·1 per cent) showed stomach lesions. Twelve diverticula (two of them associated with infiltrating lesion) and 2 infiltrating epithelial lesions appeared. In advanced stages of its development, the diverticulum may undergo malignant changes. On the other hand, the infiltrating lesion exhibits an evident neoplastic behaviour[13]. In further studies on this problem, an intramural injection of this carcinogen in the gastric antrum was given, using polyethylene glycol-400 as solvent. Pre-cancerous gastric lesions appeared in 14 out of 25 treated animals (56·0 per cent). With regard to the histogenesis of the infiltrating lesions, it originates from the deep mucosa, and one case showing perforation of muscularis mucosæ in three areas would indicate that this tumour develops from multicentric neoplastic foci[14]. However, as a detailed histogenetic study is pertinent, an experiment is now in progress. The problem of heated fats as possible carcinogens for the glandu epithelium of the stomach has also been investigate The carcinogenic activity of heated fat given ora —the physiological way—on the gastric gland mucosa of mice has not yet been demonstrated However, when a single dose (0·05 c.c.) of fat heate 361°–365° C for 30 min is injected into the wall the gastric antrum of guinea pigs, perforation muscularis mucosæ by pyloric glands appears[17]. other series the animals receiving heated fat (0·10 c. and using the same technique, all the six anim show gastric changes as epithelial hyperplasia of t mucosa, perforation of muscularis mucosæ and diverticula[18]. In both series[17,18] guinea pigs injecte with non-heated fat did not show epithelial change These observations would suggest that heated fat may play a part in the ætiology of human gastri adenocarcinoma.

At present, one experiment to study the hist pathological changes of bronchial and pulmonar epithelium in the rabbit after a single intra venous injection of 3-methylcholanthrene is in progress.

Drs. G. Contreras, Adela Ohlbaum and J. Toh (Bacteriological Institute, National Health Service and Institute of Physics and Mathematics, Universit of Chile) have been working on some aspects of th virus-cell relationship. Research has been focuse on biochemical changes related to poliovirus multipl cation in HeLa cells. First the kinetics of incorpor tion of phosphorus-32 into phosphoproteins a ribonucleic acid and the inhibitory activity of chloram phenicol were described[19]. Then the kinetics of on complete growth-cycle of poliovirus in suspende HeLa cells was investigated, the relation of it wit certain biochemical changes, and the effects chloramphenicol on the multiplication of the virus Experiments are now in progress on the kinetics the antibiotic inhibition in the HeLa cells–poliovir system.

Prof. Graciela Leyton, Dr. E. Raventos and other (Bacteriological Institute, National Health Servic and Fundación A. López Pérez), using different chen ical and immunological techniques, have been exami ing urinary proteoses in patients bearing malignan neoplasms[21].

Prof. L. Vargas and Drs. J. Rodríguez, A. Lucchir and J. Arraztoa (Departments of Physiopatholog Pathological Anatomy and Surgery, Catholic Univer sity School of Medicine) have been working on clinic chemotherapy of tumours. Thirty-six post-men pausal women were treated with hexœstrol during th terminal stages of metastatic mammary cancer. Th treatment was given after androgen therapy. I 22 patients (61 per cent) a good reaction was observe In these 22 patients the average survival-time w 27 months after the first subcutaneous implantati of hexœstrol pellets. After about the fourth to six day post-implantation, 50 per cent of the 22 wom showed a rapid decrease in size of lymphatic met stasis. The rapid regression of these metasta induced by the drug produced the so-called 'metast lysis syndrome', characterized by nausea, vomitin fever and chills. The syndrome is considered to be good reaction to hexœstrol treatment[22]. A study the effects of thiotepa on advanced breast cancer w performed. The patients received this compou after neither androgens nor œstrogens showed a therapeutic effect. A transitory regression of me stasis was noted, and the average survival time 10 months[23].

Finally, other investigations in 20 patients showing advanced cancer was done. Adrenocorticotrophic hormone, dexamethasone and 'Nitromin' were associated together as treatment. In 7 cases the complete course of the disease became known and the corresponding necropsies were performed. Each of these patients exhibited a different type of neoplasm. The therapy was well tolerated, and regression of the primary tumours or their metastases were not observed. The average survival-time was only eight weeks. Five out of the 7 patients suddenly died. The histological study of the viscera showed a diffuse tumoral infiltration, not detectable macroscopically. The spleen, which is generally not invaded in patients dying from cancer, was infiltrated in 100 per cent of the cases[14].

Prof. J. Moroder (Department of Preventive Medicine, University of Chile School of Medicine) has been working on the epidemiology of cancer. Preliminary studies on cancer mortality and morbidity in our country were reported[25,26]. Further investigation deals with statistical data of the years 1959 and 1960 [27]. These investigations have confirmed former observations on the very high incidence of stomach carcinoma. Furthermore, on January 1, 1959, the National Cancer Registry began its work.

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OBITUARIES

Sir Leonard Rogers, K.C.S.I., C.I.E., F.R.S.

Major-General Sir Leonard Rogers died in Truro, Cornwall, on September 16, 1962, at the age of ninety-four.

He was a Cornishman, born near Plymouth, and he returned to Cornwall after the death of his wife in 1951. His father was a captain in the Royal Navy, who brought up his large family in a deeply religious atmosphere. Rogers was educated at Tavistock Grammar School, Plymouth College, and St. Mary's Hospital, London, and in his schooldays he was a notable mathematician, taking several prizes. As an undergraduate he did some clinical research, published a paper, and won a scholarship in pathology. He qualified in 1891, and gained the F.R.C.S. in the following year, and at the same time passed the London M.B., B.S., with honours.

After a short period during which he demonstrated bacteriology at St. Mary's, and perhaps influenced by the fact that two of his brothers had entered the Indian services, Rogers passed the competitive examination for the Indian Medical Service. In this he hoped to find the opportunities for research which attracted him, and began his long series of investigations even in the early days when he was assigned to military duties, experimenting on the clotting of blood and inquiring into problems of malaria. As a result of this malaria work he was appointed to investigate kala azar, which was then epidemic in Assam, and which was thought to be a form of malaria. He did not succeed in differentiating the two diseases—this was done a few years later by Leishman and Donovan—but he did recognize that it was an infection associated with certain villages, and devised a method of control by removal of the people from those villages, which was very largely effective.

In 1900, Rogers (who had by then achieved the M.D. of London, and M.R.C.P.) was appointed professor of pathology in the University of Calcutta, and there he worked on amœbiasis, showing that liver abscess was due to *Entamoeba histolytica*, and proving that repeated aspiration was a much more desirable treatment than open operation. Knowing that ipecacuanha had some reputation in dysentery, but recognizing its undesirable toxic effects, he began to think about its component principles, and learned that one of them, emetine, had been found lethal to non-pathogenic water amoebae. He obtained some, tried it in amoebiasis, and thus started an effective treatment which, with its modifications, remains standard at the present time. Similarly, hearing that antimony salts had been used for African trypanosomiasis, he argued that they might be valuable in kala azar—another protozoal infection—and again he started a line of treatment which has remained standard.

These successes were achieved in clinical medicine, though he was essentially, and remained, a pathologist, and the same is true of much of his work on cholera and leprosy. In cholera he knew that infusion of normal saline gave only moderate results, and he argued that a stronger solution of salt might help to retain fluid in the body and prevent that excessive loss of fluid which is the characteristic of the disease. He therefore doubled the salt, and the treatment worked well—and once more it has remained standard practice. Towards the end of his life it became a matter of intense satisfaction to him that the Government of India accepted the advice he had long since given, that vaccination against cholera should be made compulsory for all pilgrims attending the great religious festivals, during which cholera had formerly so often been epidemic. Under British rule such a measure would probably have been unacceptable to the people.

In leprosy he started from the knowledge that chaulmoogra oil had some reputation for beneficial action, but was difficult to administer, and he isolated from it sodium gynocardate, which could be given more easily, and proved to be relatively effective. This remained the main basis of treatment until the sulphones were introduced.

Rogers undertook research on several other problems—snake venom, trypanosomiasis of horses, and other subjects—and devoted his inexhaustible energy to raising funds for a School of Tropical Medicine in Calcutta, which was opened in 1916; it remains an active institution and a permanent memorial to him.

He returned from India in 1920, was appointed medical adviser to the Secretary of State for India and physician to the Tropical Diseases Hospital; but the main interest of his later life was the British Empire Leprosy Relief Association, to which he gave large sums of his own well-earned money and time. He was president of the Royal Society of Tropical Medicine and Hygiene during 1933–35.

Rogers received many honours, as the list of his distinctions shows, and his work was well acknowledged in his lifetime, but he remained frugal in his habits, simple and even austere in his private life. He loved walking and cycling, not probably for the opportunities these gave of appreciating the beauty of Nature so much as for the release of energy they involved. Apart from his family he had few interests outside his work, but this work enraptured him, as he admitted in his autobiography, *Happy Toil*. He remained a religious man, but he also retained a critical, caustic mind, enthusiastic and resilient. His knowledge was exact, and he was at home with figures, which he could produce from memory on any public platform. He had immense innate authority, and at the same time a sense of fun, and he did not act the part of a great public figure—he was always himself, a single-minded scientist with the egocentricity which is part of genius. He was a very great man.

He married, in 1914, Una Elsie North, by whom he had three sons, who survive him, and he was ideally happy with her.

CHARLES WILCOCKS

Dr. B. P. Eddy

DR. B. P. EDDY died in Cambridge on August 11 at the age of thirty-seven, tragically cutting short a promising career in microbiology and leaving his many friends and colleagues with a deep sense of loss. Brian Pollard Eddy was born in Newcastle upon Tyne and received his education there; first at the Royal Grammar School (1936–43) and then, by virtue of an open scholarship, at King's College, Newcastle. After graduating with first-class honours in botany he was awarded the Pemberton postgraduate studentship. During its tenure for two years he did research on the formation of anthocyanins under Prof. Meirion Thomas.

In 1948 he was invited to fill a vacancy which existed for a bacteriologist at the Low Temperature Research Station, Cambridge, and he joined the staff in November of that year. While attending courses in bacteriology he completed his work on anthocyanins and was awarded the degree of Ph.D. of the University of Durham in 1949.

His work on problems relating to food began with a study of the bacterial reduction of dehydroascorbic acid. He showed that, in the bacterial cell, this depended on a rather specific activating enzyme and a particular type of cytochrome complement. He then turned to the problem of using micro-organisms for the production of new food materials. An attempt was made to produce dried 'mushroom' powder by the deep tank culture of various Basiodiomycetes Though acceptable powders were made, the desirable 'mushroom' flavour was absent. The mass cultivation of algae was also studied. *Dunaliella*, rather than *Chlorella*, was selected because it does not develop a prominent (and indigestible) cell wall and it was established that yields and growth rates were comparable with those of *Chlorella*.

Eddy's interest in the bacterial production of nitrite led him to investigate problems of meat curing. It was with this background that he edited the proceedings of the second International Symposium on Food Microbiology on the subject of "The Microbiology of Fish and Meat Curing Brines" (H.M.S.O., London, 1958). His recent activities in this sphere related to problems arising from the introduction of the new process of vacuum packing sliced bacon.

In the past few years, Eddy was especially interested in the physiology of Gram-negative bacteria. His recent revision of the genus *Aeromonas*, on which he was actively engaged at the time of his death, was probably his best known work. He published more than thirty papers, many jointly with colleagues in the Low Temperature Research Station, who will miss his co-operation. They will also miss his untiring efforts on the committee of the local branch of the Institution of Professional Civil Servants. His principal hobbies were music, of which he was passionately fond, and photography.

He was not strong physically and was for this reason exempt from War service. Temperamentally he was highly strung. However, he was throughout his university and research careers a likeable and sincere colleague, and it is as such that he is remembered.

E. C. BATE-SMITH

Mr. C. C. Calder

CHARLES CUMMING CALDER, who died in Aberdeen in April 1962, was one of a long and distinguished line of Scots who held high botanical office in India, and three of his immediate predecessors, Sir George King, Sir David Prain (later director of the Royal Botanic Gardens, Kew) and Colonel A. T. Gage were, like himself, graduates of the University of Aberdeen. Calder was born at Dunphail in Morayshire in December 1884, and was educated at Robert Gordons College, Aberdeen, and later at the University, where he took the degree of B.Sc. both in pure science and in agriculture. After graduation he went for a period of study to Berlin and on his return to Aberdeen he was for a time assistant to Prof. J. W. H. Trail, who was a close friend of King, to whom he had recommended young Prain for appointment to the Indian service. Trail no doubt influenced Calder in accepting the post of curator of the Herbarium at Sibpur in March, 1912, where he remained, apart from a short period on active service in 1918, until his appointment in January, 1923, as superintendent of the Royal Botanic Gardens, Calcutta, conjointly with the directorship of the Indian Botanical Survey. Another cognate office which he held, and to which he devoted most of his time, was that of quinologist, with responsibility for the care and distribution of the important quinine stocks for the Government of India. In his official duties he was much more concerned with administration than with scientific research, so that his publication record is rather meagre. He wrote short papers on *Oxalis* and *Vernonia*, a brief account of the vegetation of India, and, with Dr. K. P. Biswas, a handbook of the aquatic plants of India and Burma.

Calder retired in 1937, an event probably hastened by his own and his wife's poor health. He returned to Aberdeen where, during the Second World War, he concerned himself with agricultural affairs which mainly involved the direction of labour to farms. For some years before his death he was not well, and he is survived by his widow, a son and a daughter. His elder brother, who predeceased him, was Sir William Moir Calder, an eminent classical scholar, who was professor of Greek in the University of Edinburgh.

GEORGE TAYLOR

NEWS and VIEWS

Nobel Prizes:

Chemistry: Dr. M. F. Perutz, F.R.S., and Dr. J. C. Kendrew, F.R.S.

THIS year's Nobel Prize for Chemistry has been awarded jointly to Dr. M. F. Perutz, chairman of the Medical Research Council Laboratory for Molecular Biology, Cambridge, and Dr. J. C. Kendrew, deputy director of the Laboratory and head of the Structure Studies Division. This most welcome announcement follows quickly on that concerning the award of the Nobel Prize for Medicine to another member of the staff of the same Laboratory, Dr. F. H. C. Crick (jointly with Dr. M. H. F. Wilkins and Prof. W. Watson; see *Nature* of October 27, 1962, p. 319). Dr. Perutz was elected a Fellow of the Royal Society in 1954, and Dr. Kendrew in 1960. The award was made for their work on the structures of globular proteins. Both are also readers in the Davy Faraday Laboratory of the Royal Institution, London.

Physics: Prof. L. D. Landau, For.Mem.R.S.

THE Nobel Prize for Physics has been awarded to Prof. L. D. Landau, of the Institute for Physical Problems, Moscow. The award was made for his "pioneering theories for condensed matter, especially liquid helium". Prof. Landau was elected a Foreign Member of the Royal Society in 1960.

The Gairdner Foundation Awards

THE Gairdner Foundation Award of Merit has been given to Dr. F. H. C. Crick, Medical Research Council Laboratory for Molecular Biology, Cambridge, for his contributions to the solution of the genetic code. This is the first time that the Award, which is accompanied by a prize of 25,000 dollars, has been won since 1959. Four additional awards, with prizes of 5,000 dollars, have also been made to: Dr. A. H. Coons, Harvard Medical School, Boston, for his work on methods of tagging proteins with fluorescent dyes; Dr. C. Crafoord, Karolinska Institute, Stockholm, for his contributions to heart surgery and the heart–lung machine; Dr. H. G. Kunkel, Rockefeller Institute, New York City, for his contributions to the understanding of the formation and behaviour of protein molecules that are found in blood and body tissues; Dr. S. J. Sarnoff, National Institutes of Health, Bethesda, for his contributions to cardiac physiology. Established in 1957 by J. A. Gairdner, of Gairdner and Co., Ltd., Toronto, the Foundation has now conferred its awards and cash prizes totalling 150,000 dollars on 23 scientists.

Director of the Forest Products Research Laboratory: Mr. J. Bryan

MR. JOHN BRYAN has been appointed director of the Forest Products Research Laboratory of the Department of Scientific and Industrial Research. Mr. Bryan, at present deputy director of the Laboratory, succeeds Dr. A. C. Copisarow (see *Nature*, **186**, 934; 1962), who has been chief technical officer on the staff of the National Economic Development Council on a part-time basis since March, and is now to be released on full-time secondment. Mr. Bryan, who is fifty-nine, has been on the staff of the Forest Products Research Laboratory since 1926 and has been assistant director for eight years. Born and educated in Sheffield, he read civil engineering at the University of Sheffield, and worked in the field of structural steel design for three years before joining the Laboratory. After forming and leading the section working on wood preservation and later leading the section of timber mechanics, Mr. Bryan took charge of all engineering studies at the Laboratory in 1945. For some years he has worked in close co-operation with the Forestry Commission, and is chairman of a joint Department of Scientific and Industrial Research–Forestry Commission Committee on research into home-grown timbers.

Forest Products Division of the F.A.O.: Dr. N. A. Osara

DR. NILS ARTHUR OSARA, formerly director-general of the Finnish State Forestry Board, has been appointed deputy-director of the Forestry and Forest Products Division of the Food and Agriculture Organization. Dr. Osara replaces Sir Henry Beresford-Peirse, who has returned to the United Kingdom as director-general of the Forestry Commission of Great Britain. Dr. Osara was born in Ikaalinen, Finland, and attended the University of Helsinki, receiving his degree of Doctor of Forestry in 1936. During the period 1924–36, he was also employed by both the University of Helsinki and the Forest Research Institute. A war-time member of the Finnish Government, Dr. Osara was appointed Minister of Supplies and Forestry during 1943–44. From 1938 until 1948 he was professor of forest economics at the Forest Research Institute. He then became director of the Central Forestry Association, 'Tapio', from 1948 until 1952, before going on to head the Finnish State Forestry Board. From 1937 until 1952 he was also associate lecturer on forest policy at the University of Helsinki.

Physical Properties at the U.S. National Bureau of Standards : Dr. J. B. Wachtman, jun.

DR. JOHN B. WACHTMAN, JUN., has been appointed chief of the Physical Properties Section of the National Bureau of Standards, U.S. Department of Commerce. This section, which studies the physical properties of inorganic, non-metallic crystals, with particular reference to the effects of structural defects and chemical impurities, is a part of the Inorganic Solids Division. Dr. A. D. Franklin, chief of the Division, has served as acting chief of the section since it was established in March 1961. Dr. Wachtman joined the Engineering Ceramics Section of the National Bureau of Standards in 1951, and received a Meritorious Service Award from the Department of Commerce in 1960 for his research work in that area. Born in Conway, South Carolina, in 1928, he received his B.S. and M.S. in physics from the Carnegie Institute of Technology in 1948 and 1949 respectively. Dr. Wachtman was a research and teaching assistant at the Carnegie Institute of Technology from 1949 until 1951. He received his Ph.D. in physics from the University of Maryland in 1961. Dr. Wachtman is a member of the American Physical Society, the American Ceramic Society, the Society of Rheology, the Washington Academy of Sciences, Sigma Xi, and the Washington Philosophical Society.

The Christmas Lectures at the Royal Institution, London

THE Christmas Lectures for children were started at the Royal Institution, London, in 1826 and have been given every year since then. These lectures have always been well known for their vivid demonstrations and experiments. The scene of the 1961 Christmas Lectures, being given by Sir Lawrence Bragg, Fullerian professor of chemistry and director of the Davy Faraday Laboratory in the Institution, has been painted by Terence Cuneo. The picture reveals Sir Lawrence demonstrating electrostatic effects before a young audience in the world-famous and beautiful lecture theatre at the Institution. The painting was commissioned by the Royal Institution and unveiled by the president, Lord Brabazon of Tara, after one of the equally famous Friday Evening Discourses on October 12, 1962. This is the first painting of a Christmas Lecture since the well-known picture was painted of Faraday himself giving a lecture in 1856. In it (Fig. 1) Mr. W. A. Coates is seen assisting Sir Lawrence with the demonstration. Lord Brabazon of Tara is sitting in the president's chair. Also in the audience are Lady Bragg (second row, second seat), and Prof. R. King, professor of metal physics in the Institution, and Mr. K. D. C. Vernon, librarian of the Institution (left of front row, right). The head porter, Mr. T. Ryan, is standing at the door.

The National Universities Commission, Nigeria

A NATIONAL Universities Commission has been set up in Nigeria. Its inaugural meeting was held on October 11 in Lagos. Its membership is as follows: Mallam Muhammadu Tukur, Emir of Yauri (*chairman*); Mallam Abdu Gusau; Alhaji Abdurrahman Okene; Dr. S. E. Imoke, Minister of Education, Eastern Region; Mr. J. O. Udoji; Dr. F. Ogunro; Mr. G. A. Konolafe; Mr. S. O. Wey, Permanent Secretary, Prime Minister's Department; Mr. A. Ikoku; Mr. E. E. Esua; Mr. E. A. Osindero. The Commission has a full-time secretary of senior status, Mr. Okoi Arikpo; he is a graduate of the University of London (University College) and a barrister. He

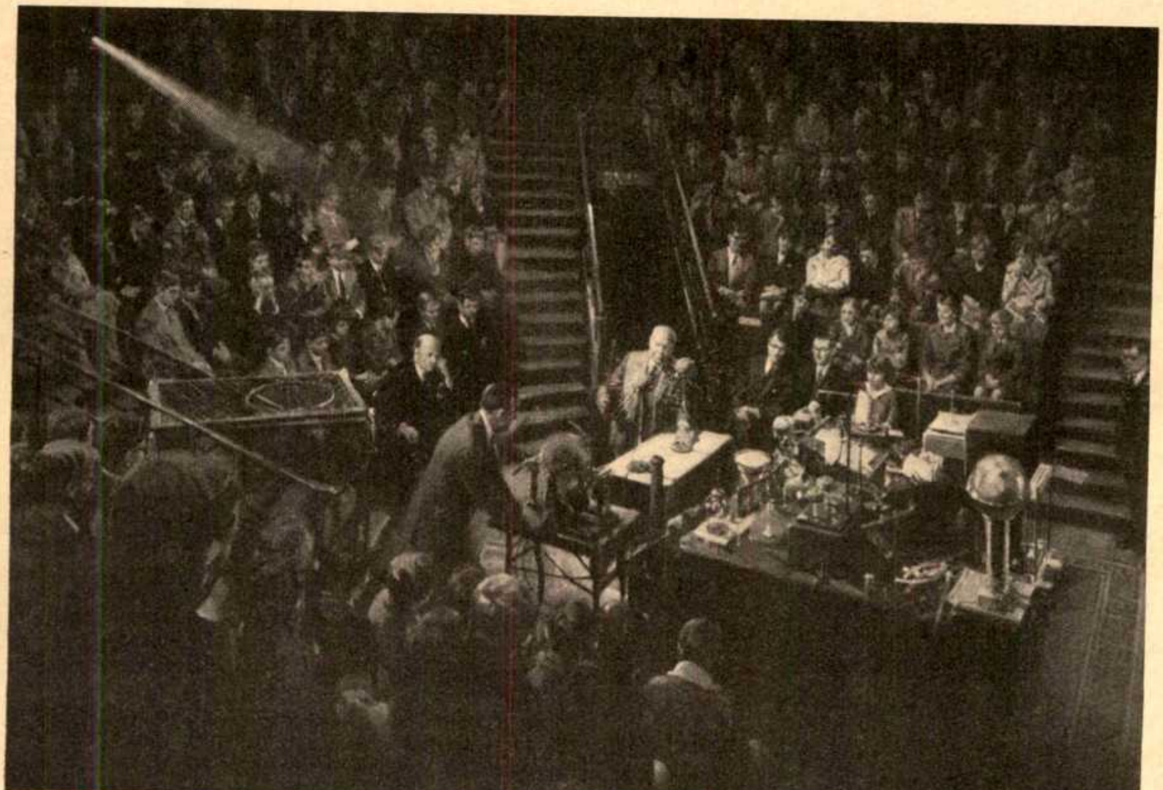

Fig. 1. Photograph of the painting. (*By courtesy of the Royal Institution and The Times*)

was formerly a member of the Council of Ministers, and a lecturer in anthropology in University College, Ibadan. He delivered the second series of Lugard Lectures in 1957, which were published as a pamphlet, *Who are the Nigerians?* The Commission is directly under the Federal Prime Minister and was appointed by him. The Prime Minister has invited Sir Eric Ashby, Master of Clare College, Cambridge, to act as honorary adviser to the Commission. The Commission has wide terms of reference, which include advising the Federal Government on the financial needs of university education in Nigeria, assisting in planning the balanced and co-ordinated development of the universities, receiving annually a block grant from the Federal Government and allocating it to the universities with such conditions attached as the Commission may think advisable, and making such other recommendations to the Federal Government or to universities relating to higher education as the Commission may consider to be in the national interest.

The National Bureau of Standards Stratton Awards

Dr. James R. Wait, Dr. Peter L. Bender and Raymond L. Driscoll are the first recipients of the newly established Samuel Wesley Stratton Award. These awards are given in recognition of outstanding contributions by scientists at the National Bureau of Standards, Washington. Each award consists of a bronze plaque and 1,500 dollars, and is named after the first director of the National Bureau of Standards. In 1901, Dr. Stratton organized the Bureau as a unique scientific institution and, during a 21-year tenure of office, firmly established its position in the scientific and industrial community. Dr. Stratton left the Bureau in 1922 to become the ninth president of the Massachusetts Institute of Technology. Dr. James R. Wait was cited for "his contributions to a better understanding of the mechanisms of electromagnetic radiation and radio wave propagation". Dr. Peter L. Bender and Raymond L. Driscoll were honoured for "their contributions to precision electromagnetic measurement and, particularly, the determination of the gyromagnetic ratio of the proton". Their work has provided a better standard for magnetic fields and made possible more accurate values for many other fundamental constants of physics.

The National Institutes of Health London Office

The National Institutes of Health, United States Public Health Service, has opened an office in London (24–31 Grosvenor Square, W.1) in connexion with its grant and fellowship programmes in the United Kingdom, as part of the Institutes' Office of International Research. Prof. Willoughby Lathem is the scientific representative in the London office. He is on leave of absence from the University of Pittsburgh, where he is associate professor of medicine, and will represent the National Institutes of Health in negotiations and consultations with British and international scientific organizations, collect information on the character and extent of medical and biological research, advise grantees and assist the Institutes' advisory groups by providing information concerning research projects proposed by British institutions for support from the Institutes.

The Optical Society of America: Awards

At the forty-seventh annual meeting of the Optical Society of America, held at Rochester during October 3–5, the Frederic Ives Medal for 1962 was conferred on Dr. Max Herzberger, senior research associate in charge of optical research in the Physics Division of Eastman Kodak. Dr. Herzberger was cited for "his outstanding contributions to optics and image formation". Born in Germany, he worked with the Leitz, Wetzlar and Zeiss Companies, and joined the Eastman Kodak Co., Rochester, in 1935. Also at the meeting Prof. Alfred Kastler, professor of physics at the University of Paris, received the first award of the C. E. K. Mees Medal, an award recently established by the Optical Society for recognition of a scientist, regardless of nationality, who has made outstanding contributions to the science of optics. Prof. Kastler is well known for his contributions to research in spectroscopy, especially spectra of the night sky and the sodium line at twilight, in atomic excitation by electron impact, and, more recently, in optical pumping.

Elections

At the Rochester meeting Prof. Richard C. Lord, professor of chemistry and director of the Spectroscopy Laboratory at the Massachusetts Institute of Technology, was named president-elect of the Optical Society of America. Prof. Lord is also president of the Commission on Molecular Spectroscopy of the International Union of Pure and Applied Chemistry. He is well known for his work on far-infra-red spectroscopy, the spectra of deuterium compounds, and the calculation of the thermodynamic properties of gases and crystals from spectroscopic data. In addition to Prof. Lord, the following were announced as directors of the society: Dr. John A. Sanderson, superintendent of the Optics Division, U.S. Naval Research Laboratory; Dr. W. Lewis Hyde, director of development, J. W. Fecker Division, American Optical Co.; and Dr. George Wald, professor of biology and an authority on physiological optics at Harvard University. The new officers will assume their duties after January 1, 1963. Dr. Lord will serve as president-elect until January 1, 1964, when he begins his term as president. Dr. Stanley S. Ballard, chairman of the Department of Physics at the University of Florida in Gainesville, will assume office as president on January 1, 1963. Dr. David L. MacAdam, of the Eastman Kodak Co., is now president.

The River Lee Basin

The Lee drains a small area (548 square miles) but is important because of its proximity to north London with its growing population and the increasing need of water for domestic consumption and for the disposal of sewage. Furthermore, the Lee Valley contains a large area of glasshouses requiring watering, and irrigation is of great value for increasing the yield of crops and potatoes for the London market which tends to augment the abstraction of river water in dry spells during the summer. In an area of about 23 in. of rainfall per annum and a large loss by evaporation, the Surface Water Survey is requested to assess the demand and to install more gauges to estimate the potential supply. Gauging stations measure the flow of the Mimram at Panshanger Park, of the Rib at Wadesmill, of the Ash at Mardock and Easneye as well as of the Lee at Fieldes Weir and of Pymmes Brook at Edmonton. The mean annual run-off from the whole drainage area is reported as 5·4 in. per annum, and only about half this is expected to be usable because suitable sites for reservoirs are few and because of the small rainfall and the excessive loss by evapotranspiration. The abstraction of ground water from the chalk has risen steadily during the past five years, and a recent report dealing with the River Lee Basin makes suggestions for re-charging this underground reservoir, the level of which is falling alarmingly (*River Lee Basin.* Hydrological Survey. Hydrometric Area No. 38. London: H.M.S.O., 1962. 9*s.* 6*d.*). Some importation of water from outside the hydrometric area, it is hinted, will be needed.

Food Preservation Quarterly

THE canning of water for emergency use is discussed by D. J. Casimir in a recent issue of *Food Preservation Quarterly* (22, No. 2; June 1962). Of the six different treatments which were compared, it was found that the simplest (involving boiling without additions, hot filling and no subsequent retorting) gave a satisfactory product after storage for three years at 100° F. Among other articles in this issue are the following: accelerated freeze-drying, by J. D. Mellor; a discussion of the mechanism and control of freezer burn, by G. Kaess and J. F. Weidemann; some chemical aspects of food processing, by C. O. Chichester; the use of bulk bins for fruit storage, by E. G. Hall (and Mellor); and the internal corrosion performance of electro-tinplate cans, by E. G. Davis. The journal is published by the Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia.

The National Central Library, London

THE forty-sixth annual report of the National Central Library for the year ended March 31, 1962, records some progress in planning the new building in Store Street and it is hoped that actual building can commence by January 1963 (Pp. 26. London: The National Central Library, 1962). Inter-lending again increased, issues from or through the Library being 87,011 compared with 90,612 in 13 months of 1960–61. Of these, 16,667 were from or through university libraries, 9,953 special outlier libraries, 2,159 Government departments, 3,380 industrial and research organizations and 36,617 public libraries. Loans to libraries overseas totalled 3,699 lent and 2,550 borrowed, while 633 photo-copies were supplied and 786 obtained from abroad. Outlier libraries now number 352. Of 7,546 volumes added to the stock, 4,080 were purchased, including 851 British Government publications which are now all being purchased. Stress is laid on the part played by university libraries in meeting the needs of other libraries. The present book fund of the Library is about £9,000, which has to cover books for adult classes as well as binding, but when adequate funds are forthcoming the Trustees consider the Library could function as an effective national lending library. This would be mainly in the humanities, and would not duplicate the large number of books and periodicals already available for loan from other libraries. Besides additional funds for purchase of books and periodicals, additional housing space will also be required.

Ramsay Memorial Fellowships for Advanced Students of Chemistry

APPLICATIONS for two Ramsay Memorial fellowships for advanced students of chemistry will be considered in February 1963. One of the fellowships will be limited to candidates educated in Glasgow, but they can apply to be considered for either fellowship. The value of each fellowship will be £750 per annum, to which may be added a grant for expenses of research not exceeding £150 per annum, and will normally be tenable for two years. Further information can be obtained from the Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College, Gower Street, London, W.C.1. Completed application forms must be received not later than December 31.

Stimulated Emission of Radiation

A SHORT course of four lectures on masers is to be held at Battersea College of Technology during November and December, commencing November 22. The title of the course is to be "Stimulated Emission of Radiation". The lecturers will include Drs. G. D. Sims (University College, London), J. C. Walling (Mullards), O. S. Heavens (Royal Holloway College) and Mr. M. S. Lipsett (Imperial College of Science and Technology); the course is under the direction of Mr. G. R. Parlett. The topics to be included are radio and optical frequency masers, their developments and applications. The first lecture will be in the form of an introduction. Further information and application forms can be obtained from the Secretary (Spectroscopy Courses), Battersea College of Technology, London, S.W.11.

Announcements

MR. R. L. MICHAELSON, controller of administration at Elliot Automation, Ltd., has been elected president of the British Computer Society. Last year, Mr. Michaelson was chairman of the Executive Committee of the Society.

A MEETING on "The Viscosity and Visco-Elasticity of Inorganic and Organic Glasses", arranged jointly by the Society of Glass Technology and the British Society of Rheology, will be held in the University of Sheffield during January 8–10. Further information can be obtained from Mr. D. Hawksworth, Society of Glass Technology, Thornton, 20 Hallam Gate Road, Sheffield, 10.

A SHORT course of lectures on "Problems of Radioactivity at Water and Sewage Works", arranged by the Manchester College of Science and Technology, will be held in the College during January 10–11. The programme will include lectures on: radioactivity; radiation effects; radiation measurement; radiochemical analysis; radioactive waste disposal by discharge to sewers; problems at waterworks; environmental discharge of radioactive waste; and films will also be shown. The fee for the course will be £7. Further information and forms of application can be obtained from the Registrar, Manchester College of Science and Technology, Manchester 1.

A MEETING of the Illuminating Engineering Society will be held on December 11 at the Federation of British Industries, 21 Tothill Street, London, S.W.1. The programme will include the following papers: "Colour Perception and Colour Rendering", by Mr. R. W. Brocklebank and Mr. M. H. Wilson (Goethean Science Foundation); "Colour Rendering Tolerances and the Colour Rendering Properties of Light Sources", by Dr. B. H. Crawford (National Physical Laboratory); "Practical Problems of Specifying and Measuring Colour Rendering Properties", by Mr. G. T. Winch. Further information can be obtained from the Secretary, the Illuminating Engineering Society, 32 Victoria Street, London, S.W.1.

SOVIET VIEWS ON VITAMIN B_{12}

By Prof. J. BIELY

Department of Poultry Science, University of British Columbia, Vancouver, Canada

IN the summer of 1961 I attended the fifth International Congress of Biochemistry held in Moscow, U.S.S.R. As a result I was able to establish friendly relations with a number of prominent Russian investigators in the field of vitaminology from whom I have since received a number of very interesting monographs. These cover research work of which I had no previous knowledge, although they contain a wealth of original information. They do not contain summaries in English, French or German, languages with which English-speaking people are familiar, which is most regrettable because most of the published papers deserve much wider recognition than they now receive, since they are available only in the Russian language.

As one means of overcoming the difficulty I suggest that some of the more significant papers be translated into English and published. This would make available to English-speaking people some knowledge of the vast research work being carried on in the U.S.S.R.

I recently received a monograph entitled *Vitamin Resources and their Uses* published by the Institute of Biochemistry which was named after Prof. A. N. Bach*. This is the fifth volume in a series. It contains 311 pages and deals with 28 different topics. The introduction is written by Dr. V. N. Bukin and Mme. L. Y. Areshkina. I think the best way of reviewing the contents of this monograph is by way of a translation (more or less free) of this introduction, which gives a general idea of the subject-matter covered. The majority of the papers are of a highly technical nature, and it is very gratifying indeed to see the number of references made to foreign publications, particularly those in English. There are, of course, many references to Russian authors.

"The high biological activity of vitamin B_{12} excited unusual interest, initiating an enormous flood of publications dedicated to the study of the various aspects of the problem.

"This type of work began in the U.S.S.R. in 1952 when in the Vitamin Laboratory of the Institute of Biochemistry, named after A. N. Bach, the first crystals of vitamin B_{12} were received and turned over to sixteen clinics for experimentation.

"In the course of these experiments, significant results were obtained in the clinic of the Central Institute of Hæmatology and Blood Transfusion, and other clinics treating hæmatopoietic disorders. At the same time it was established that, over and above participation in hæmatopoiesis, vitamin B_{12} also plays an important part in the functions of the liver and nervous system.

"Since then a series of biochemical, technological and clinical researches were twice discussed at symposia on vitamin B_{12} in 1958 and 1960. Simultaneously with clinical experiments with the crystalline preparation, tests were also made of less-refined preparations to be used with livestock, produced by the same laboratory and the All-United Vitamin Institute. The combined results of these tests are reported in two bulletins.

"The results of all this research were published in a journal *Vitamin B_{12} and its Clinical Uses*, (Medgiz, 1956), which also included tests in the field of biosynthesis and technology in the production of vitamin B_{12}.

"The contents of this monograph therefore express in various ways the different trends of the experiments. There is a definite reason for this kind of grouping of related works as the mutual familiarization of specialists of different branches of knowledge, working on one and the same subject, is a source of incentive to new methods and expressions of new areas of study and use of the vitamin. The articles in the monograph are grouped in the following basic divisions: biochemistry of vitamin B_{12}: its biosynthesis and technology of production; chemical, microbiological and biological methods of determination; the use of vitamin B_{12} in clinics; its use in livestock breeding. In each of these divisions new experimental results appear as well as conclusions and recommendations of a scientific or practical nature, and methods of testing are given in such detail that these could be reproduced in other laboratories.

"A large part of the monograph is set aside for the reporting of work on the biosynthesis of vitamin B_{12} and this is very gratifying. Over and above the scientific interest in this work, this has an independent practical meaning since on an adequate synthesis depends the health and potential productivity of livestock. Unfortunately, one cannot say the same of research dedicated to the study of the biochemical functions of vitamin B_{12}. These works are no less important, but they are few; it is essential to develop research in this direction.

"A whole series of papers in the monograph is dedicated to research in the uses of vitamin B_{12} for the clinical treatment of serious illnesses.

"In these articles there appears an exceedingly important and new proof, obtained in the course of experimentation on animals (S. V. Andreyev) and in the clinic (a series of authors), of the outstanding role of vitamin B_{12} in the treatment of severe traumas of nervous, muscular, vascular and bone tissues. This material could be the starting point for extremely important biochemical research of normal and traumatized tissue.

"Finally, there are two articles showing the vast effect of the use of vitamin B_{12} on livestock. One especially interesting fact determined in the course of this work is the interrelation and synergism of the growth-stimulating action of vitamin B_{12} and antibiotics. Quantitative interrelation in this connexion has not as yet been studied although it would appear to be most important from a practical stand-point and also in explaining the mechanics of the growth-stimulating action of antibiotics.

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* *Vitamin Resources and Their Uses*—Vitamin B_{12}—its biosynthesis, functions and uses. Foreword by Dr. V. N. Bukin and Mme. L. Y. Areshkina. (Published by the Bach Institute of Biochemistry, Moscow, U.S.S.R.) 1.25 dollars.

"We hope that the publication of this monograph will assist in developing further theoretical research and a wider application of vitamin B_{12} in clinical work and on livestock with the view that all the valuable qualities of this vitamin may be most fully utilized for the protection of animal health and ensuing material benefits."

In view of the present interest in vitamin B_{12} I think it would be well worth while if some agency could undertake to provide an English translation of this monograph and also perhaps of the four which preceded it.

I thank Mrs. Pauline Renacre, 205 East 29th Ave., North Vancouver, for her assistance with the translation, and Mrs. A. McCullough, secretary in the Faculty of Agriculture, for help in editing the manuscript.

GLOBAL FALL-OUT IN AUSTRALIA DURING 1960–61

DURING the years 1960 and 1961, continuous monthly sampling of fall-out over Australia for gross radioactivity was maintained by the Atomic Weapons Tests Safety Committee at eight stations: Perth, Adelaide, Melbourne, Hobart, Sydney, Brisbane, Townsville and Darwin. The Committee's network of 24 stations taking daily samples was closed down in April 1960, when no active samples had been detected for more than a year. In the past, this monitoring programme, and, especially, the daily sampling, provided information on tropospheric and early stratospheric fall-out over Australia from nuclear weapon tests held overseas. Accordingly, when the Soviet Union resumed testing of high-yield weapons in the atmosphere in September 1961, the daily sampling was recommenced using the 24-station network to give representative measurements for the regions inhabited by the majority of the Australian population.

The observations of gross radioactivity in fall-out are complementary to data obtained by the Committee in its extensive and continuous survey of strontium-90 in the Australian environment. Results from this survey to the end of 1960 have already been published in detail. A report by Drs. J. R. Blake, L. J. Dwyer, J. R. Moroney, D. J. Stevens and Prof. E. W. Titterton* presents the results of total β-activity measurements on the monthly samples taken from January 1960 until December 1961, and on the daily samples taken from September 15 until December 31, 1961, following the resumption of weapon testing by the U.S.S.R.

During the two years for which results are presented, the U.S.S.R. tested about 50 weapons in the atmosphere from September to November 1961; France tested three weapons in the atmosphere over the Sahara Desert in February, April and December 1960; late in September 1961, the United States commenced a new programme of underground testing at the Nevada test site.

The major weapon tests by the U.S.S.R. were carried out in the vicinity of Novaya Zemlya in the Arctic; the United States Atomic Energy Commission has estimated the total explosive yield for the series to be about 120 megatons and the fission yield at about 25 megatons. This corresponds to an injection of radioactivity into the atmosphere of the northern hemisphere in the order of twice that which resulted from the weapon tests conducted by the U.S.S.R. in the Arctic in October 1958; those tests produced, during 1959, marked increases in radioactivity in the biosphere of the northern, but not of the southern, hemisphere. France tested only low-yield nuclear weapons of estimated total yield considerably less than 1 megaton. No global fall-out has arisen from the underground weapon tests being carried out by the United States.

Results show that the level of total β-activity in fall-out over Australia continued to decrease during the period 1960–61. The data give no indication of the arrival of debris from the French tests during this period or the major U.S.S.R. series of September–November 1961. Some fall-out over Australia of long-lived fission products from these Soviet tests may be expected in the spring of 1962.

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* *Austral. J. Sci.*, **24**, No. 12 (June 1962).

NORWAY INSTITUTE OF ATOMIC ENERGY

AN illustrated survey of the work of the Institute for Atomenergi (IFA) at Kjeller and Halden, Norway, during 1961, together with details of the organization and an abstract of the accounts for the budget year 1961, are given in the recent report*. Mr. Gunnar Randers continued as managing director with Mr. O. R. Kåsa as assistant director and project manager of the Halden Project.

The main facility for physics research and isotope production is the 10-year-old 450-kW heavy-water reactor *Jeep* at Kjeller. The reactor, which is operated continuously, except for routine stops for isotope insertion and removal, and the semi-annual inspection of the fuel element canning, released 130·3 MWD of heat during 1961, bringing the total up to 680·5 MWD since the reactor commenced operation. At the summer inspection the aluminium canning of one fuel element showed several cracks. This was the first time that such damage had been detected. Various improvements in the reactor system and auxiliary equipment were made during the year. In order to compensate for the neutron loss due to the slightly thicker walls of the new reactor tank installed during 1960 and to improve neutron flux conditions, some fuel elements consisting of 1·7 per cent enriched uranium oxide are to be installed and the first of these, installed in November 1961, has already improved the flux and reactivity conditions.

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* Institutt for Atomenergi, Kjeller. Survey of Activities, 1961. Pp. 24. (Kjeller: Institutt for Atomenergi, 1962.)

The report states that one of the most significant events of the year under review was the commissioning during June, and the official inauguration on November 29, of the zero energy reactor *Nora* and of the fuel processing pilot plant. The reactor was established as a joint IFA–IAEA undertaking and was designed and built by NOR–ATOM *A/S*. The research programme has been approved by the *Nora* Committee under its chairman, Dr. R. Ramanna of India. For this programme three fuel charges are available, the first a 3 per cent enriched fuel charge provided by the U.S. Atomic Energy Commission, the second a reference charge of 2·5 tons of natural uranium metal, and the third a charge of elements from the Halden second core. The research programme is concentrated on the physical aspects of spectral shift controlled reactor systems.

In the spring of 1961 the design of the *Jeep II* reactor was reviewed in order to simplify the design and bring down costs; to introduce a flux trap; and to consider the use of the principle of spectral shift for long-term reactivity control. The reactor will be built and started as a heavy-water reactor and will be housed in a thermally insulated steel containment shell 22·4 m high and 22 m in diameter to withstand a pressure of 0·15 kg/cm^2. The erection of the shell and control building was started in the summer of 1961. The reactor will be moderated and cooled by heavy water and fuelled by slightly enriched UO_2 canned in aluminium tubes. *Jeep II* is planned to go critical during 1965, and it is estimated that the thermal neutron flux at a power-level of 2 MW in the centre of the reactor will be about $1 \cdot 5 \times 10^{13}$ neutrons/cm^{2}sec.

The experimental programme at the Halden reactor continued with the first fuel charge up to May, when the reactor was shut down prior to the insertion of the second, enriched core. During the second phase of the programme the reactor will be operated at 20 MW instead of 6 MW at 155° C. The programme so far includes the determination of data on various reactor physics parameters, such as temperature and water-level coefficients, and the critical buckling as a function of moderator temperature has been calculated. Modifications, partly based on experience gained during the operation of the reactor, which will be introduced for operation at the higher power, include the installation of a new steam transformer to be operated in parallel with the original one, and a tertiary loop with a new steam generator for dissipation of the steam produced.

During 1961 two new wings of the Netherland–Norwegian Reactor School were taken into use, thus increasing the capacity of the school considerably by providing facilities for experimental work in reactor engineering, physics and radiochemistry. The nine weeks standard course in fundamentals of nuclear engineering was held twice, and during March a course on analogue computer techniques and their application to nuclear engineering and chemical processing problems was held for the staff at Kjeller and Halden.

The report concludes with details of the safety work and health physics; the technical information service including a list of Kjeller Reports and Halden Project Reports issued during the year; international co-operation; and the numbers and distribution of the staff at the Institute on December 31, 1961.

PUBLICATIONS OF THE NATIONAL PHYSICAL LABORATORY, TEDDINGTON

FOUR new booklets in the series *Notes on Applied Science* of the National Physical Laboratory, Teddington, have recently been published*. The first, No. 25 in the series, entitled *Hydrometers and Hydrometry*, deals with basic considerations of air buoyancy, surface tension and the spacing of the scale; the general use of hydrometers with examples; the verification and adjustment of hydrometers with particular reference to practice at the National Physical Laboratory; and types of hydrometer scale and the relationship between scales. It is emphasized that ordinary hydrometers of constant mass are convenient instruments for the measurement of the density and specific gravity of liquids, and that greater accuracy can be obtained by their use than is generally realized. Though *British Standard* 718: 1960 contains considerable information on hydrometers and hydrometry, several interesting points outside the scope of the *Standard* are dealt with in the booklet.

The information given in No. 27, *Inspection of Gauging Dimensions Involving Linear and Angular Measurements*, is intended to assist engineering inspectors in checking gauges, jigs and workpieces, involving combined measurements of length and angle. The use of the Universal Measuring Block manufactured by the Pitter Gauge and Tool Co. Ltd. is described, and full particulars of the measuring equipment associated with it are given together with the types of measurement which can be made with the equipment. A principal purpose of the booklet is to present a general procedure for the type of measurement in which the required dimension must be obtained by calculation from indirect measurements of the work. Examples of both the simpler direct measurement technique and of the indirect measurement procedure are given and fully explained. The derivation of the associated trigonometrical formulæ required in the calculations are set out in an appendix.

Measurements of frequency with an accuracy approaching one part in 10^{10} are now required not only for precision laboratory measurements but also for routine operations. The techniques which make such measurements possible are briefly discussed in No. 28 of the series, entitled *The Measurement of Frequency*, where an account is given also of the more usual types of frequency meter utilizing a resonant circuit. The standard of frequency at the National Physical Laboratory is a cæsium atomic resonator and this is used to calibrate the quartz clock working standards. These in turn monitor the carrier frequencies of a number of transmitting stations, with the call sign *MSF*, which are operated from the Laboratory from the British Post Office Station at Rugby. Details of these transmissions and of the more powerful carrier wave of the station *GBR*, which is controlled by the *MSF* standard, are given in the booklet. Separate sections deal with frequency synthesis and measurement and with cavity-resonator frequency meters and their testing and calibration. These meters are most useful in a wide field of applications where an accuracy of about one part in 10^4 is adequate.

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* Department of Scientific and Industrial Research. National Physical Laboratory. *Notes on Applied Science*.

No. 25: *Hydrometers and Hydrometry*. By J. B. Rands and P. H. Bigg. Pp. iv+31+1 plate. 2*s*. 6*d*. net.

No. 27: *Inspection of Gauging Dimensions Involving Linear and Angular Measurements*. By Dr. J. C. Evans and C. O. Taylerson. Pp. v+42. 3*s*. 6*d*. net.

No. 28: *The Measurement of Frequency*. By Dr. L. Essen, E. G. Hope and K. Morris. Pp. v+17. 1*s*. 9*d*. net.

No. 29: *Physical Photometry*. By Dr. B. H. Crawford. Pp. iv+16. 1*s*. 9*d*. net.

(London: H.M.S.O., 1962.)

The fourth booklet, No. 29, *Physical Photometry* by Dr. B. H. Crawford, describes those procedures in photoelectric photometry which have their worth in precise measurement and which are in regular use at the National Physical Laboratory. Photometry is essentially the measurement of radiant energy by a selective receiver, the human eye, but individual eyes vary and it is assumed that there is a statistically satisfactory 'standard eye'. A value for the photopic (light-adapted) standard observer was accepted in 1933 by the International Committee on Weights and Measures and for the scotopic (dark-adapted) by the Commission International de l'Éclairage in 1951. These values are tabulated in the booklet. After a general examination of the photoreceptors in present-day use, the spectral correction of vacuum emission photocells and of radiation thermopiles and bolometers is discussed in some detail. Finally, there are two sections dealing with amplifiers and photometers.

S. Weintroub

PETROLEUM CHEMISTRY: U.S.S.R.

THE past decade has witnessed a spectacular commercial development of the petroleum industry in the manufacture and marketing of petroleum-derived organic and inorganic chemicals in Great Britain. Julian M. Leonard, in a recent presidential address to the Institute of Petroleum (*J. Inst. Pet.*, **48**, 271, 1962), quotes figures showing that the share of petroleum as raw material for the chemical industry has increased from about 9 per cent in 1949 to 47 per cent in 1959, at present running at more than 50 per cent and anticipated to reach 65 per cent when 1962 figures are available. In the United States the production of petrochemicals was a much earlier development than in the United Kingdom and it is estimated that to-day some 80 per cent of organic chemicals manufactured there are based on petroleum. It is doubtful whether the petroleum technologist, not directly concerned with modern refinery flow-sheets, is himself aware of the phenomenal expansion in this new field and of its technical and economic significance in the future of the world's chemical industry. Certainly the layman is even less informed on this subject, probably because it is not one that can be easily propagated in the popular Press.

The starting point of petrochemicals, as Leonard stated, is the complex of naturally and artificially produced petroleum gases from which are synthesized ethylene, propylene, butadiene, benzene, toluene, xylene, etc. Some of the more important products stemming from these foundations are synthetic rubber, synthetic fibres, detergents, thermoplastics, and agricultural insecticides and fertilizers, many of these replacing materials formerly derived from purely natural, mainly vegetable resources. The possibility of producing edible oils and fats from petroleum, although not yet matured on a large scale, is none the less practicable and in itself opens up another highly specialized prospect in the chemical industry.

In the academic and technical worlds concerned with petrochemicals there has naturally evolved an extensive literature during the course of development of this fascinating subject, especially in the United States and the United Kingdom. Western European countries have also contributed their share. In these spheres language is no bar to the past or present records of research, discovery or progress. But the same cannot be said of published work in the U.S.S.R., which despite its magnitude and value, must necessarily remain a 'closed book' to all but those chemists who can read the Russian publications *per se*. The increasing amount of research in the U.S.S.R. on the nature and configuration of petroleum hydrocarbons, composition of sulphur and nitrogen-bearing compounds, and on conversion processes used in the manufacture of petrochemicals, is of such a volume that it has led to the launching in the U.S.S.R. of a new periodical *Neftekhimiya—Petroleum Chemistry*, which aims at co-ordinating the work of "investigators on the chemistry of petroleum and on the petroleum chemicals industry". This is the quote from the foreword to the English version of *Petroleum Chemistry: U.S.S.R.*, a quarterly published by Pergamon Press for Pergamon Institute, Oxford, of which the first volume and number* has just appeared in the United Kingdom, under the editorship of D. L. Samuel with translator B. J. Hazzard. The former writes: "Such work is of great interest to all workers in similar or related fields and they will doubtless be well pleased with the opportunities of reading of the work of their Russian colleagues, made possible by the issue of an English edition. The papers of greatest interest to western research workers are translated in full and an abstract provided of those of lesser appeal".

The first volume of this English publication contains translations of twenty-five original papers by Russian scientists of considerable academic and technical importance in the modern realm of petroleum chemistry in general and petrochemicals in particular. Abstracts of other relevant papers in *Neftekhimiya*, running to a total of thirty-eight (of which the publishers are prepared to supply full English translations), conclude what, in future numbers, is obviously destined to become a most valuable medium of international exchange of knowledge of petroleum chemistry conceived in the fullest sense of its modern terms of reference.

H. B. Milner

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* *Petroleum Chemistry: U.S.S.R.*, 1, No. 1; 1962. Published quarterly. Translation editor, D. L. Samuel. Pp. 1–218. (London and New York: Pergamon Press, 1962.) Annual subscription-rate £30 (85 dollars).

THE ROWETT RESEARCH INSTITUTE

IN these days when research tends to be so highly specialized, it is obviously difficult for the director of a research institute to write a report which will be interesting and understandable throughout, even to readers reasonably conversant with the general trend of the institute's work. This is particularly so where the institute is a large one containing a number of departments each applying the techniques and using the jargon of a different science. The difficulty is not decreased when the approach to the practical objects of the institute is in large measure through basic research on the underlying scientific problems.

A report appealing to the non-specialist, and understandable throughout by a well-informed reader, would require to discuss each topic with some fullness and to include some account of previous work on the subject. This would mean, especially for a large institute, an annual report of considerable bulk and cost, and from year to year these would be largely repetitive as the work summarized one year would have to be repeated the next.

In an endeavour to solve this problem, recent annual reports of the Rowett Research Institute have each contained two main sections. Summaries have been given of all the papers published during the period under review. These summaries have been arranged under appropriate headings, and full references given to the original publications. This enables any specialist to discover new work which may interest him. The other main section has consisted of a couple of reviews, each by some member of the staff of the Institute, of a topic on which he is an acknowledged authority. Each review naturally deals at some length with work carried out at the Institute, but has not been limited to that work and has presented a critical survey of the whole subject in the light of present knowledge. Consequently, over the past few years, a number of valuable reviews has appeared within these annual reports.

The current annual report of the Rowett Research Institute, which covers the year 1960–61, again contains these two main sections*. The review articles this year are on "Calorimetry, 1952–1961" by J. D. Pullar, and on "Epithelial Form and Function in the Intestine" by B. F. Fell. These well-written articles will be very useful to anyone wishing to follow recent advances in these subjects.

In the past, one drawback to the form of report prepared by the Rowett Research Institute, based on summaries of published papers, has been that work appears in these reports only a considerable time after it has been carried out. In the present report an effort has been made to meet this difficulty by extending the summaries so as to give some account, not only of published papers, but also of others 'in the press' or in preparation. Though this perhaps does not completely solve the problem, it does succeed in bringing the report more up to date and of increasing its interest.

The research work described in this report extends from basic research, such as that on the use of hydrogen-bond-breaking extraction solvents and fractionation procedures for the characterization of non-protein forms of bound amino-acids in leaves and seeds and the determination of their metabolic significance, to the demonstration that it is possible to fatten beef cattle on a diet of 85 per cent bruised barley and 15 per cent of a protein-rich-mineral-vitamin supplement containing sodium bicarbonate and no roughage. As an example of work of great interest from both the theoretical and practical points of view, reference may be made to the elucidation of the relationship between deficiency of copper in lambs and 'swayback'. It had already been shown that deficiency in copper accompanied by ataxia could be produced in lambs by feeding molybdenum and sulphate supplements to their ewes. The affected lambs showed demyelination in the spinal cord motor tracts and other lesions of the central nervous system. It has now been found that cytochrome oxidase, which in all probability contains copper as an essential constituent, is deficient in the brains of lambs suffering from 'swayback'. This reduced activity of the enzyme is associated with copper-levels in brain tissue of less than three parts per million. Animals with abnormally low contents of copper in the liver may or may not suffer from 'swayback', but in the non-affected animals with abnormally low copper in the liver it was found that the brain level was invariably more than the critical three parts per million. It is suggested that cytochrome oxidase assay may provide a rapid method for confirming suspected cases of 'swayback'. This investigation into copper, cytochrome oxidase and 'swayback' is but one example of the many items which a reader browsing through this report will come across to stimulate his interest and curiosity.

W. O. KERMACK

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* The Rowett Research Institute. Annual Report on Animal Nutrition and Allied Sciences, Vol. 18, 1962. Pp. 80. (Bucksburn, Aberdeen: The Rowett Research Institute, 1962.) 10s.; 1.40 dollars.

FRAGMENTATION OF METEOR BODIES

By DR. B. LEVIN

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ACCORDING to the conventional physical theory of non-fragmenting meteors, if to neglect deceleration, the form of the light curve $m = m(H)$ does not depend on the initial mass M_0 and velocity v_0 of the meteor body and on the zenith distance z of its radiant, but only on parameter μ, which characterizes the relation between the changes of the frontal cross-sectional area S and mass M in the course of the ablation of the meteor body:

$$S \sim M^{\mu} \qquad (1)$$

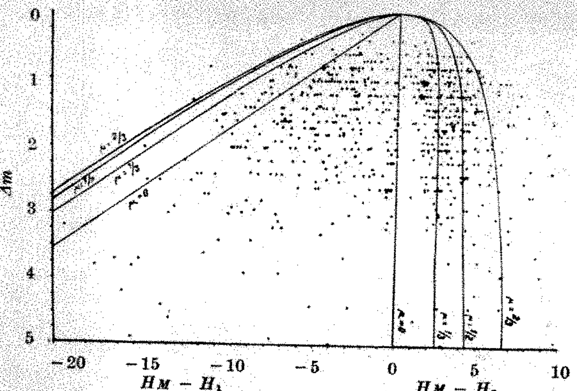

Fig. 1. Comparison of photometric data for photographic meteors with theoretical light curves for different values of parameter μ

Denoting I and m the luminous intensity and magnitude of a meteor on the height H at which the air density $\rho = \rho_0 e^{-H/H^*}$ and marking with the subscript 'M' the values of these quantities in the point of maximum light we have (ref. 1):

$$\frac{I}{I_M} = \frac{\mathrm{d}M/\mathrm{d}t}{(\mathrm{d}M/\mathrm{d}t)_M} = \mu^{-\mu/1-\mu} \frac{\rho}{\rho_M}\left[1 - (1-\mu)\frac{\rho}{\rho_M}\right]^{\mu/1-\mu} = e^{\Delta H/H^*}\left[\frac{1}{\mu} - \left(\frac{1}{\mu} - 1\right)e^{\Delta H/H^*}\right]^{\mu/1-\mu}$$

(where $\Delta H = H_M - H$). Or:

$$\Delta m = m - m_M = 1{\cdot}09\left\{\frac{\Delta H}{H^*} + \frac{\mu}{1-\mu}\ln\left[\frac{1}{\mu} - \left(\frac{1}{\mu} - 1\right)e^{\Delta H/H^*}\right]\right\} \qquad (2)$$

Other authors have considered only the case $\mu = 2/3$, which corresponds, in particular, to a spherical body which does not change its form during ablation. Really on the average it is $\mu < 2/3$. The corresponding light curves lie inside the curve for $\mu = 2/3$ (Fig. 1).

Hawkins and Southworth[2], in their analyses of 360 meteors photographed by Super-Schmidt cameras, showed that almost all points of appearance and disappearance of meteors lie within the theoretical curve for $\mu = 2/3$, that is, the observed length of meteor trails is shorter than the theoretical one. Ananthakrishnan[3] removed the systematic deviation for the points of disappearance calculating the theoretical curve, adopting an *ad hoc* hypothesis that the luminosity factor $\tau \sim \rho v$ (instead of the conventional $\tau \sim v$). But this systematical deviation can be removed on the basis of the conventional theory if we take a light curve for $\mu \approx 1/2$ (Fig. 1). The systematic deviations for the points of appearance remain because they are due to fragmentation of meteor bodies.

The conventional physical theory of meteors, when $\tau \sim v$, gives the following expression for maximum luminous intensity:

$$I_M \sim M_0 v_0^4 \cos z \qquad (3)$$

By means of this formula the absolute maximum photographic magnitudes m_M of meteors given by Hawkins and Southworth[2] were reduced to m_M^*, corresponding to $M_0 = 1$ g, $v_0 = 10$ km/sec, $\cos z = 1$ (the data on the masses of meteor bodies were kindly sent us by Hawkins and Southworth prior to publication. These masses were obtained by the photometric method and expressed in the Opik-Jacchia scale). For 358 meteors (for 2 meteors the masses are lacking):

$$\overline{m_M^*} = +\,0^m{\cdot}66.$$

The mean square deviation is great and amounts to $\pm\,0^m{\cdot}62$.

When the fragmentation decreases the path-length, L, of the meteor its light energy must be radiated on a shorter path. The increase of the mean light intensity is directly proportional to the decrease of the path-length. If to approximate the light curve by triangle

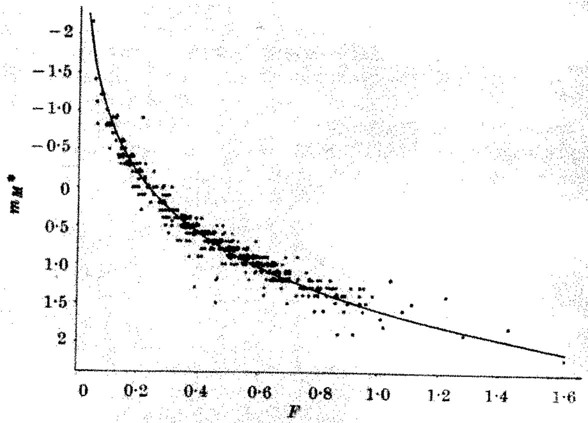

Fig. 2. Reduced maximal magnitudes of meteors versus fragmentation factor F

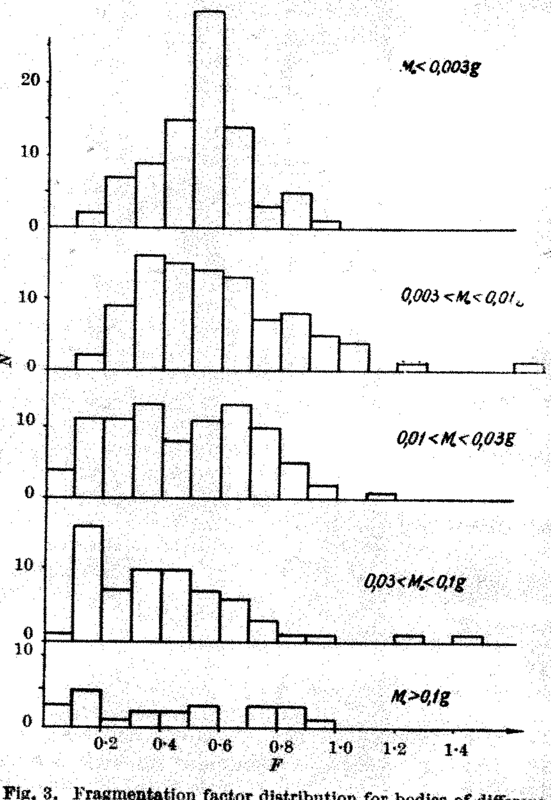

Fig. 3. Fragmentation factor distribution for bodies of different mass

with a height I_M and a base L the same becomes true also for a maximum intensity I_M.

Let us introduce a fragmentation parameter, F, which is defined for each meteor according to the equation:

$$F = \frac{L_{\text{obs}}}{L_{\text{theor}}} = \frac{(H_1 - H_2)_{\text{obs}}}{(H_1 - H_2)_{\text{theor}}} \qquad (4)$$

$(H_1 - H_2)_{\text{theor}}$ is obtained, for the Δm value observed for a given meteor, from the theoretical light curve for $\mu = 1/2$. Only few meteors have $F \approx 1$, but for more than 90 per cent the length of a trail is markedly shortened by fragmentation.

Fig. 2 gives a graph of the dependence of $m_M{}^*$ on F. All points lie in a narrow band along the curve which corresponds to the equation:

$$m_M{}^* = 1{\cdot}60 + 2{\cdot}5 \log F \qquad (5)$$

The mean square deviation from this curve is only $\pm 0^{\text{m}}16$ or about 4 times smaller than without allowance for fragmentation and even smaller than the error of measurements of m_M which is $\pm\ 0^{\text{m}}3$ according to Hawkins and Southworth. The excellent representation of the observations by equation (5) is somewhat unexpected because $(H_1 - H_2)_{\text{theor}}$ should be obtained not for the observed Δm but for its unknown theoretical value.

Equation (5) means that for the meteors produced by fragmenting meteor bodies equation (3) must be changed into:

$$I_m = \frac{M_0 v_0{}^4 \cos z}{F}$$

If to adopt the same correction $+\ 1^{\text{m}}8$ to transfer photographic magnitudes into visual, as for brighter meteors, it follows from equation (5) that a non-fragmenting meteor body which produces a meteor of zero visual magnitude during vertical motion with $v_0 = 40$ km/sec must have an initial mass $M_0 = 0{\cdot}09$ g and when a fragmentation is intense and $F = 0{\cdot}1$ a ten times smaller mass, $M_0 = 0{\cdot}009$ g, is sufficient. To a mean value $m_M{}^* = 0^{\text{m}}66$ corresponds $M_0 = 0{\cdot}04$ g which is close to $M_0 = 0{\cdot}055$ g obtained earlier[1] from the analysis of 117 meteors photographed by conventional cameras.

For meteor bodies of different masses their distribution according to F is found to be different (Fig. 3). For massive bodies a maximum lies in the region of $F \approx 0{\cdot}1 \div 0{\cdot}2$, and for small bodies it is in the region $F \approx 1/2$. The evidence that the big bodies produce more fragments than the small ones was found earlier for Jiacobinid meteors[1]. The dependence of fragmentation on the mass of meteor bodies influences the magnitude distribution of meteors. Even for shower meteors one cannot suppose that the magnitude distribution of meteors depends only on the mass distribution of meteor bodies. All previous determinations of the mass distribution based on this supposition must be revised. The results based on radio-echo observations also need revision.

[1] Levin, B. Yu., *Physical Theory of Meteors and Meteoric Matter in the Solar System* (Moscow, 1956).

[2] Hawkins, G. S., and Southworth, R. B., *Smithsonian Contrib. Astrophys.*, **2**, 349 (1958).

[3] Ananthakrishnan, R., *Nature*, **187**, 675 (1960); **190**, 896 (1961).

MICROSCOPIC EXAMINATION OF HIGHLY RADIOACTIVE FALL-OUT PARTICLES

By T. MAMURO, A. FUJITA, T. MATSUNAMI and K. YOSHIKAWA

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IT is well known that highly radioactive fall-out particles were found throughout Japan, resulting from the series of large-scale nuclear test explosions carried out by the U.S.S.R. during September and October 1961[1-5]. Towards the beginning of November 1961, when abnormally high concentrations of radioactivity in the atmosphere were observed at our Laboratory, we were able to find and collect many highly radioactive particles with the aid of a Geiger–Müller survey meter in various places, for example, on the roof, on the rain collector, on the surface of leaves, on polyethylene bottles or polyvinyl chloride sheets which were left outdoors. Since the rainfall on November 17, it became very difficult to find any of these particles where rain-water had washed away dusts; however, we were able to continue to find such particles on dust films formed inside buildings into which the wind blew freely. Especially were we able to collect many particles in the oil tank of a multipanel air-filter which is set by the air-feeding apparatus of a building in our Laboratory.

Highly radioactive particles were isolated from among non-radioactive dust particles after elaborate procedures. They were immersed into a very small drop of cedar oil and photomicrographs of them were taken. Figs. 1–21 are examples of these photographs. Fig. 22 is a photograph of the scale, one division of which is 10μ. Photographs designated by P were taken focusing at the perimeter of particles, showing the shape of the particle contour clearly, but the front side only vaguely. Photographs designated by F and M were taken in order to show the change in depth of the surface state of particles, focusing on the front side of particles and on the middle part between the front side and the perimeter, respectively.

We observed microscopically more than fifty highly radioactive particles. Most of them were quite, or nearly, spherical. There were, however, a few particles which deviated appreciably from the spherical (Figs. 11 and 14). The surface of some particles was irregular and creased. Several particles were coated with colourless amorphous materials like frosting (Figs. 8 and 9). These materials were assumed to be non-radioactive because they had the same appearance as that of non-radioactive air-borne dust particles.

Almost all the particles were yellowish or reddish brown; however, there were some colourless, golden or black particles. Isolation procedure was more difficult for colourless particles than for those which were coloured, because the recognition of a colourless particle under a microscope was more difficult.

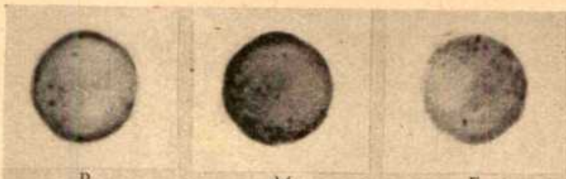

P M F

Fig. 1

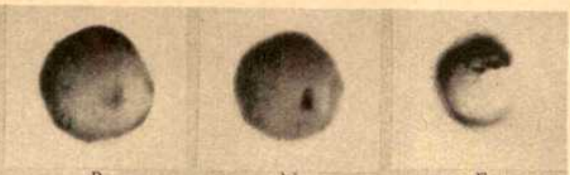

P M F

Fig. 2

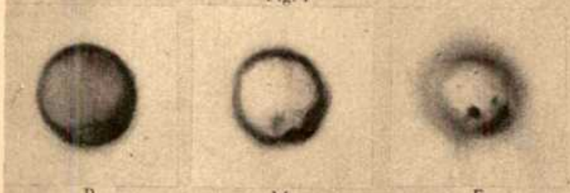

P M F

Fig. 3

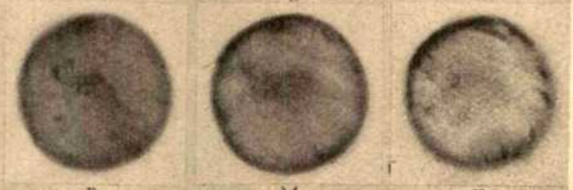

P M F

Fig. 4

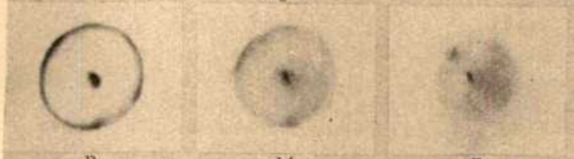

P M F

Fig. 5

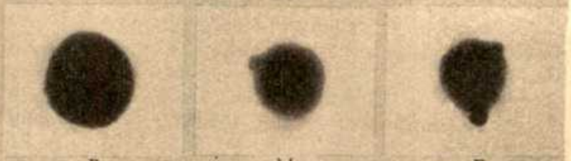

P M F

Fig. 6

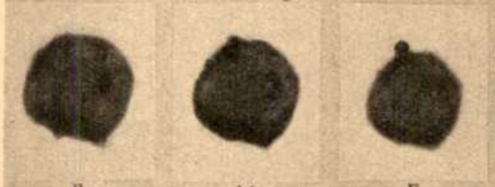

P M F

Fig. 7

P M F

Fig. 8

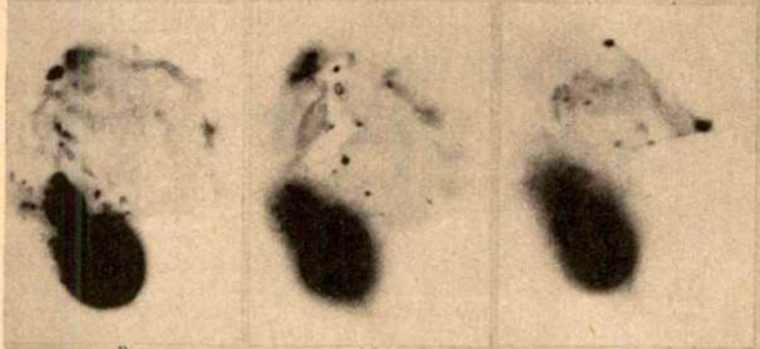

P M F

Fig. 9

Fig. 10 (P)

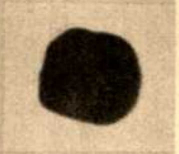
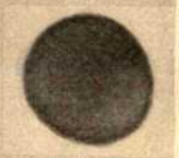


Fig. 11 (P)

Fig. 12 (P)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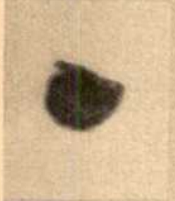
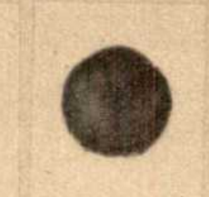


Fig. 14 (P)

Fig. 15 (P)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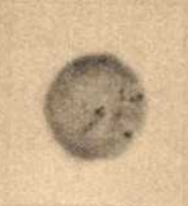
Fig. 17 (P)

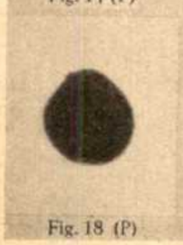
Fig. 18 (P)

Fig. 19 (P)

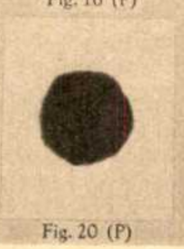
Fig. 20 (P)

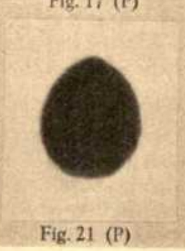
Fig. 21 (P)

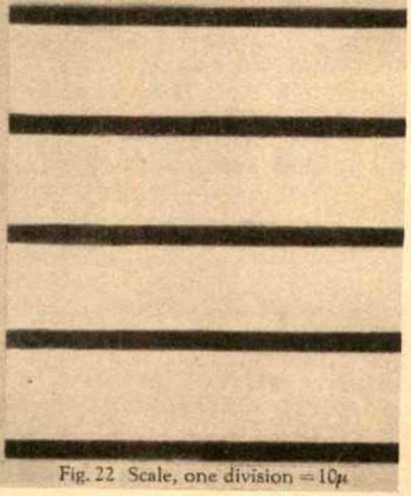
Fig. 22 Scale, one division = 10μ

Table 1. CHARACTERISTICS OF HIGHLY RADIOACTIVE PARTICLES

Fig. No.	Particle diameter (μ)	Colour	Shape	β-Activity at the end of June 1962 (mμc.)	Estimated β-activity at the beginning of November 1961 (mμc.)	Sampling place
1	10·1	Golden	Spherical	1·1	24	Air-filter
2	10·4	Yellowish brown	Nearly spherical	1·4	25	Air-filter
3	8·7	Golden	Spherical	0·87	16	Air-filter
4	13·9	Golden	Spherical	4·8	87	Air-filter
5	9·8	Colourless	Spherical	1·0	18	Air-filter
6	9·0	Reddish brown	Nearly spherical	0·73	13	Air-filter
7	10·1	Reddish brown	Nearly spherical	1·2	22	Air-filter
8	11·8	Black	Spherical, coated with amorphous materials	1·1	20	Indoors
9	8·4	Reddish brown	Spherical, coated with amophous materials	0·35	6·3	Indoors
10	11·3	Black	Spherical	1·3	23	Air-filter
11	9·0	Reddish brown	Appreciably deviated from sphere	0·77	14	Air-filter
12	9·2	Reddish brown	Ellipsoidal	0·91	16	Air-filter
13	12·1	Yellowish brown	Spherical	0·97	17	Air-filter
14	6·9	Reddish brown	Like a beam	0·32	5·8	Air-filter
15	10·1	Yellowish brown	Ellipsoidal	0·88	16	Air-filter
16	10·4	Reddish brown	Spherical	1·3	24	Air-filter
17	10·1	Colourless	Spherical	1·8	32	Air-filter
18	8·1	Reddish brown	Nearly spherical	0·68	12	Air-filter
19	8·7	Dark brown	Ellipsoidal	0·46	8·3	Air-filter
20	8·7	Reddish brown	Spherical	0·55	10	Air-filter
21	10·4	Black	Oval	0·61	11	Air-filter

It has been reported that division of radioactivity seems to happen during the handling of fall-out particles by a needle[2,6]. We experienced such a phenomenon twice. The larger radioactivity was separated and confirmed to be due to a spherical radioactive particle; the smaller radioactivity, on the other hand, was divided further into two or more smaller radioactivities which gave so low counting-rates that they could not be separated. The photographs as shown in Figs. 6–9 suggest that such division of radioactivity was caused by the separation of two or more closely adjacent radioactive particles by the impact of a needle. All the highly radioactive particles that we could isolate and observe microscopically seemed to be so hard that even the impact of a needle could not crack them into two or more particles.

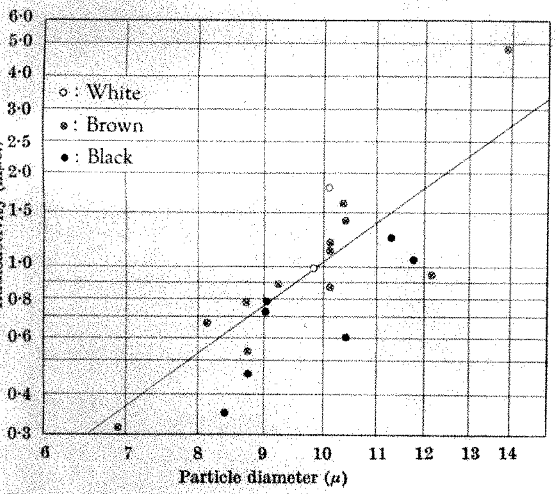

Fig. 23. Relation between diameter and β-activity of the particles

Table 1 presents a summary of the results of observation on twenty-one highly radioactive particles the photomicrographs of which are given in Figs. 1–21. Fig. 23 shows the relation between diameter and β-activity of the particles. A straight line of gradient 3 is drawn for comparison. Activity is roughly proportional to volume, as expected[7]. It seems that particles of light colour have larger specific activity than those of dense colour and that the conditions of formation differed from one to another.

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PREFERENTIAL ADSORPTION OF NORMAL HYDROCARBONS ON CAST IRON

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IN the course of work on the determination of heats of preferential adsorption of surface active compounds and hydrocarbons on metal surfaces, it was noticed that normal hydrocarbons gave exceptionally high heats of adsorption on an iron powder containing 1·1 per cent carbon. Further work revealed that even larger heats of adsorption are obtained for a cast iron containing 3·5 per cent carbon.

The work was carried out in a flow calorimeter described previously[1]. The procedure used was to inject different organic substances into a carrier liquid (n-heptane) flowing through a bed of cast iron powder. The injected substances come into contact

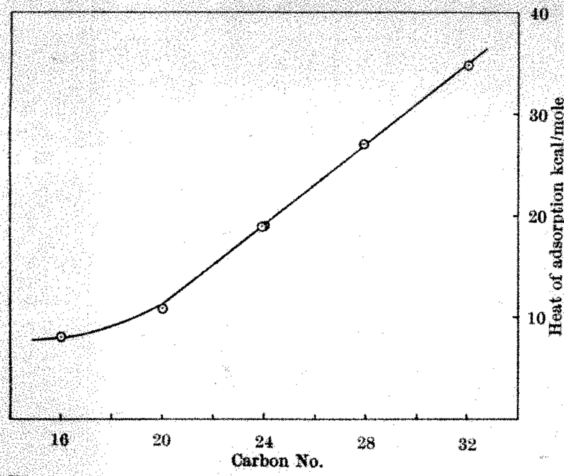

Fig. 1. Heat of adsorption of normal hydrocarbons from n-heptane on iron at 25° C

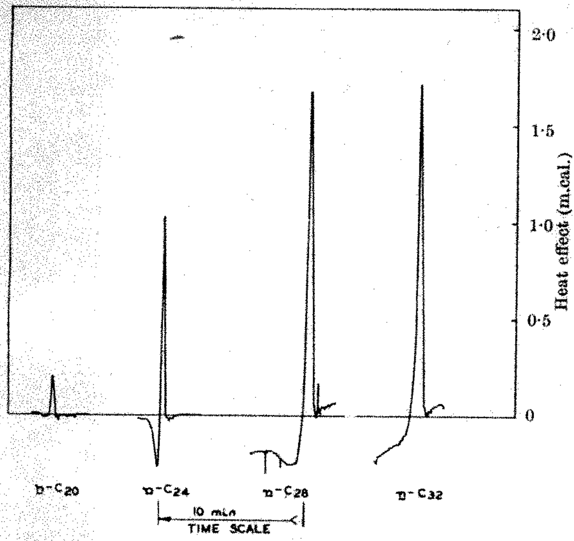

Fig. 2. Adsorption of normal hydrocarbons on iron. 0·05 mg quantities of the hydrocarbons injected into n-heptane carrier

with the powder and are adsorbed from the carrier liquid. Equilibrium conditions are not attained because the carrier desorbs the adsorbed solute after the solution has passed through the iron. The adsorption process is rapid (10–15 sec) and is accompanied by a positive heat effect. Desorption takes place more slowly (normally 1–15 min) with a corresponding negative heat effect. The heat effects are reproduced for calibration purposes by an electrical coil immersed in the iron powder with the carrier flowing through it. The flow-rate of the carrier was fixed at 0·4 ml./min.

Adsorption of normal hydrocarbons and mineral oil fractions on cast iron was also studied by replacing n-heptane as the carrier liquid by 1 per cent weight solution of the compounds dissolved in n-heptane. The solute is then adsorbed until an equilibrium is reached and the heat effect produced corresponds to the adsorption of the equilibrium amount of solute. The amounts of the solutes adsorbed were found by determining the decrease in their concentration when 0·5 ml. of the solutions were placed in contact with 2 g of the iron powder. The determinations were carried out by using the flow calorimeter as a detector for the solutions injected into the carrier liquid. The calorimeter was calibrated by injecting known amounts of the solute into the carrier. A similar procedure described previously[1] was used to estimate the proportion of solute adsorbed from solution when it was injected into the carrier liquid.

Results obtained for different normal hydrocarbons are given in Figs. 1 and 2. The results in Fig. 1 are for the adsorption on the iron powder with a carbon content of 1·1 per cent. In each experiment the iron was saturated with a 1 per cent solution of a normal hydrocarbon. It can be seen that the heat evolved during the adsorption procedures increases linearly with the carbon number of the hydrocarbons between C_{20} and C_{32}. The order of the heat effects is comparable with the heats of crystallization of the normal hydrocarbons. The experiments with branched hydrocarbons, such as squalane, gave very small heat effects (1–2 per cent of the effects produced by n-C_{24} to n-C_{32}). Therefore the iron powder adsorbed the normal hydrocarbons selectively. In Fig. 2, the peaks represent heat effects produced when 0·05 mg quantities of the normal hydrocarbons were injected (in the form of 1 per cent solutions in n-heptane) into the carrier. Only a part of the injected hydrocarbons was adsorbed. The heats of adsorption calculated from these peaks are therefore lower than the heats calculated from the results of the experiments in which the iron was saturated with the hydrocarbon solutions.

The heat evolved on adsorption of n-dotriacontane on iron containing 1·1 per cent carbon is represented in Fig. 3. Before the adsorption of n-dotriacontane on the iron had begun, 0·1 mg of n-hexadecanol was injected into the carrier as an internal standard. The heat evolved when the alcohol was adsorbed on the clean iron was 3·5 millicalories. After desorption of the alcohol the iron was saturated with a 1 per cent solution of n-dotriacontane. Injection of 0·1 mg of the alcohol into the solution produced a very small heat effect. Evidently the adsorbed hydrocarbon formed a layer on the iron surface, which prevented to a large extent the adsorption of the alcohol. Afterwards, the adsorbed dotriacontane was desorbed with n-heptane and a corresponding negative heat effect recorded. The desorption process was relatively very slow. After 50 min of passing n-heptane through the iron powder, 0·1 mg of the alcohol

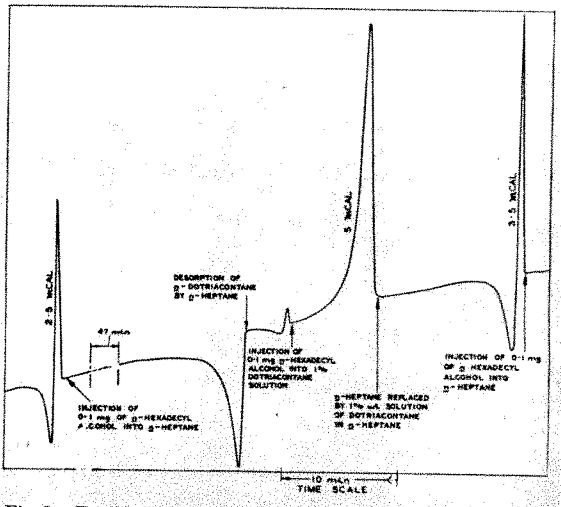

Fig. 3. Equilibrium adsorption and desorption of n-dotriacontane on iron

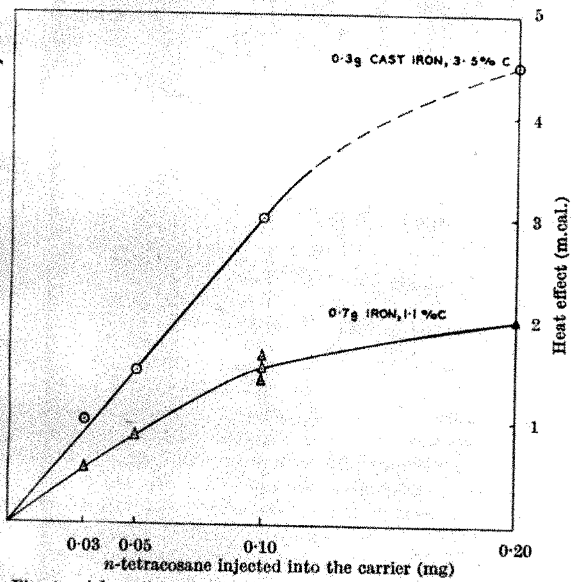

Fig. 4. Adsorption of tetracosane on iron powders with different carbon contents

was injected into the carrier and the heat evolved was only about 70 per cent of the heat produced when the alcohol was adsorbed on the clean iron. When the same adsorption-desorption cycle was carried out with *n*-tetracosane, the hydrocarbon became almost completely desorbed after about 5 min and then the injection of the alcohol gave the same heat effect as that produced before the adsorption run was begun.

Thus the rate of desorption of *n*-dotriacontane from the iron powder is considerably slower than that of *n*-tetracosane. The same conclusion can be reached by examining the peaks reproduced in Fig. 2, where the negative heat of desorption is much sharper for n-C_{24} than the heats for n-C_{28} and n-C_{32}. For *n*-eicosane the desorption is so fast that it overlaps to a considerable extent with the positive heat of adsorption and thus a very small peak is recorded. The same applies also to *n*-hexadecane.

In Fig. 4, the heat effects produced on injection of *n*-tetracosane into *n*-heptane and its subsequent adsorption on to iron with 1·1 per cent carbon content are compared with the heat effects produced by the adsorption on a cast iron with a carbon content of 3·5 per cent. Similar injections on an iron powder with a very low carbon content produced negligible heat effects. It appears therefore that the preferential adsorption of normal hydrocarbons on cast iron is connected with its carbon content. It is possible that the effects observed are chiefly adsorptions on carbon similar to those reported by Breshchenko[2] and by me[3].

I found recently that many fractions of lubricating oils are strongly adsorbed on cast iron, and that this is connected with the presence in the oils of molecules with long normal hydrocarbon chains. This observation has an obvious relevance to the mechanism of lubrication of cast iron by mineral oils.

I thank the Chairman and Directors of the British Petroleum Co. for permission to publish this article.

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ROLE OF OXYGEN IN THE PRIMARY FORMATION OF H AND H_2O^- IN THE RADIATION CHEMISTRY OF WATER

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A GENERAL phenomenon[1-9] of the radiation chemistry of air-free aqueous solutions of organic compounds is the dependence of the yields of hydrogen on solute concentration and *p*H. It is now fairly well established that $G(H_2)$ decreases with increase (above $\sim 10^{-2} - 10^{-1}$ M) in solute concentration and with decrease in the hydrogen ion concentration. An interpretation[2-5] has been provided to account for the effect of solute concentration and *p*H. More recently, attention has been focused on the observation that the yields of hydrogen in neutral air-free solutions of organic solutes are well above the 'molecular' yield of H_2, $GH_2 = 0{\cdot}45$ for γ-rays. The actual yield was found to be dependent on the particular solute used and could be as high as 2·5–3·0. H_2O^-, the alkaline form of hydrogen atom formed by the radiation[2,3,10], cannot dehydrogenate an organic solute (RH_2), but in the absence of electron-acceptors it dissociates slowly to give an H atom ($H_2O^- \rightarrow H + OH^-$), which can then dehydrogenate. In the presence of quantities of electron scavengers, which could be the solute itself[3], initial impurities in the water[8], products from the radiation[6], or added second electron-accepting solutes[6,8,9], $G(H_2)$ reaches a low limiting value of $1{\cdot}00 \pm 0{\cdot}05$.

Allan and Scholes[6] have suggested that the yield of H_2 above 'molecular' results from reducing radicals generated initially in the acid form, H atoms:

$$H_2O^* \rightarrow H + OH \qquad (1)$$

followed by $H + RH_2 \rightarrow H_2 + RH$. Hence $G(1) \sim 0{\cdot}55$ or ~ 20 per cent of the total yield of reducing species in neutral water. Recent[10] kinetic evidence shows that in the radiolysis of water molecular hydrogen is formed by two mechanisms. The main portion results from recombination of H_2O^- and about 22 per cent of the total H_2 via recombination of H atoms generated initially in the 'spurs' of the radiation.

The formation of H atoms directly from water and their presence in the bulk of the solution was recently questioned[3,11]. The reasoning was based on the results of the relatively simple and extensively studied radiolysis of oxygenated aqueous solutions of hydrogen peroxide. Barr and Allen[12] had indicated from their work that the basic and acid forms of H behave very differently in their competition between oxygen and hydrogen peroxide, and later Czapski and Allen[11] showed on the basis of such a competition that all the reducing species (100 ± 5 per cent) must be present in their basic form, H_2O^-.

The conclusions from the O_2–H_2O_2 and the organic systems are each quite valid and correct in themselves. It is suggested that the apparent difference is due to the presence of oxygen. Oxygen affects (see following) the precursor of the directly produced H atom such that H_2O^- is formed quantitatively instead of H. This suggestion is substantiated as follows:

(*a*) In the radiolysis of air-free neutral H_2O_2 solutions, on the basis of reactions (2)–(4), $G(-H_2O_2) = 4\cdot70$ and

$$H_2O^- + H_2O_2 \rightarrow OH + H_2O + OH^- \tag{2}$$

$$OH + H_2O_2 \rightarrow HO_2 + H_2O \tag{3}$$

$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \tag{4}$$

$$H + O_2 \rightarrow HO_2 \tag{5}$$

$G(O_2) = 2\cdot55$, taking $G_{\text{red}} = 2\cdot85$, $G\ \text{OH} = 2\cdot25$ and $GH_2O_2 = 0\cdot70$. However, if $G(1) = 0\cdot55$, and since $\frac{k(H+O_2)}{k(H+H_2O_2)} \simeq 3{,}000$[11], according to reactions (2)–(5), $G(-H_2O_2) = 3\cdot60$ and $G(O_2) = 2\cdot00$. Hochanadel[13] found the initial yield of $G(-H_2O_2) = 3\cdot7$ at 10^{-4} M and 4×10^{-3} M H_2O_2, in good agreement with assumption of direct formation of H atoms. This indicates, furthermore, that the apparent difference mentioned here is not a property of organic systems but applies to both organic and inorganic solutes irradiated in air-free solutions.

(*b*) Barr and Allen[12] interpreted their results in the irradiations of H_2–O_2–H_2O_2 solutions by a mechanism consisting of reactions (2) and (4)–(7).

$$OH + H_2 \rightarrow H + H_2O \tag{6}$$

$$H_2O^- + O_2 \rightarrow HO_2 + OH^- \tag{7}$$

This mechanism gave a satisfactory explanation to their results. They expected, however, the rate of consumption of oxygen to be constant and equal to $\frac{1}{2}(G_{\text{red}} + G_{\text{OH}}) = 2\cdot55$. Starting with known initial concentrations of O_2 they actually found that the initial yield of $G(-O_2)$ was very close to 2·55, but that the maximum dose required for complete consumption of oxygen and start of the chain-decomposition of H_2O_2 was greater than anticipated, making $G(-O_2)$ at maximum dose $\leqslant 2\cdot15$ (ref. 12) as compared with 2·55. This difference is here interpreted to mean that initially when the solutions have a relatively large $[O_2]$ reaction (1) does not take place, but that at doses near maximum, that is, when $[O_2]$ is very low, H atoms can be formed via reaction (1). It follows that in the latter case the extent of reaction (2) will be reduced, that of (5) and (4) increased, resulting in a continued increase in the formation of H_2O_2 and therefore an increase in the maximum dose required before H_2O_2 is rapidly decomposed by a chain reaction. This means that the ratio of initial O_2 concentration to the maximum dose, expressed in molecules per 100 eV, that is $G(-O_2)$ would be smaller by a G-value of up to 0·55 ($=G(1)$). A micromolar of O_2 seems sufficient for reaction (2), (5) or (7) to take place, but between 10–100 μM are necessary for reaction with the precursors of directly formed H atoms.

If H atom is produced from dissociation of an excited water molecule, H_2O^* could be formed either by direct excitation or as a result of ion neutralization[14]. The role of oxygen may be in intercepting the returning electron and preventing ion neutralization. But it is clear that this electron is not the same as the 'thermal' electron, H_2O^-, since high concentrations (up to 10^3–10^4 times $[O_2]$) of good scavengers for H_2O^-, for example, chloroacetic acid[3], cannot compete with O_2 in this electron capture process, though:

$$\frac{k(H_2O^- + O_2)}{k(H_2O^- + \text{ClAc})} = 3\cdot4 \text{ (refs. 3, 11)}$$

An alternative interpretation, which at present is considered much more likely, is the quenching by oxygen of excited water molecules leading to electron transfer:

$$H_2O^* + O_2 \rightarrow H_2O^+ + O_2^- \tag{8}$$

The electron once formed in water will reduce O_2 or migrate to other acceptors, on the basis of concentration and rate constants of competing solutes. Although oxygen is an efficient trap for the electron, having a reaction rate constant $\geqslant 10^9$ l./mole-sec (H_2O_2, H^+ are about equally efficient), the migration of the electron is still considered to occur. This is because the electron, with its great mobility and great selectivity to traps, will not 'stick' except in a fully competitive way.

Oxygen dissolved in water[15] or organic solvents[16] is known to cause an ultra-violet absorption spectra, in the case of water starting below 220 mμ. This has been interpreted by Mulliken *et al.*[16] to a charge-transfer interaction between oxygen and the solvent. It is also well known that oxygen quenches fluorescence[17] very efficiently, and Weiss[18] has explained such quenching in polar solutions by a similar mechanism. The life-times of the excited water molecules are probably[14,16,19] sufficiently long, thus providing ample probability for encountering oxygen molecules.

In conclusion, H and H_2O^- can both be formed directly in the radiolysis of oxygen-free water. In neutral solution $GH = 0\cdot55$ and $GH_2O^- = 2\cdot30$. In the presence of oxygen, all the reducing species formed ($G = 2\cdot85$) are present as electrons. The independent formation of the two possible reducing species in neutral water, one capable of dehydrogenating (the 'acid form') and the other of reacting with electron acceptors (the 'alkaline form') provides further evidence that the main reducing specie is the hydrated electron, H_2O^-.

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HYDROGEN BONDING IN PHENOLS

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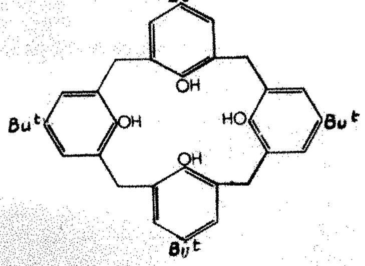
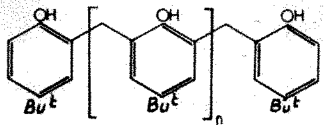
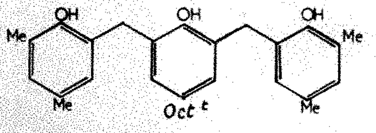
CONFORMATIONAL analysis of fairly rigid molecules such as those of the steroids and triterpenoids is well established, but comparatively little attention has been given to the stereochemistry of more flexible systems and of molecular aggregates in solution. As part of a general investigation[1] in this field we have examined the infra-red absorption spectra of a number of polyphenolic compounds (I, II, III and IV) of the novolak[2] type. Our results (Table 1, Fig. 1) show that in carbon tetrachloride solution these flexible molecules, in which the phenolic nuclei are linked by methylene bridges, assume well-defined, intramolecularly hydrogen-bonded conformations, both as monomers and, in certain cases, as intermolecularly bonded dimers. These conformations are represented in stylized form in Fig. 2 and a drawing of the Dreiding molecular model of a single example is reproduced in Fig. 3.



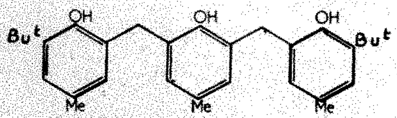
(I)



(II)
n = 0, 1, 2, 3, 4



(III)



(IV)

Oct^t = $-C(CH_3)_2CH_2C(CH_3)_3$

The experimental approach[3] distinguishes non-bonded from bonded hydroxyl groupings by comparing the lowered hydroxyl stretching absorption frequency of the latter with the non-bonded value of about 3,600 cm^{-1}. Further, hydroxyl absorption frequencies due to intermolecular hydrogen bonds differ from those pertaining to the intramolecular type in being dependent on concentration, and the number of non-bonded hydroxyl groupings may be inferred from the intensity of the 3,600 band relative to the intensity of the same band in simple phenols[4]. Certain stereochemical requirements have to be met by conformations for which reasonably strong (ν_{OH} ~ 3,300 cm^{-1}) hydrogen bonds involving phenolic hydroxyls are postulated: approximate coplanarity of the C—O—H bond with the benzene ring[4]; and O····O distance[5] of approximately 2·7 Å; and the hydrogen atom must lie somewhere near a line joining the two oxygens. (The exact positioning is not certain. The lone pairs on the receptor oxygen probably induce a pseudo-tetrahedral disposition with respect to the latter, though there is likely to be more trigonal character in phenolic bonding than that of alcohols owing to electron delocalization involving the aromatic nucleus.) The weaker, OH···· benzene ring, type of hydrogen bond is unlikely to compete, except in unusual steric situations.

The cyclic tetranuclear novolak (I) represents, perhaps, the simplest case of those listed in Table 1.

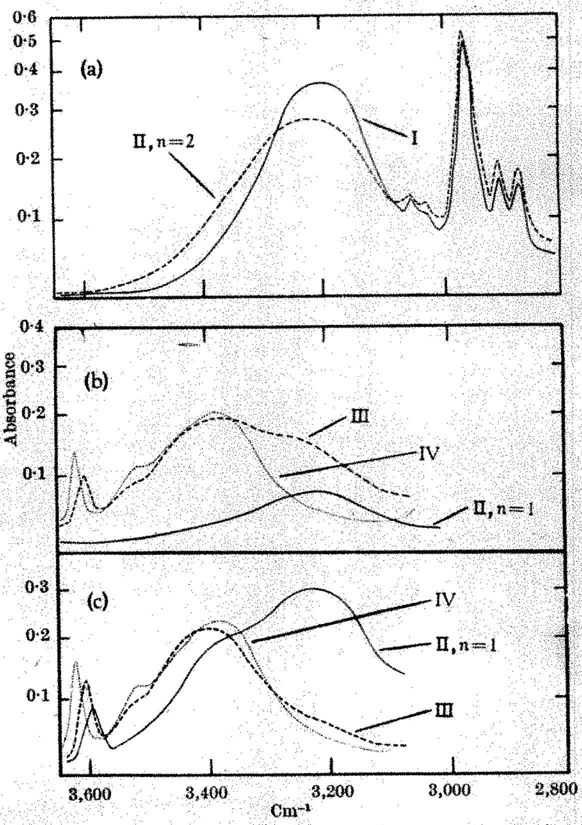


Fig. 1. Hydroxyl stretching absorptions, in carbon tetrachloride of the novolaks I, II (n=1 and 2), III and IV. Concentrations: (a) c. 0·3 mM, (b) c. 15 mM except for II, n=1 saturated solution, and (c) c. 0·5 mM. Cell paths: (a) 20 mm, (b) 0·5 mm, and (c) 20 mm

Table 1. HYDROXYL STRETCHING FREQUENCIES, ν(OH) IN CARBON TETRACHLORIDE

	Results at two different concentrations										Assignments		
Compound	Conc. (mM)	Cell path (mm)	ν (cm^{-1})	$\Delta\nu^{a}_{1/2}$ (cm^{-1})	ε^{a}	Conc. (mM)	Cell path (mm)	ν (cm^{-1})	$\Delta\nu^{a}_{1/2}$ (cm^{-1})	ε^{a}	Hydrogen bonding	Hydroxyls involved	Species (Fig. 2)
I	0·27	20	3,200	(190)	670	Concentration independent					Intra	4	*C*
II, $n=2$	0·32	20	3,215	(280)	440	Concentration independent					Intra	4	*D*
II, $n=3$	0·30	20	3,200	(280)	610	Concentration independent					Intra	5	*F*
II, $n=4$	0·20	20	3,190	(280)	650	Concentration independent					Intra	6	*G*
II, $n=0$	25·80	0·5	3,606	26	60	0·7	20	3,606	28	155	Free	1	*A*
			3,470	*sh*	—			3,470	(110)	110	Intra	1	
			3,260	(260)	175			3,250	—	65	Inter	4	*E*
II, $n=1$	Sat. Soln.	0·5				0·49	20	3,596	36	80	Free	1	*B*
								3,394	—	110	Intra	2	
			3,220	*br*	—			3,220	(230)	290	Inter	6	*H*
III	16·20	0·5	3,606	40	110	0·47	20	3,606	28	120	Free	1	*B*
			3,520	*sh*	—			3,520	*sh*	—			
			3,390	—	220			3,400	(180)	200	Intra	2	
			3,220	—	160						Inter	6	*H*
IV	15·50	0·5	3,622	25	165	Concentration independent					Free	1	*B*
			3,520	*sh*	—								
			3,350	(190)	240						Intra	2	
Phenol	2,500	0·05	3,611	19	—	100	0·51	3,611	19	—	Free	1	*L*
								3,485	—	—	Inter	1	
			3,350	(280)	—						Inter	3	*K*(?)

Values in parentheses are approximate; —, not measured; *sh*, shoulder, *br*, broad band. Intensities are given as apparent extinction coefficients, [ε^a], (l. mole^{-1} cm^{-1}) rounded to the nearest 5 units and measured from a solvent–solvent base-line superimposed on the record of the spectrum of the solution (determined with solvent in the reference beam). Recording conditions are set out fully in reference 1. Gifts: Prof. A. Zinke, compounds I and II, $n=0$, 1, 2, 3, and 4; Mr. A. R. Philpotts, Dr. H. C. Bailey, and Mr. P. A. Jenkins of the Distillers Co., Ltd., compound III; and the American Cyanamid Co. compound IV.

From an examination of the Dreiding model, it would appear that the most stable conformation should be that having a closed ring of hydrogen bonds, with each of the hydrogen atoms between two oxygen atoms as indicated diagrammatically (*C* in Fig. 2). The absorption data are in complete accord as the only hydroxyl absorption in dilute carbon tetrachloride solution is the broad, intense, symmetrical band near 3,200 cm^{-1} (Fig. 1*a*) which was concentration independent over the range examined (since a closely similar band was observed for the solid state, the crystal may well consist of an array of such basin-shaped molecules). Most interestingly, the acyclic tetra-, penta- and hexa-nuclear novolaks (II, $n = 2$, 3, and 4) absorb in a similar manner and it would seem, therefore, that in solution these substances exist as the 'cyclic' intramolecularly hydrogen-bonded monomers *D*, *F* and *G*, respectively. The similarity in absorption pattern of the cyclic (I) and the acyclic (II, $n = 2$) tetranuclear novolak is illustrated in Fig. 1*a*. The increased width and asymmetry of the band of the latter compound is probably a result of the increased mobility of the molecular backbone. Dimeric association at the low concentrations examined is conceivable but unlikely in view of the ease with which the models of the monomers can be induced to take up the conformations illustrated (Figs. 2 and 3).

Unlike the preceding compounds, the di- and tri-nuclear novolaks (II, $n = 0$ and 1) show concentration dependent absorptions implying the existence of self-association even at very low concentrations. This behaviour (Table 1 and Fig. 1*b*, *c*) is almost certainly due to monomer–dimer equilibria and arises from the inability of the di- and tri-nuclear monomers (*A* and *B*, respectively) to form closed rings of hydrogen bonds in the manner already discussed for the monomers of the tetra-, penta- and hexa-nuclear compounds (*D*, *F* and *G*, respectively). Examination of Dreiding models suggests that relatively unstrained closed rings of hydrogen bonds are possible for the di- and tri-nuclear dimers, the former species (*E*) being closely analogous to the tetranuclear compound (*D*), and the latter (*H*) to the hexanuclear compound (*G*). Although both *E* and *H* apparently persist to very low concentrations (about 1 mM), the trinuclear dimer (*H*) seems the more strongly associated on the grounds of both ν_{max} and the relative intensities of the monomer and dimer bands.

We have been able to examine the effect of ortho-substitution of the terminal rings of the trinuclear novolak. As anticipated, introduction of the methyl groupings (compound III, Fig. 1*b*, *c*) greatly reduced dimer formation, while no dimer at all could be detected in fairly concentrated solution of the di-*t*-butyl compound (IV). Models of the type *H* dimer of compound (III) show pronounced interaction between the ortho methyls and it is conceivable that other cyclic conformations (for example, *I* and *J*), where some of the benzene rings are orientated in opposing senses and the methyl groups are directed away from each other, may be more stable.

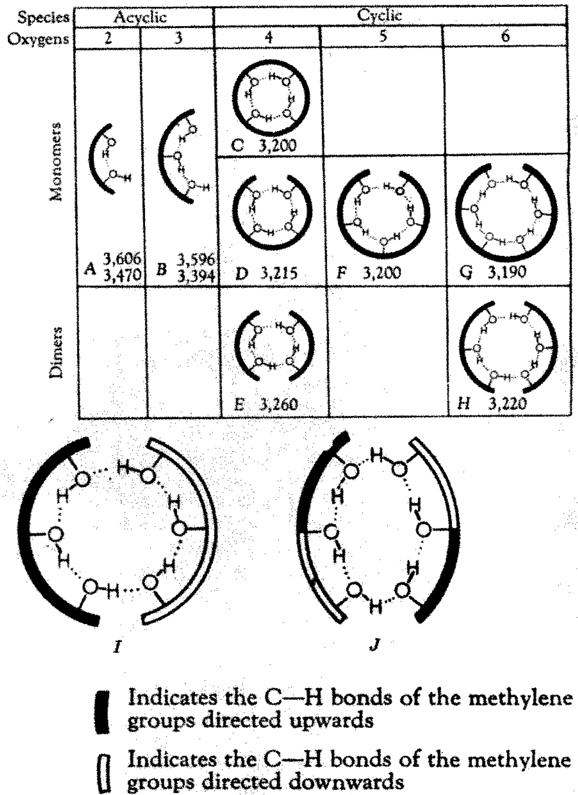

Species
Oxygens
Acyclic
Cyclic
2
3
4
5
6
Monomers
Dimers
C 3,200
A 3,606 3,470
B 3,596 3,394
D 3,215
F 3,200
G 3,190
E 3,260
H 3,220
I
J
Indicates the C—H bonds of the methylene groups directed upwards
Indicates the C—H bonds of the methylene groups directed downwards

Fig. 2. Summary of assignments for the novolaks I and II, $n=0$–4. Values quoted are the frequency measurements in cm^{-1}

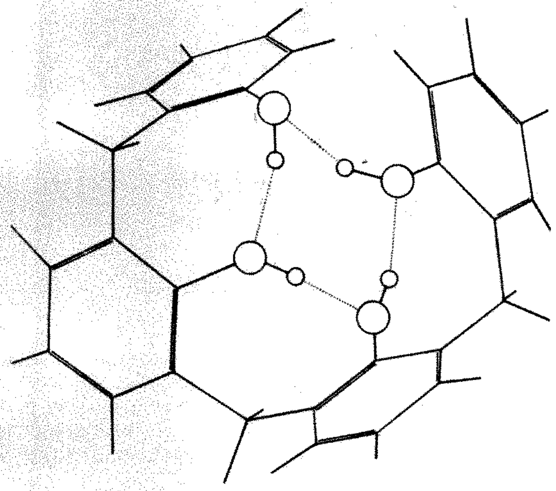
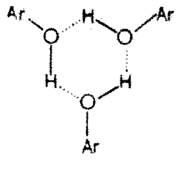


Fig. 3. Planar projection of the Dreiding molecular model of the postulated conformation (D) of the acyclic tetranuclear novolak II, $n=2$. The large and small circles indicate the oxygen and hydrogen atoms of the hydroxyl groups respectively. The dotted lines indicate hydrogen bonds. The para-t-butyl groups have been omitted

Coggeshall[6] and, later, Goddu[7] have examined the hydroxyl stretching region of a number of alkyl substituted dinuclear novolaks (*bis*-phenylol alkanes) related to (II, $n = 0$) but the concentration and frequency-ranges covered were narrow and these workers did not elucidate the monomer–dimer relationships established in the present work. However, it is clear from Coggeshall's data for an ortho-di-t-butyl substituted novolak that dimeric association is absent in quite concentrated (0·5 M) solutions in carbon tetrachloride (cf. II, $n = 0$). The *cis* and *trans* forms postulated by Coggeshall to explain the non-bonded and bonded hydroxyl absorptions are now unnecessary, but the peculiar effect reported[6,7] for the dinuclear novolaks substituted on the central aliphatic carbon requires elucidation.

The absorptions ascribed to the monomers (species B) of the three trinuclear novolaks merit some comment. First, the trend in the free hydroxyl frequencies: no *ortho* substituent 3,596, ortho methyl 3,606, and *ortho* t-butyl 3,622 cm^{-1}. The values for the corresponding *cis*-conformations (OH directed towards the alkyl grouping) of single *ortho*-alkyl substituted phenols[4,8,9] are 3,612, 3,621 and 3,647 cm^{-1}, respectively. (The *ortho* methyl value given is that for 2,6-dimethylphenol since only a single unresolved band at 3,613 cm^{-1} is observed for 2-methylphenol itself.) The lower values of the novolak monomers undoubtedly register the electronic effect[10] of the intramolecular hydrogen bond on the terminal hydroxyl. The same effect can be detected in the data given by Coggeshall[6] and Goddu[7]. Secondly, the broad bands assigned to the intramolecular bonded hydroxyls show some sign of sub-structure and, indeed, frequency differences might be expected between the two hydroxyl groups involved. Kuhn and Bowman[11] sought an indication of such behaviour in their somewhat parallel study of simple acylic triols.

It is interesting to compare the hydrogen bonding shifts, $\Delta\nu$, for the monomers of the series of novolaks (II, $n = 0$, 1, 2, 3, and 4) given in Table 2. The considerable increase in $\Delta\nu$ on going from II, $n = 1$ to II, $n = 2$ must certainly reflect the increased effectiveness resulting from the formation of the cyclic system (D) of hydrogen bonds. The simple addition of a fourth oxygen to the first three would otherwise be of little consequence. Numerous workers[3,4,8,11] have discussed the nature of the association equilibria present in solutions of alcohols and of phenols. Both cyclic (for example, K) and acyclic (for example, L) hydrogen-bonded species have been proposed. In the case of phenol itself the bands at 3,485 and 3,350 cm^{-1} (Table 1) are generally held to be due to dimers (L) and trimers, respectively, and on steric grounds it seems likely that the latter might well be cyclic (K). On the other hand, the hydroxyl region of a concentrated (2·5 M) solution of phenol in carbon tetrachloride closely resembles that of a very dilute solution (Fig. 1c) of a trinuclear novolak where the acyclic monomer predominates, and it is tempting to propose that the phenol trimer is also acylic. This argument is unsatisfactory, however, as the novolaks are more closely analogous to the *ortho* alkyl-substituted phenols for which different values for ν_{OH}[dimer] and ν_{OH}[trimer] are quoted[4,9]. In fact, the $\Delta\nu$ values for phenol are dimer (equivalent to species A, Table 2) 126 cm^{-1} and trimer 261 cm^{-1}. The relatively large increase from 126 to 261 cm^{-1} is a strong indication that the band centred at 3,350 cm^{-1} is mainly due to cyclic trimer. Acyclic trimer or higher polymers may well be present, but a cyclic tetramer could not be a major contributor.



K

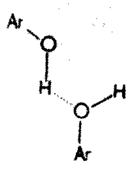


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Table 2. $\Delta\nu$ VALUES FOR THE NOVOLAKS II, $n=0$, 1, 2, 3, AND 4

n	0	1	2	3	4
Species assigned (Fig. 2)	A	B	D	F	G
No. of hydroxyls involved	2	3	4	5	6
$\Delta\nu$ (cm^{-1})	141	217	397	411	421

Where $\Delta\nu = \nu_{OH}[\text{non-bonded}] - \nu_{OH}[\text{bonded}]$.
$= 3{,}611 - \nu_{OH}[\text{bonded}]$.

It would seem desirable to extend the foregoing approach to measurements in more polar solvents, so that closer analogies may be found for the more general problems of the conformations of polymer molecules in solution and of molecular specificity in biological systems.

We thank Mrs. F. Lawrie for the infra-red measurements, Prof. A. Zinke for his co-operation in supplying the series of para-t-butyl substituted novolaks, and Mr. A. R. Philpotts and his colleagues for samples and helpful suggestions. One of us (T. C.) thanks the Scottish Education Department for financial assistance.

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SMALL-ANGLE DIFFRACTION OF POLYETHYLENE

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M. KUROKAWA
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SEVERAL investigators have reported that small-angle diffraction is noted on the meridional direction at the drawn filaments of synthetic polymers. Applying the Bragg equation, long periods are calculated from the interference maxima, and these periods are interpreted as corresponding to the periodic alternations of crystalline and amorphous regions[1-4]. Regularity is limited to short range, so in general only first-order diffraction is noticed; but at the suitably prepared specimens two or three higher-order diffractions appear weakly[5]. It has also been shown that these long periods can be increased as the specimens are annealed at higher temperature[1-4]. Recently, we have found new forms of small-angle diffractions in Ziegler polyethylene, which are quite different from ordinary small angle diffractions.

Samples used were undrawn filaments of Ziegler polyethylene (commercial name, 'Hizex'), which were drawn about seven times at 20° C or in boiling water and then annealed at various temperatures. Pellets of Ziegler polyethylene (mol. wt. 66,000) were used, from which films were made by melt compression as usual, and these films were drawn and annealed in the same way.

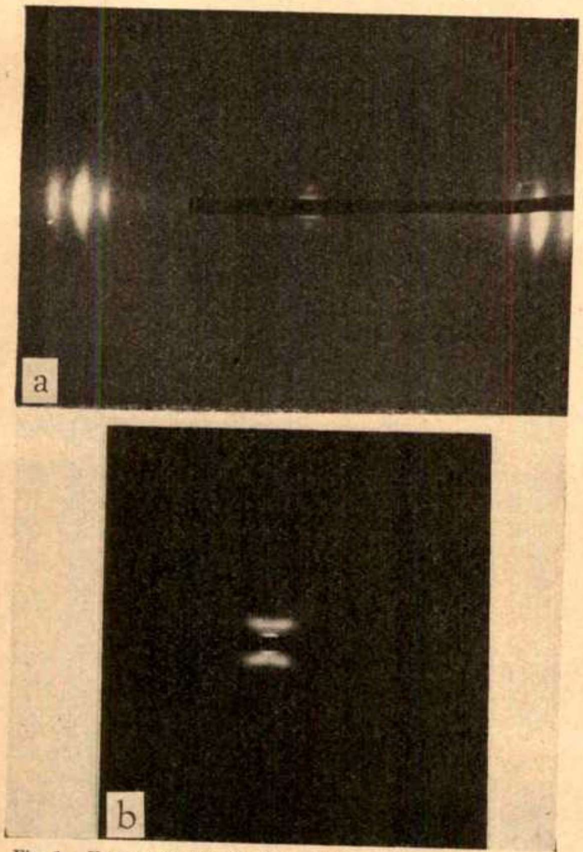

Fig. 1. X-ray photographs of polyethylene filaments drawn at 20° C and annealed at 120° C. *a*, Wide angle; *b*, small angle, corresponding to the shielded centre of *a*

Small-angle diffraction patterns were photographed by copper *K* radiation with pin-hole slits, at distances of 22·2 cm and 7·0 cm, and exposure times of 5–10 h. Small-angle scattering curves during annealing were recorded with a Geiger–Müller counter spectrometer, equipped with a small electric furnace in order to anneal the samples.

Specimens drawn at 20° C showed the ordinary off-meridional four-point patterns, and their long period and scattering intensities could be increased as the specimens were annealed at high temperatures. Together with these ordinary long-period diffraction we observed new small-angle diffraction patterns followed by several higher-order ones on the meridian after annealing at temperatures above about 90° C. Specimens drawn in boiling water also showed the ordinary off-meridional four-point diffraction and new on-meridian diffraction after annealing at about 120° C. The new small-angle diffractions did not appear at the drawn, but unannealed, specimens. These new small-angle diffractions and the ordinary ones are shown in Fig. 1, and Bragg spacing and observed intensities of these new diffractions are given in Table 1. We can conclude that these two forms of diffraction have different origins, because the new diffraction patterns appear on the meridian and their periods are independent of the annealing temperature (Table 2), while ordinary small-angle diffraction patterns are off-meridional four points and their periods are temperature dependent.

Table 1

Order	Period (Å)	Intensity (observed)
1	48·1	8
2	24·1	1
3	16·2	2
4	11·9	Weak
5	9·2	Weak

Table 2

Sample annealing temperature (°C)	Ordinary small-angle diffraction		New small-angle diffraction period (Å)
	Long period (Å)	δ*	
Drawn at 20, unannealed	163	34	—
65	178	34	
95	198	37	47·1
110	217	37	47·0
120	246	37	48·1
125	304	38	47·7
Drawn in boiling water,			
120 (contractible)	242	37	47·6
120 (not contractible)	228	Streak on meridian	48·2

* δ, Angle between diffraction spots and meridian.

Considering that the results of the new periods are independent of the annealing temperature, that several higher-order diffractions can be observed, and that their appearance are sensitively affected with the incident angle of X-ray to the specimens, we may

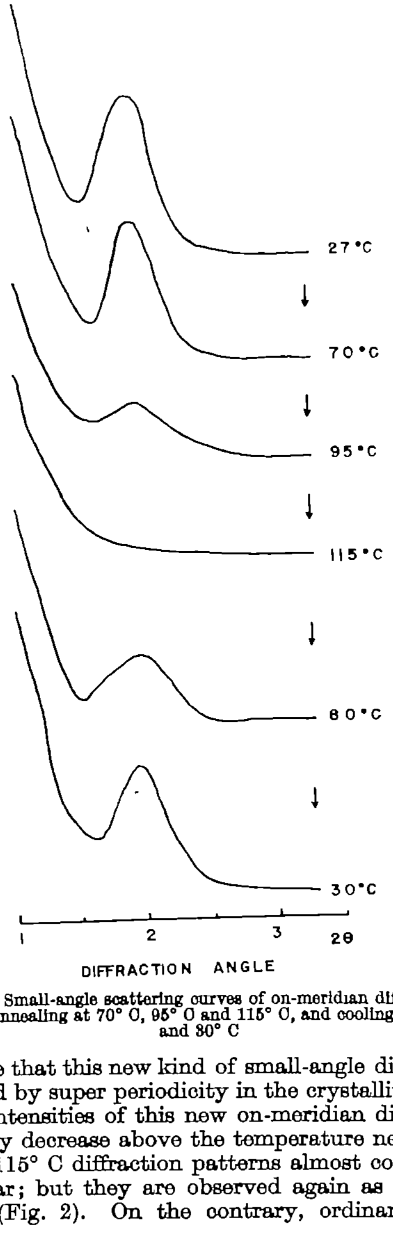

Fig. 2. Small-angle scattering curves of on-meridian diffractions during annealing at 70° C, 95° C and 115° C, and cooling at 80° C and 30° C

conclude that this new kind of small-angle diffraction is caused by super periodicity in the crystallites.

The intensities of this new on-meridian diffraction gradually decrease above the temperature near 80° C and at 115° C diffraction patterns almost completely disappear; but they are observed again as they are cooled (Fig. 2). On the contrary, ordinary small-angle diffraction patterns do not become extinct during annealing, or rather scattering intensities increase at high temperature. The extinction of Bragg diffraction patterns at high temperature are also noted on 6·10 nylon. In this case (001) diffraction becomes extinct at a temperature a little below the melting-point; but (002) diffraction does not. This means that owing to the active thermal vibrations of molecules in the crystallites, difference of electron distribution between (001) and (002) planes will disappear on the average, and consequently diffractions of odd order will become extinct.

It is well known that single crystals of polyethylene are precipitated from dilute solution, and the molecules are folded in these lamellar crystals[6,7]. We made the film by melt compression from the aggregates of single crystals precipitated from 0·02 per cent hot xylene solution of the same Ziegler polyethylene filaments. The film was drawn about seven times and then annealed at 120° C. These films did not show the new on-meridian small-angle diffractions.

The specimens used in this experiment are somewhat plasticized, but new small-angle diffractions will not be caused by the plasticizers themselves, because the diffraction patterns disappear at high temperature and they do not appear at the drawn specimens until they are fully relaxed by annealing.

From the above results and the fact that the dimensions of unit cell of these drawn and annealed polyethylene are equal to the value of normal orthorhombic structure, super-periodic structure in the crystallites, corresponding to the on-meridian diffraction, will result neither from the periodic distribution of branching nor from the pseudo-structure arising from the stretching[8], but from the periodic defects or distortions of chain in the crystallites. At present we cannot explain the detailed nature of them or the effect of plasticizer on these super-periodic structures if it is present.

We thank Prof. Horio of Kyoto University for his advice and Mr. Ban for his assistance.

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INHIBITION OF BACTERIAL GROWTH BY MAGNETIC FIELDS

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THE results of investigations of the effect of magnetic fields on the growth of bacteria have so far been inconclusive. Leusden[1] found that the growth of coliform organisms and staphylococci was not affected by exposure to magnetic fields. Jennison[2] grew 25 species of bacteria, yeasts and moulds on solid media between the poles of an electromagnet which provided a homogeneous field of 3,000 œrsteds. After 48-h exposure, he checked the cultures for size of colony, staining reaction, pigment production, and spore formation. He found no difference between magnetized cultures and controls. However, Magrou and Manigault[3] showed that tumour production by *Bacterium tumefaciens* on a strain of *Pelargonium zonale* was retarded by magnetic fields.

The work reported here was begun by growing a nutrient broth culture of *Serratia marcescens* between the tapered constant gradient pole caps (*V*-4089-1) of a Varian 4-in. electromagnet. The average field strength was 15,000 œrsteds, having a constant gradient of 2,300 œrsteds/cm throughout the culture medium. The paramagnetic strength of this field was 34·5 mega-œrsteds^2/cm. (One mega-œrsted^2/cm exerts on 1 c.c. of a substance of a paramagnetic susceptibility of 10^{-6} an accelerating force of one dyne in the direction of the gradient vector.)

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Standard culture tubes containing 5 ml. of nutrient broth were inoculated with one drop of a 24-h broth culture from a 1-ml. pipette. One tube was incubated in the magnet and the other, serving as a control, was incubated outside the magnet. Both cultures were grown for 10 h at 27° C.

At hourly intervals, 0·1-ml. samples were taken and diluted by serial 10-fold dilutions. 0·1-ml. of the last two dilutions was surface plated on nutrient agar. The plates were incubated at 27° C for 48 h and the resulting colonies counted.

The magnetized and control cultures were found to have approximately the same number of cells and the same growth-rate up to 6 h. At 7 h the magnetized culture showed a lower plate count than the controls. This difference was greatest at 8 h and diminished up to 10 h when magnetized and control cultures had approximately the same number of cells.

A similar procedure was followed with a culture of *Staphylococcus aureus* except that the incubation temperature was 37° C. Under these experimental conditions, there was no effect on the growth of the organism. The experiments were repeated using special pole caps on the electromagnet (Fig. 1). These pole caps, consisting of a wedge, opposite to a concave piece, produced an average gradient of 5,200 œrsteds/cm. The gradient in the culture medium was nowhere less than 3,000 œrsteds/cm and reached at some parts 8,000 œrsteds/cm. The average paramagnetic strength of the field was 78 mega-œrsteds^2/cm, or more than twice as strong as the one previously used.

A difference in the number of cells was evident from 3 to 6 h, when the magnetized culture showed a higher plate count and higher growth rate than the control. However, at 6 h inhibition of this growth began and reached its maximum at 7 h, when the plate count of the magnetized culture fell below that of the control. This difference gradually diminished up to 9 h, when the magnetized and control cultures had approximately the same number of cells. Thus *S. aureus* was maximally inhibited around 6–7 h, as was *S. marcescens*.

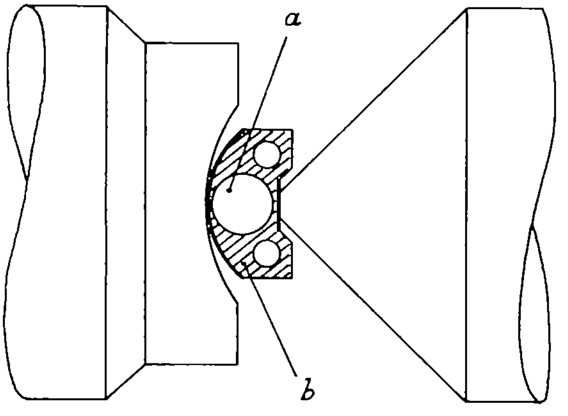

Fig. 1. Culture tube (*a*) in thermostat (*b*) between the pole caps of the electromagnet, in a field of 15,000 œrsteds and 5,200 œrsteds/cm average gradient

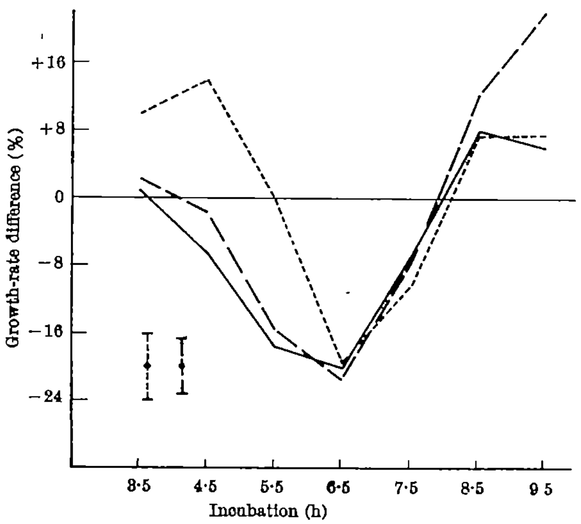

Fig. 2. Difference of the relative hourly growth-rate between magnetized and control culture. Dashed line, *Serratia marcescens*; dotted line, *Staphylococcus aureus*, full line, difference curve computed from equations (1) and (2). Standard errors are indicated

The effect of the magnetic field is more clearly seen if the difference between relative hourly growth rate of magnetized and relative hourly growth rate of control cultures is plotted, as shown in Fig. 2. The curve for *S. marcescens* is the average of three experiments; the curve for *S. aureus* is the average of two experiments. The minimum of the curve is in both cases significant to a probability-level better than 1 : 1,000,000. The higher growth rate for the hours preceding the minimum is significant for *S. aureus* but not significant for *S. marcescens*, whereas the higher growth rate shown for the hours following the minimum is significant for both cultures.

These results indicate that magnetic fields of a paramagnetic strength as used do affect the growth of bacteria. The inconclusive results of previous workers may have been due to the low paramagnetic strength of their fields.

Microscopic examination of samples taken from the magnetized cultures showed no morphological changes.

The exact physiological mechanism which causes the inhibition is unknown. Nor is it clear why *S. aureus* required a more inhomogeneous field than *S. marcescens* to be inhibited. The fact that the inhibition was observed in a highly inhomogeneous magnetic field, and particularly that the inhibition of *S. aureus* was found in a field of the same average strength as with *S. marcescens* but at a higher paramagnetic strength, indicates that the inhibition may be due to a paramagnetic phenomenon. The small magnitude of the inhibition which we observed even with fields of great inhomogeneity may be due to the fact that bacteria do change their position relative to the direction of the magnetic field and gradient vector. This constant changing of position, due to convection currents and Brownian motion, would tend to compensate all first-order reversible cumulative effects of the field, and only second-order reversible cumulative effects, or immediately permanent effects, may exert themselves. An exception would be if a bacterium as a whole were oriented through the magnetic field. This could occur if the bacterial cells were paramagnetic and, furthermore, if they were non-spherically shaped, like *S. marcescens*. This question needs further investigation.

A further interpretation of the growth-curve can be achieved through its mathematical analysis. One can describe the main part of the growth-curve of bacteria, that is the phase of logarithmic increase, the phase of negative growth acceleration, the maximum concentration, or stationary phase, the death-phase and the logarithmic death-phase by the equation:

$$N = N_0 \exp A\ [1 - \exp(-it) - \exp d(t - \delta)] \quad (1)$$

where N_0 is the number of viable cells at the onset of the logarithmic increase phase, chosen as the zero point of the time-scale (t equals incubation time less lag time); A is a constant determining the height of maximum concentration, or stationary phase, and depends among others on the total available volume; i is a parameter which determines the slope of the logarithmic increase phase and is a measure of the fission rate (minus the small proportion of cells which failed to survive); d is a parameter determining the slope of the logarithmic death-phase; it is a measure of all detrimental factors appearing during the further history of the culture and includes such factors as change in *p*H, accumulation of toxic products, exhaustion of nutrient material and others, which are the reasons for the increase in the death-rate of cells; δ is the total length of the growth-curve, after which the number of viable cells is reduced anew to N_0; it is also a measure of the time-lag, after which the effect of the detrimental factors become dominant. The growth-curve of *S. marcescens* under our experimental conditions can be well approximated with the values $A = 4$; $i = 0{\cdot}25$/h; if δ is adequately long, the second term in the exponent has no bearing on the shape of the curve up to the maximum stationary phase.

It is reasonable to assume that the magnetic field can be considered as a factor which either decreases the fission rate or increases the death rate of the cells. The circumstance that in the beginning of the logarithmic increase phase the magnetic field did not affect adversely the growth rate would indicate that its effect would be more of the nature of a detrimental factor, increasing the death rate. It manifests itself when the number of cells approaches the maximum concentration and the fission rate gradually decreases. Similar to the effect of other detrimental factors, this could be expressed through a term like the second term in the exponent in which the parameter m is a measure of the death rate caused by the magnetic field and a time-lag μ is the necessary time for the biomagnetic effect to develop. If the magnetic field is the predominant detrimental factor, the logarithmic death-phase will set in much earlier and the number of viable cells begins to drop before the usual maximum concentration is reached. It is, however, possible that while the death rate of the earlier generations increases, the new generations become better adapted to withstand the detrimental factor of the magnetic field. Depending on when, and to what degree, this resistance is building up in the later generations, it can more or less compensate the increased death rate of the earlier generations. It may be that actually the total number of viable cells will continue to increase and merely a temporary inhibition in the growth rate will be observable.

The observation that the growth rate after its minimum at 6·5 h returned quickly to its original value, and furthermore, that eventually the same maximum concentration value was reached, as without magnetic field, suggests that the adaptation of the later generation must have been more or less complete. We do not want to use the term 'magneto-resistant mutant' since no attempts were made to verify the presence of a genetic change. Mathematically, the growth-curve of these resistant cells could be described by equation (1), in which the zero point of the time-scale starts with a time ν later, which time is necessary that the number of resistant viable cells reach the starting value N_0. Assuming that the fission rate of the resistant cells is the same as that of the original population, the total number of viable cells is given by the equation:

$$\begin{aligned} N_m = {} & N_0 \exp A\ [1 - \exp(-it) - \exp m(t - \mu)] \\ & + N_0 \exp A\ [1 - \exp\ -i(t - \nu) - \exp d(t - \delta)] \quad (2) \end{aligned}$$

The first term in this equation describes the growth-curve of the original population, the second term that of the magneto-resistant population with the meaning of the parameters as discussed here, whereby it was assumed that the effect of the magnetic field on the death rate, overrides all other detrimental factors. From equations (1) and (2) we can compute the relative difference in growth rate between magnetized and control cultures. This is shown in Fig. 2, where the full line was computed using the previously given values for A and i and taking $\nu = 1$ h; $\mu = 5{\cdot}5$ h and $m = 1$/h. As we can see it follows well the experimental curve of *S. marcescens*. The *S. aureus* curve, which in the beginning shows a somewhat enhanced growth rate in the magnetized culture, can be explained by a slightly higher fission rate of the resistant cells.

We wish to stress that in both cultures the increase of the growth rate after the minimum can only be explained if magneto-resistant cells are formed. From the fitted values of the parameters we may conclude that after 5·5 h of incubation in the field, the number of resistant and non-resistant viable cells became equal and that after 7·5 h of incubation the number of non-resistant viable cells was again reduced to N_0. The other possible solution, as already mentioned, would be that the magnetic field does not kill the cells, but merely temporarily inhibits their division until they become adapted to the new environment, the magnetic field. We could think of a process similar to that occurring during the lag phase when cells inoculated from an old culture do not start to divide at once, but first grow in size and are rejuvenated and fitted for reproduction.

However, the analogy ends here. At the time when the first signs of inhibition appear, the majority of the population consists of 5th–8th generation cells. It is somewhat difficult to understand that cells multiplying in the field for 5–8 generations without being affected should abruptly cease to divide in order to adapt themselves to the field and thereafter resume their original fission rate. In our opinion the first solution is more probable: the magnetic field does not affect the fission rate, but has a mortal effect (presumably caused through a paramagnetic phenomenon). During the incubation in the magnetic field, a strain resistant to this mortal effect develops. The mortality rate of the non-resistant strain rapidly increases in younger generations, clearing the biological space for the multiplication of the resistant cells.

The experiments were performed at the University of Illinois College of Pharmacy and supported in part by a graduate research grant of the University of Illinois and in part by the Biomagnetic Research Foundation.

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DEHYDRATION-INDUCED SYNTHESIS OF NUCLEIC ACIDS AND CHANGING OF COMPOSITION OF RIBONUCLEIC ACID: A POSSIBLE PROTECTIVE REACTION IN DROUGHT-RESISTANT PLANTS

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DEHYDRATION has diverse effects on plant cells, and the extent in the response of plants to drought varies in different plants. The tolerance of a plant to dehydration changes with age[1] as well as with the rate of loss of water[2]. Recently, the existence of a relationship between a liponucleic acid fraction and relative drought resistance was demonstrated[3].

The possible relations between nucleic acids and drought tolerance have now been more closely examined. This article will be confined to the Oleaceae of which two systematically close genera were selected: the drought-tolerant *Olea europea* L. var. Souri and the drought-sensitive *Ligustrum sinensis*.

Leaves from the central part of one-year old branches were slowly dehydrated at 30° C. At various time-intervals four replicate leaf samples were removed and analysed for nucleic acids. The detailed assay procedures for nucleic acids, RNA and DNA extraction and fractionation, their hydrolysis and ion-exchange chromatography have all been described[4,5].

The RNA composition of both the olive and ligustrum leaves were found to be of the *G*–*C* type.

Fig. 1 demonstrates the changes in the nucleic acid content in olive leaves with progressing dehydration, and the same is shown for the ligustrum leaves in Fig. 2. The response of nucleic acids in olive leaves is interesting because it shows that on dehydration both DNA and RNA accumulated rather intensively. In contrast, in the ligustrum leaves, the DNA-level remained fairly constant at any level of dehydration while the level of RNA increased only slightly.

A similar increase in the RNA-level with dehydration has previously been found by Gates *et al.*[6] and Kessler[3] in intact tomato plants subjected to soil drought; but this initial increase was followed by a pronounced decrease in the RNA-level.

That in the drought-sensitive ligustrum leaves the RNA-level did not decline as in the intact tomato plants[3,6] might be related to the fact that in the present case we are dealing with an excised tissue from which no outward translocation is possible.

DNA synthesis occurs in non-dividing cells, continuing after the cessation of mitosis[8]. DNA, as well as RNA, are also synthesized in rooted leaves where growth is based only on cell enlargement and not on cell division[9]. In the present case, DNA synthesis was initiated in non-dividing olive leaves under the effect of water stress, and this 'induced' DNA synthesis was paralleled by an intensive accumulation of RNA even during a fairly high degree of dehydration.

The drought-induced synthesis of nucleic acid reminds, to a certain extent, Stocker's 'restitution' theory, in which he assumes an over-compensation of the plant metabolism during a slow water removal through which the plant enters a hardened condition[10,11].

Of particular interest was the fact that, in olive leaves, the newly accumulated RNA in the dehydrating leaves was differently composed than the RNA of fully turgorized leaves (Table 1). The $\frac{G+C}{A+U}$ ratio of ligustrum-RNA is about 1·04 in the fully hydrated leaves. On dehydration, there was a slight tendency of this ratio to decrease, being at all levels of dehydration near 1·00.

A different picture was obtained with the olive leaves. In this case the $\frac{G+C}{A+U}$ ratio increased on dehydration from 1·07 to 1·38. These data indicate that the drought-resistant olive leaves not only accumulated RNA on dehydration but also synthesized RNA molecules of different types as judged by the increasing $\frac{G+C}{A+U}$ ratio.

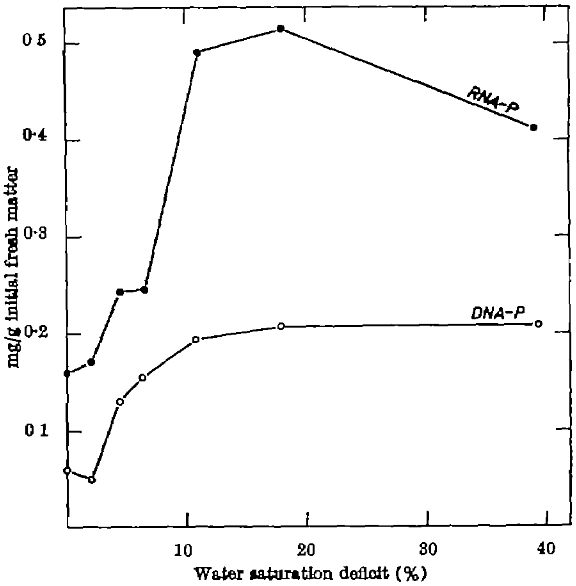

Fig. 1. Concentration of RNA and DNA in olive leaves at different levels of dehydration

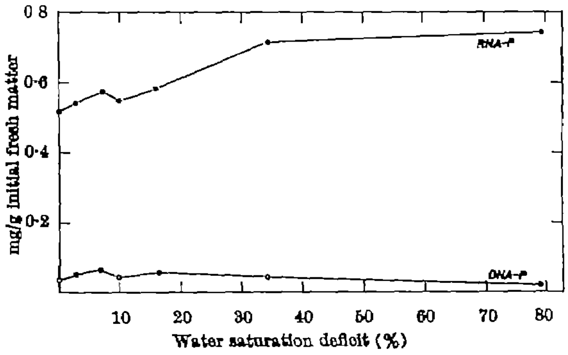

Fig. 2. Concentration of RNA and DNA in ligustrum leaves at different levels of dehydration

Table 1. NUCLEOTIDE COMPOSITION OF RNA'S FROM OLIVE AND LIGUSTRUM LEAVES AT DIFFERENT LEVELS OF DEHYDRATION

% Water loss	Mol. (%)				Purines / Pyrimidines	(Guanylic acid + Cytidilic acid) / (Adenylic acid + Uridylic acid)
	Cytidilic acid	Adenylic acid	Uridylic acid	Guanylic acid		
			(1) Olive			
0	24·2	26·5	21·8	27·5	1·17	1·07
5	24·5	25·7	20·8	29·0	1·21	1·15
11	24·6	23·6	20·8	31·0	1·20	1·25
39	24·9	21·9	20·2	33·0	1·21	1·38
			(2) Ligustrum			
0	24·1	24·9	24·1	26·9	1·07	1·04
6	24·2	25·1	24·4	26·3	1·06	1·02
11	24·1	25·5	24·4	26·0	1·06	1·00
35	24·1	25·3	24·2	26·4	1·07	1·02

The increase in the $\frac{G + C}{A + U}$ ratio indicates the formation of $(G + C)$-rich RNA molecules. The configuration of RNA in plants has not yet been fully elucidated. If we assume that leaf-RNA is double stranded then the 'dehydration-induced' synthesis of $(G + C)$-rich RNA molecules might become of particular significance. The G–C pairs, which are more hydrogen bonded, are denser[12] and have a higher thermal stability[13–15]. Their structure might, therefore, be more stable; in particular, in those cases where drought is also combined with high temperatures.

On the basis of these results it might be postulated that the extent of the resistance of plants to unfavourable conditions as drought and low temperatures is related, at least partially, to either one of the following factors: (1) a higher $G+C$ content of its RNA[7]; (2) its capacity to synthesize $(G+C)$-rich RNA molecules under the stimulus of slight stress conditions. This latter process might well constitute a basic part of hardening reactions.

Note added in proof. In a recent communication it has been reported (West, S. H., *Plant Physiol.*, Supp. 37, ii; August 1962) that, similarly to our present results, total RNA increased when water was limited.

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DEVELOPMENT OF LYSOZYME RESISTANCE IN *MICROCOCCUS LYSODEIKTICUS* AND *SARCINA LUTEA*

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THE development of lysozyme-resistance in *Micrococcus lysodeikticus* was described almost simultaneously by reports from our laboratory[1] and from Brumfitt, Wardlaw, and Park[2]. Afterwards, we demonstrated that our resistant cells were white in colour and devoid of carotenoids[3–6]. In the work described by Brumfitt, Wardlaw and Park[2], and in later work by Brumfitt[7], it was established[8] that the cells examined by the latter workers retained pigmentation and were therefore different cells than those we have obtained in the course of our experimental work. The object of this article, therefore, is to present information on how we have obtained resistant cells, to describe the means by which lysozyme develops resistance in the culture, to corroborate previous findings with regard to resistance and pigmentation in a second strain of organisms, and finally to make suggestions concerning the differences between resistant cells obtained by us and those obtained by Brumfitt and co-workers.

The conditions used for growth of cells and other manipulations have been described previously[1,3,9–11].

Fig. 1 shows the course of development of lysozyme resistance as a function of transfer (sub-culture). The level of sterile lysozyme was 0·1 μg/ml. liquid culture. It is shown that nearly complete resistance (white cells) is obtained rapidly under these conditions (in the initial culture a 40–60 h lag phase is encountered). At the stage of the 7th transfer, lysozyme was omitted from some of the cultures and these afterwards reverted to the yellow, lysozyme-sensitive strain. The resistant, white cells were morphologically identical to the lysozyme-sensitive, yellow cells as examined by Gram-staining. The resistant cells, therefore, appeared to be derived from the starting culture. Ultra-violet irradiation of the white, resistant strain gave rise to 2 yellow strains that conformed to biochemical patterns demonstrated by a strain obtained from ultra-violet treatment of the sensitive parent strain[12].

Lysozyme appears to select the resistant mutant simply by lysing the sensitive cells and permitting the white, resistant organisms to grow out (Table 1).

The value of checking this phenomenon in another strain of organisms (*Sarcina lutea*, A.T.C.C. 272)

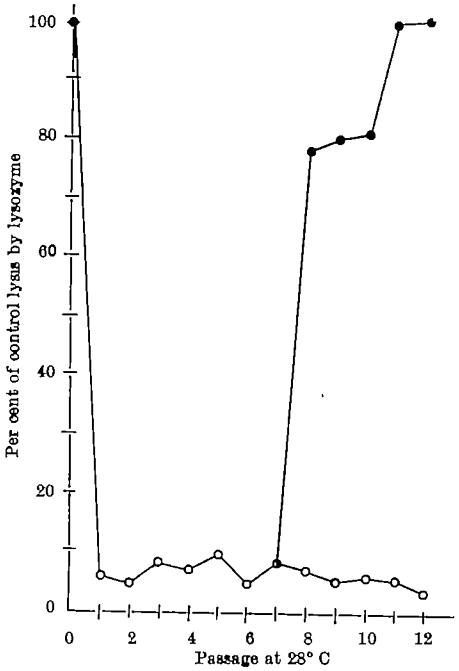

Fig. 1 Course of development of lysozyme resistance in liquid cultures of *M. lysodeikticus* and subsequent reversion to lysozyme-sensitive cells when lysozyme was withdrawn from the medium. Open circles represent cultures with lysozyme; closed circles represent cultures without lysozyme

became obvious. The features of resistance development in *S. lutea* were similar in stepwise experiments to *M. lysodeikticus* except that a more marked intermediate condition was apparent where cells remained yellow and partially resistant to lysozyme up to 1 μg lysozyme per ml. medium. At high concentrations of the enzyme, pure white cells were obtained that were not lysed by 50 μg lysozyme in the assay system. Pure white, resistant cells can be obtained using high levels of lysozyme in the culture (10 μg/ml.) in a one-step experiment and these cells grow out after a lag period when this high amount of lysozyme is combined with the yellow culture.

The content of extractable carotenoid pigments in *S. lutea* in various stages of resistance is illustrated in Fig. 2. This pattern is similar to that previously observed with *M. lysodeikticus*.

The relationship of cell whiteness to resistance and cell yellowness to sensitivity may now be discussed. In view of the facts that Brumfitt *et al.*[2,7,8] developed lysozyme-resistant cells (on agar media) and these cells lost little or no pigmentation, this question deserves some attention. Diphenylamine inhibits carotenogenesis in various organisms[13,14] presumably by interfering with cytoplasmic dehydrogenases and flavin enzymes that catalyse the oxidation of precursors to carotenoid pigments[15-17]. This activity is reversible when diphenylamine-treated cells are sub-cultured without addition of diphenylamine. We investigated the action of this compound on *S. lutea* in relation to the resistance of the resulting cells to lysozyme. Cells carried through several platings from single colonies were used ensuring that no white mutants were detectable by our procedures. Growth of this organism was studied with levels of diphenylamine from 10^{-4} to 5×10^{-4} *M*. The highest level inhibited both rate and extent of growth by about 80 per cent while the lowest level resulted in growth inhibition of 10 per cent. Table 2 shows that inhibition of carotenoid formation does not seriously alter sensitivity to lysozyme in the lysozyme-sensitive organism. This means that carotenoid *per se* is not directly related to lysozyme substrate, a conclusion which is supported by the facts that lysozyme substrate resides primarily in the cell wall and carotenoids are found in the protoplast membrane. It has now become obvious that the cultures studied by Brumfitt and co-workers did not contain the white mutant that is tenaciously associated with our cultures and with *M. lysodeikticus* A.T.C.C. 4698 and many others[13]. We therefore conclude that development of lysozyme-resistance in our case is by selection and that development

Table 1. DEVELOPMENT OF LYSOZYME RESISTANCE BY LYSOZYME IN LIQUID CULTURES OF *M. lysodeikticus*

	Type of inoculum used	Development of white, lysozyme-resistant cells
I.	*M. lysodeikticus*, yellow, lysozyme-sensitive, wild strain	yes
II.	2 replatings of single colony from I, yellow, lysozyme-sensitive	yes
III.	5 replatings of single colony from I, yellow, lysozyme-sensitive	no
IV.	Pure replated white, lysozyme-resistant strain	yes
V.	Pure replated yellow, lysozyme-sensitive cells mixed and equilibrated with pure replated, white lysozyme-resistant cells (10 sub-cultures)	yes (50% smaller lag than I)

Table 2. LYSABILITY BY LYSOZYME AND CONTENT OF CAROTENOID PIGMENTS IN DIPHENYLAMINE-TREATED CELLS OF *S. lutea*

Type of cell	Lysability by lysozyme Δ % *T* 30–60 sec.	Carotenoid content. Absorbancy at peak wave-lengths 413 mμ	438 mμ	467 mμ
Normal	3·0	0 33	0 46	0·43
Grown in 1×10^{-4} *M* diphenylamine	3·0	0·18	0·17	0·11
Grown in 2×10^{-4} *M* diphenylamine	2·7	0 08	0 07	0·05

Absorption spectra of extracts of diphenylamine-grown cells resemble the spectrum of lysozyme-resistant cells (Fig. 2).

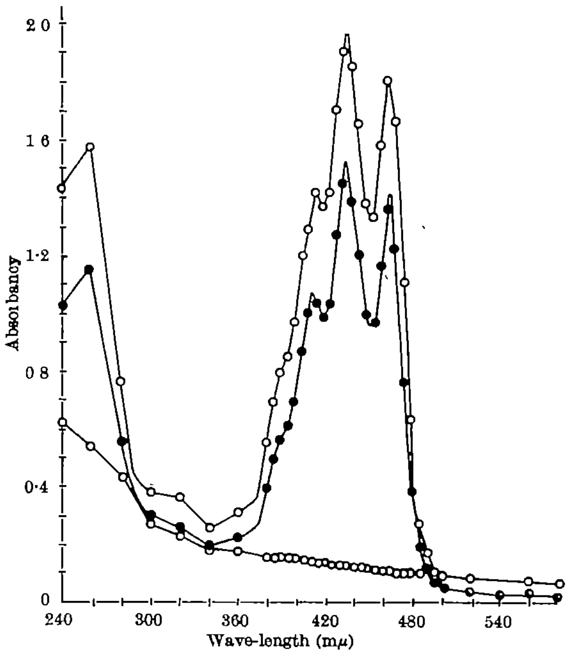

Fig. 2. Spectra of boiling methanol extracts of *S. lutea* in various stages of resistance to lysozyme. Upper curve . normal culture, no lysozyme added ; middle curve ; culture partially resistant to lysozyme ; lower curve . culture fully resistant to lysozyme (no lysis observable in assay system with 50 μg egg white lysozyme added to system). Little difference in absorbancy at maxima exists between the upper curves when absorbancy is based on dry weight of cells

Table 3 EFFECT OF STREPTOMYCIN SULPHATE ON THE DEVELOPMENT OF LYSOZYME RESISTANCE IN *M. lysodeikticus*

Inoculum	Streptomycin sulphate added to culture (mg %)	Crystalline lysozyme added to culture (μg/ml.)	Result
NR*	—	—	Excellent growth–yellow culture
NR-SS†	—	—	Excellent growth–yellow culture
NR	6	—	20 h lag period–yellow culture
NR-SS	6	—	Excellent growth–yellow culture
NR	—	0 2	10 h lag–good growth–white culture
NR-SS	—	0·2	No growth in 54 h
NR	6	0·2	No growth in 54 h
NR-SS	6	0·2	No growth in 54 h

* Refers to artificially mixed pure strains of yellow, lysozyme-sensitive cells and white, lysozyme-resistant cells equilibrated through 5 sub-cultures at 30° C All reported yellow cultures are sensitive to lysozyme. All white cultures are resistant to lysozyme. All cultures were identical morphologically after Gram stain.

† Refers to equilibrated strains above trained on increasing levels of streptomycin sulphate added to the culture to a final level of 6 mg per cent followed by 3 sub-cultures at this level at 30° C.

of resistance in pigmented cells on agar medium may well occur by a biochemical effect on the cytoplasm. We have not been able to reproduce the latter phenomenon in liquid culture with pure, sensitive strains (Table 1), although the existence of coloured resistant mutants (light yellow, red, peach, and pink) of *M. lysodeikticus* has been shown and these strains have been isolated[12]. Stechschulte has shown by indirect selection by replica plating that development of lysozyme resistance on agar media was due to spontaneous mutation[12].

The effect of streptomycin sulphate on the growth of the normal and resistant strains of *M. lysodeikticus* was studied. Both the rate and extent of growth is much more severely affected by the antibiotic in the case of the white, resistant strain. It is likely that the white, resistant mutant could be quantitatively removed from the culture by the use of streptomycin in amounts (10–60 μg/ml.) that would permit considerable survival of the sensitive strain.

This observation was extended to an examination of the effects of streptomycin sulphate on the development of lysozyme resistance in the artificially equilibrated, normal yellow and resistant white strains of *M. lysodeikticus* (Table 3). This shows that streptomycin sulphate caused a long lag period in the growth of the culture not previously trained on streptomycin (formation of streptomycin resistance). This level of streptomycin virtually abolishes the white, resistant mutant. When a low level of lysozyme is added to the culture the resistant cells are rapidly obtained after a brief lag in cultures that were not subjected to streptomycin. The streptomycin-trained cells could not produce the white, resistant cells in 54 h of growth, indicating that the resistant mutant had been destroyed and that the action of lysozyme is one of selection. It is clear that when both streptomycin and lysozyme are added to the culture of trained (to streptomycin) or untrained cells that outgrowth of the white, resistant form fails to occur within 54 h. This indicates that the antibiotic interferes with selection of the mutant by lysozyme either through pretreatment or *de novo*.

In view of the findings presented here, we are re-studying the question of whether the chemical composition of cell walls obtained from white, resistant mutants compared with the yellow, sensitive organism will yield telling chemical information with regard to lysozyme action.

This investigation was supported by research grant *G*-15854 from the U.S. National Science Foundation.

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INCORPORATION OF 1-O-PHOSPHORYL-2-DIMETHYLAMINOETHANOL AND PHOSPHORYLCHOLINE INTO THE PHOSPHOLIPIDS OF BRAIN AND LIVER DISPERSIONS

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THE enzymatic reactions for the formation of phosphatidylcholine and phosphatidylethanolamine by the transfer of phosphorylcholine and phosphorylethanolamine to a D-1,2-diglyceride have been well established by the work of Kennedy[1]. Evidence for another pathway to phosphatidylcholine, probably involving the stepwise methylation of phosphatidylethanolamine, has been demonstrated by Greenberg *et al.*[2,3] and Artom and Lofland[4]. Small amounts of the intermediate monomethyl- and dimethylaminophospholipids have, indeed, been found in liver tissue[5] (see also Hall and Nyc[6]). Free 2-dimethylaminoethanol has also been observed in animal tissues[7], and since this can be phosphorylated *in vivo* and *in vitro*[8,9] it seemed of considerable interest to see if the phosphorylated ester could be incorporated into a phospholipid.

1-*O*-Phosphoryl-2-dimethylaminoethanol and phosphorylcholine labelled with phosphorus-32 were prepared by methods similar to that of Riley[10] and coupled with cytidine-5′-monophosphate to give the appropriate cytidine diphosphate base by the procedure of Kennedy[11]. The nucleotides had the expected molar ratio of cytidine to phosphorus and yielded phosphorylated base and cytidine-5′-monophosphate in a stoichiometric ratio when hydrolysed with acid.

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Dispersions of rat cerebral cortex and liver were prepared in potassium chloride–sodium fluoride as described by Dawson[12] and the equivalent of 100 mg of tissue incubated in the medium indicated in Table 1. After incubation the total lipids were extracted and washed by the method of Folch *et al.*[13].

Table 1. INCORPORATION OF THE RADIOACTIVITY OF CMP-^{32}PC AND CMP-^{32}PD INTO THE LIPIDS OF BRAIN CORTEX AND LIVER DISPERSIONS

Exp. No.	Tissue	Total lipid P extracted (μg)	Per cent incorporation from CMP-^{32}PC	Per cent incorporation from CMP-^{32}PD
1	Brain	125	10·6	—
		125	—	16 5
2	,,	100	8·3	—
		105	—	13·3
3	,,	80	10·5	—
		128	—	16·5
4	Liver	83	10 0	—
		80	—	19·5

Each incubation contained the equivalent of 100 mg of tissue in a medium containing: 0 053 M NaCl, 0·019 M KCl, 0·0118 M KH_2PO_4, 0·0136 M $MgCl_2$, 0·0155 M NaF, 0·025 M $NaHCO_3$, 0·049 M pyruvate, 0·054 M fumarate, $3·2 \times 10^{-5}$ M cytochrome *c*, 0·001 M adenylic acid, 7×10^{-5} M CMP-^{32}PC (367×10^3 c./100 sec/μmole) or 7×10^{-5} M CMP-^{32}PD (213×10^3 c./100 sec/μmole).

Gassed with 95 per cent O_2–5 per cent CO_2. Incubation period 1 h at 37°.

In Table 1 it can be seen that, for both brain and liver dispersions, 13–19 per cent of the radioactivity of the cytidine diphosphate 2-dimethylaminoethanol (CMP-^{32}PD) was incorporated into tissue lipids, an amount corresponding to about 0·32 μmoles of phosphorylated base/g fresh weight/h. The incorporation of the radioactivity of the cytidine diphosphate choline (CMP-^{32}PC) was somewhat lower, possibly because of dilution of the added nucleotide with endogenous material. When the free [^{32}P] phosphate esters were incubated with the dispersions the incorporation of radioactivity into lipids was only about 0·1 per cent of the material added.

Chromatography, on a column of aluminium oxide, of the lipids extracted after incubation with the two different nucleotides gave the result indicated in Fig. 1. Incubation with CMP-^{32}PC yielded a radioactive lipid quantitatively eluted with chloroform/methanol (1:1 v/v) behaving, as expected, like phosphatidylcholine[14]. With CMP-^{32}PD as substrate, the presence of 4–6 per cent water in the chloroform/methanol (1:1 v/v) was required to elute the radioactive lipid obtained. Changing the eluant to ethanol/chloroform/water (5:2:2 v/v/v)[15] brought off non-radioactive phospholipid which was a mixture of ethanolamine and inositol-containing lipids. The behaviour on an alumina column of the lipid obtained on incubation of tissue with CMP-^{32}PD was that expected of a phosphatidyl-2-dimethylaminoethanol. In further experiments an amount of this lipid material containing 2 μg phosphorus and 1,860 counts/100 sec was mixed with an amount of synthetic distearoylphosphatidyl-2-dimethylaminoethanol ≡ 20 μg phosphorus (kindly supplied by Prof. E. Baer). After adsorption on alumina (Fig. 2) all the radioactivity and added phospholipid were eluted almost coincidentally with methanol–chloroform–water; the slight difference might be due to the saturated fatty acids in the synthetic material.

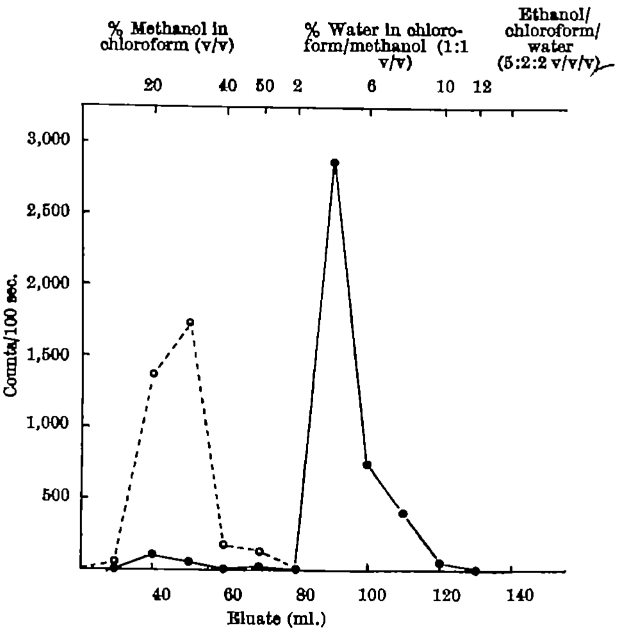

Fig. 1. Chromatography on alumina of the lipids obtained after the separate incubation of brain tissue with the radioactive nucleotides (Table 1, Exp. 1). An amount of lipid ≡ 60 μg phosphorus was applied to a 1-g column (0 6 × 15 cm) and the elution carried out in a step-wise fashion. Broken line, incubation with CMP-^{32}PC; full line, incubation with CMP-^{32}PD

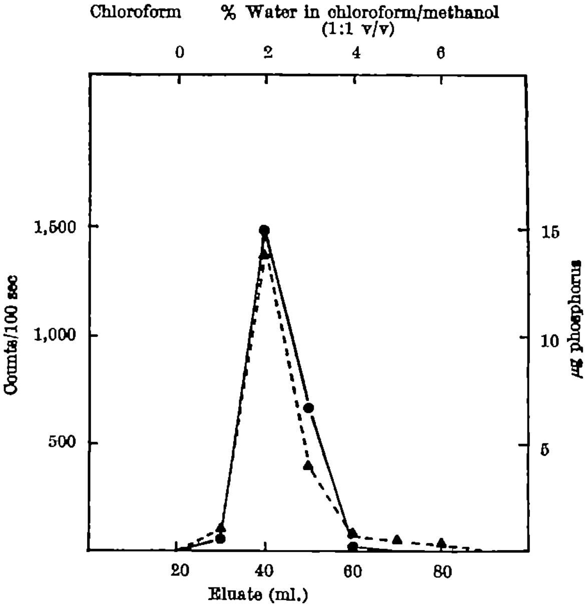

Fig. 2. Chromatography on alumina of synthetic distearoyl phosphatidyl-2-dimethylaminoethanol with the radioactive lipid obtained after incubation of brain tissue with CMP-^{32}PD. The radioactive fraction (2 μg phosphorus and 1,860 c./100 sec), obtained in an experiment similar to that shown in Fig. 1, was mixed with synthetic lipid (20 μg phosphorus), re-adsorbed on a column (0·5 g and 0·6 × 7 cm) and eluted as indicated. Full line, μgP; broken line, counts/100 sec

Paper chromatography of the total lipid extract on the silicic acid paper in the chloroform/methanol (4:1 v/v) solvent of Lea, Rhodes and Stoll[16] or the di-isobutylketone/acetic acid/water (40:20:3 v/v/v) solvent of Marinetti[17] gave only one radioactive spot which behaved similarly to the synthetic lipid. 'Thin layer' chromatograms run on plates of 'Silica gel *G*' (Merck) by Mr. J. Chamberlain of the Department of Anatomy gave similar results. When the radioactive fraction obtained from an alumina column (Fig. 2) was freed from cerebrosides with 'Florisil' and run in these chromatographic systems with increasing amounts of the synthetic lipid, the results were again consistent with the conclusion that incubation of CMP-^{32}PD with a dispersion of brain or liver leads to the formation of radioactive phosphatidyl-2-dimethylaminoethanol.

These findings strongly suggest that phosphatidyl-2-dimethylaminoethanol can be formed in tissues by a pathway analogous to that described for lecithin by Kennedy. It may have some bearing on the observation of Artom[9] and Bremer and Greenberg[3] that, although such a dimethylaminophospholipid could be methylated in the presence of liver microsomes to yield lecithin, methylation of the ethanolamine-containing lipids was not observed.

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ROD RECEPTOR POTENTIAL FROM THE RETINA OF THE NIGHT MONKEY

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METHODS of isolating and identifying receptor potentials in monkey retinæ have been reported[1]. The most general method of isolation is insertion into the eye of a polished steel rod, which is pressed on the retinal vessels where they enter the eye at the optic disk; the retinal circulation may thus be completely clamped, leaving the choroidal circulation intact. This method was first applied in *Cynamolgus* and *Rhesus macaque* monkeys[1], which are diurnal species with a pure cone fovea. Intraretinal microelectrodes were used for recording the local ERG(LERG) from a small retinal area immediately surrounding the electrode. This LERG, like that of the cat, proved to consist of four major components[1–3]. When the retinal circulation was clamped, both the *b*-wave and DC component were completely abolished. These components are from cells of the inner nuclear layer[1,3]. The *c*-wave from the pigment epithelium was not abolished, but in diurnal monkeys the *c*-wave is quite small; thus essentially complete isolation of the fourth component was attained. This component was stably maintained by the choroidal circulation and was identified by rigorous criteria as a receptor potential. The pure cone receptor potential was obtained by recording from the central fovea, while stimulating with small light spots. This pure cone response rises rapidly to a plateau, which is well maintained during long stimuli, and after the stimulus this response decays rapidly to the baseline. The leading edge of the rising cone potential contributes the *a*-wave of the LERG, while the decay of the cone potential contributes the initial phase of the *d*-wave.

Similar isolation of the pure rod receptor potential was next desired. Preliminary investigation of the cat indicated that the time-course of the rod potential is slower than that of cones, particularly in the decay phase[1]. The cat retina is not ideal, however, for this problem; the *b*-wave is not entirely eliminated by clamping the retinal circulation, the *c*-wave is exceptionally large, and cones are present in addition to the rods. Thus the South American night monkey, *Aotus trivirgatus*, a strictly nocturnal species, was investigated.

Experiments were performed on 20 adult animals. Although the body-weight was only 0·5–1·0 kg, the eye diameter was about 18 mm, which was more than adequate for our procedures. Recording methods were basically as described for microelectrode work in the retina of the intact cat eye[4]. Anæsthesia was maintained by intraperitoneal injections of thiopental sodium. A device was used for insertion of three needles into the eye. One needle was used for a tungsten microelectrode, with tip diameter less than 0·5μ, the retinal depth of which was controlled by a hydraulic electrode advancer. A chlorided silver reference electrode was inserted through the second needle into the vitreous humour. The steel rod for clamping the retinal circulation was inserted through the third needle. A light stimulator provided retinal stimuli which were well focused and readily controlled in size and position. Responses were led to a cathode follower and amplified by conventional direct-coupled methods.

Fig. 1 shows responses under normal conditions, and with the retinal circulation clamped, when the electrode was near the retinal side of Bruch's membrane. The normal record shows a positive *a*-wave and negative *b*-wave. When the stimulus terminates there is no *d*-wave, defined as a negative deflexion followed by a positive deflexion. Instead there is only a positive deflexion, as found in the cat, which has been shown due to termination of the negative DC component from the inner nuclear layer[2,3]. There is also a slow positive potential which long outlasts the stimulus, consisting partly of the *c*-wave.

When the retinal circulation was clamped, the *b*-wave was abolished. The positive deflexion after the stimulus was also abolished, indicating elimination of the negative DC component. The remaining response was a summation of the positive receptor

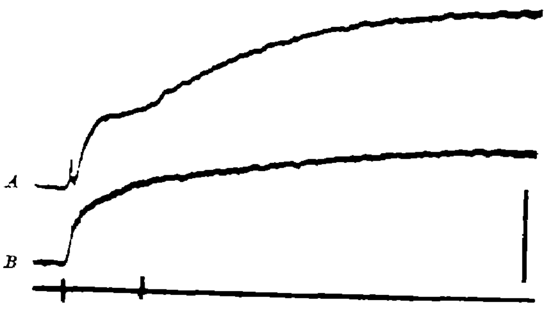

Fig. 1. LERG's recorded by a microelectrode adjacent to the retinal side of Bruch's membrane. Record *A* made under normal conditions, and in *B* the retinal circulation was clamped. The retinal stimulus spot was about 1·5 mm in diameter, focused and centred on the electrode. Stimulus intensity was maximum for the optical system, stimulus duration was 320 msec, and the repetition-rate was one every 30 sec. Positive potentials shown upward, both here and in Fig. 2. following the convention for ERG work. Calibrating voltage signal 1·0 mV

potential and positive *c*-wave. This response shows a rapid initial rise, the latency of which corresponds to that of the *a*-wave in the normal LERG. When the stimulus terminates, however, there is no sign of a rapid negative deflexion; thus the rod potential must decay very slowly. Since the receptor potential and *c*-wave are both positive in polarity with this method of recording, the general form of the rod potential is not entirely clear. This may be shown better by recording the ERG of the entire retina with extra retinal leads. For this type of recording the active electrode was a chlorided silver wire in the vitreous humour, and the reference electrode was a chlorided silver wire in the mouth. It has been shown previously that the same basic components are found in the LERG and ERG[3]. It has also been shown that all components except the *c*-wave invert polarity between the LERG and the ERG[3]. Thus in the ERG the receptor potential is negative, but the *c*-wave is positive.

Fig. 2 shows that the normal ERG consists of a negative *a*-wave, positive *b*-wave, and a negative deflexion after the stimulus. There is also a slow negativity during and following the stimulus. When the retinal circulation was clamped, both the *b*-wave and the negative deflexion after the stimulus were abolished. The remaining potential shows an initial rapid rise of negativity, the leading edge of which corresponds in time to the *a*-wave of the ERG. The negativity throughout this response must be due to the receptor potential, which is sufficiently large to mask the positive *c*-wave. Thus the rapid initial rise is clearly the rising phase of the rod potential. It is also clear that when the stimulus terminates this rod potential has considerable magnitude and decays very slowly. Fig. 2 likewise shows that the rod potential appears to increase in amplitude when the retinal circulation is clamped. This effect is particularly noteworthy in the decay phase, where results of eliminating the *b*-wave and DC component should be minimal, but it is not clear whether clamping the retinal circulation produces a true increase of the rod potential. Evidence for such an effect is much clearer for cones, especially in the fovea, where a tentative interpretation has been offered[1]; these effects are of special interest and require further study.

After the initial rapid rise of the rod potential in Fig. 1, the secondary phase of slow rise is probably due at least partly to the *c*-wave. An even more marked phase of secondary slow rise is evident in Fig. 2, where it is undoubtedly due partly to recording a summation of receptor potentials which have different latencies because of variations in stimulus intensity to different retinal areas[3]. It is not yet known whether a secondary phase of slow rise is a true characteristic of the rod potential. During stimuli of several seconds duration, the amplitude of the rod potential is well maintained; this is true in both intraretinal and extraretinal records. The time-course during long stimuli appears more variable, however, than that of the cone potential. The *c*-wave must be eliminated to determine further details concerning the time-course of the rod potential.

Identification of the rod potential of the night monkey, as a true response of the receptors themselves, has been made by the same criteria used for cones[1]. The only exception is that the night monkey has no fovea, for comparison of receptor potential amplitudes between the fovea and periphery. It is also pertinent, from the point of view of identification, that the same procedure has been used for isolating both rod and cone responses. *S*-potentials of mammalian retinæ, which are single unit slow potentials from the inner nuclear layer[5], have now been found to survive clamping of the retinal circulation in all four species studied. Receptor potentials are readily distinguished from *S*-potentials, however, since the latency of the receptor potential has been shorter than that of the *S*-potential in all cases noted. Apparently the cells which generate *S*-potentials are unique, among cells of the inner nuclear layer, in the ability to maintain their functions from the choroidal circulation. A previously considered possibility, that the *b*-wave and DC component of the LERG might arise from the same cells which generate *S*-potentials[3], must therefore be abandoned.

Svaetichin has concluded, from work in fish and frog, that the receptor potentials of rods and cones are opposite in polarity[4]. This conclusion was based on evidence interpreted to indicate that the *a*-wave was a cone response and the *b*-wave was a rod response. This conclusion must be rejected, at least for the mammalian retina. The *b*-wave does not arise from receptors[1,3]. With any given method of recording, the polarities of rod and cone potentials are the same; also in both cases there is an initial rapid rise, the leading edge of which contributes an *a*-wave. Both types of receptor potential are likewise well maintained during long stimuli. After the stimulus, however, the cone potential decays rapidly while the decay of the rod potential is delayed

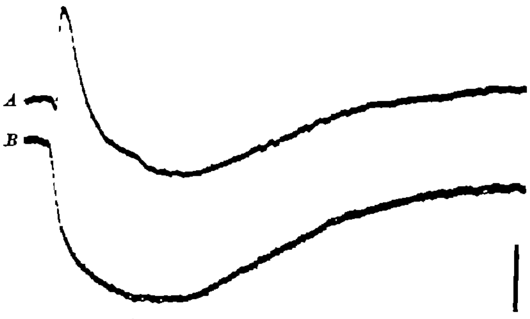

Fig. 2. ERG's recorded between an active electrode in the vitreous humour and a reference electrode in the mouth. Record *A*, normal conditions; record *B*, retinal circulation clamped. Stimulus was a retinal spot about 4·0 mm in diameter, of maximum intensity for the optical system, 320 msec in duration, and repeated once every 30 sec. Calibrating voltage signal 0·3 mV.

and very slow. It may therefore be noted that the mammalian retina is another example of a sensory system containing both fast and slow receptors. In this case the fast receptors are the cones, while the slow receptors are the rods, and the distinction is clearest in terms of the decay rates. Similar principles may be expected in retinæ of lower vertebrates, and some recent observations suggest they pertain in fish[7].

The rapidly rising phase of the rod receptor potential inverts polarity within the retina during electrode withdrawal. Maximum amplitude of this rapidly rising phase, of positive polarity, is found near the distal tips of the receptors. During electrode withdrawal this response declines to zero, becomes negative, and maximum amplitude of the negative response is found near the proximal receptor terminals. Similar findings have been reported for the cone potential[1]. The time-courses of the positive and negative potentials, at different retinal levels, appear to be identical; this is true in both rod and cone cases. These findings provide the key for a working hypothesis concerning the electrical activity of rods and cones. Assuming that the receptor potential is due to a depolarization, which seems required by general principles of receptor action, these findings indicate that the depolarization is initiated at the presynaptic membrane of the receptor-bipolar synapse. The positive potential of identical latency, recorded from near the distal tip, may be explained as resulting from the electric field which this depolarization produces along the receptor axis. Depolarization of the presynaptic membrane may be assumed to be associated with release of a transmitter from the synaptic vesicles which have been described at the receptor-bipolar synapse[8]. Hence this depolarization is probably the electrical end-product of receptor activity. The minimum latency of the receptor potential, as determined to date, is about 1·7 msec. This is too long for a response which directly reflects photochemical isomerization, and indicates an intervening process. The mode of conduction from the outer segment to the proximal receptor terminal must therefore be considered. Evidence for impulse activity in mammalian visual receptors has been sought, under a variety of theoretically favourable conditions, but no sign of impulses produced by any portion of the receptor has ever been obtained. The possibility of a depolarization which is electrotonically conducted in the orthodromic direction also seems definitely excluded by the findings reported here. Thus neither of the known modes of conduction, involving the outer membrane of the cell, appears to apply in the case of retinal receptors. Serious consideration must therefore be given to the possibility that conduction from the outer segment to the proximal terminal is by some type of activity in intracellular fibrils oriented along the receptor axis. Such fibrils have been described in monkey rods and cones by Cohen[9], who also finds them in mouse rods and speculates concerning a conductive function[10]. The conduction time in these fibrils may account for the latency of the receptor potential.

In the night monkey slender 'rudimentary' cones have been found scattered among the rods[11]. Walls considers that this retina evolved from a diurnal type, and that these receptors are in a transitional stage of evolution from cones to rods[11]. Since no rapid decay phase can be detected in the isolated receptor potential of the night monkey, its receptor potential must be due entirely to rod activity. Thus the 'rudimentary' cones may be responding with the rod type of potential, although retaining some histological characteristics of cones. On the other hand, typical cone responses may occur in receptors which are too sparsely scattered to give a detectable contribution to the massed receptor potential. In either case the night monkey appears to be an excellent animal for the study of pure rod activity by means of the isolated but massed receptor potential. Whether scattered functional cones are present must be determined by other methods.

It has also been found that the pure rod and pure cone forms of receptor potential are the extremes between which many combinations may be obtained. In a response from a mixed population of receptors, the relative contributions of cones and rods should be indicated by the relative amplitudes of the rapid and slow decay phases of the receptor potential. The cat retina is dominated by rods, but also contains cones[12]. The cat's receptor potential is predominantly like that of the night monkey, but after the stimulus a small phase of rapid decay has now been detected. Although small in amplitude it has proved entirely reliable and undoubtedly represents decay of the cone potential in this animal. In diurnal monkeys the relative contributions of cones and rods to the response may be varied readily by comparing foveal with peripheral responses, and by varying the stimulus spot size while recording from the fovea These methods of altering the proportions of responding cones and rods have produced the predicted effects on relative amplitudes of the fast and slow decay phases of the receptor potential. The described form of rod potential has therefore been detected in all four species studied, and the described form of cone potential has been found in all of these except the night monkey. This makes it very likely that the reported differences in decay rate, of rod and cone potentials, represent fundamental and quite general differences between the receptor potentials of rods and cones. It is also evident that relative amplitudes of the rapid and slow decay phases, in the isolated receptor potential, provide a method for study of relative cone and rod activity.

The major qualitative differences between rod and cone ERG's occur in that portion of the response following termination of the stimulus. These differences may now be understood from the decay rates of rod and cone potentials. In typical cone ERG's, after the stimulus there is a rapid positive deflexion preceding a negative deflexion (the *d*-wave)[13]. This *d*-wave is found in the retinæ of diurnal monkeys, where it has been shown due to rapid decay of the receptor potential, followed by decay of the DC component from the inner nuclear layer[1]. In Fig. 2 the initial positive deflexion does not occur; this absence of the *d*-wave is typical of rod ERG's (ref. 13), and is undoubtedly because there is no rapidly decaying phase of the rod potential. On the other hand, the long-lasting negativity following the stimulus, as noted in Fig. 2, appears to be found only in rod ERG's. This is clearly due to the prolonged decay phase of the rod potential.

The problem of how off-responses are generated, in neurones of the second order and higher levels, may also prove related to the decay rates of receptor potentials. In the night monkey the dominant response of ganglion cells, evoked by large stimuli, is an on-response. Off-responses have been observed in the light-adapted state, but these are rare by comparison with the cat. These rare off-responses may be mediated by scattered functional cones, and future study of this problem is required.

The different decay rates of rod and cone potentials are probably the basis for certain functional differences between rods and cones which have long been known from psychophysical studies. The rapid decay of the cone response is theoretically advantageous for following flickering light stimuli. It is probably the physiological basis of the well-known fact that the fusion frequency of cones is higher than that of rods, which means that cones have a superior ability to resolve stimuli in time. Considering the fact of physiological nystagmus, this superior time resolution of cones may also confer some degree of superiority for spatial resolution. On the other hand, the very slow recovery of the rod potential must contribute to the greater susceptibility of rods to light adaptation, as determined by the elevation of threshold for a stimulus delivered shortly after terminating the adapting light.

Since some of the functional differences between rods and cones appear to be determined by the decay rates of rod and cone potentials, the form of the receptor potential suggests itself as a criterion for determining whether receptors are functionally rods or cones. In certain cases such a criterion may be crucial. For example, duplicity of function has recently been demonstrated in the retina of the nocturnal gecko[14]. This animal shows no Purkinje shift; also the receptors appear to be a single histological type, and are believed to be rods which have evolved from cones[14]. The ERG of this animal shows a well-developed *d*-wave, however, and the functional duplicity includes a higher fusion frequency in the photopic state[14]. Interpretation of these facts, in view of the decay rates of rod and cone potentials, suggests that functional differences may occur among receptors which show no differences in structure or contained photopigments.

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INDEPENDENCE OF WHOLE BLOOD AND HÆMOGLOBIN SOLUTION OXYGEN DISSOCIATION CURVES FROM HÆMOGLOBIN TYPE

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IN the transfer of oxygen across the placenta of the human being and of a variety of animal species an important role has been ascribed to the differences in the oxygen affinity of fœtal and maternal blood. The higher affinity of fœtal blood for oxygen has been considered an advantage in the transfer of oxygen from the maternal to the fœtal circulating blood streams across a barrier, the placenta.

It is commonly assumed that the differences in the physiological characteristics of two different populations of red cells are adequately accounted for by finding differences in the amino-acid sequence of the two hæmoglobins within the cells. In point of fact, this assumption has not been restricted to discussions of adult and fœtal hæmoglobin types alone. In spite of the general acceptance of this assumption evidence has accumulated that does not warrant unquestioned acceptance of such an explanation for the difference in the positions of maternal and fœtal whole blood oxygen dissociation curves.

This evidence can be summarized as follows:

(1) Oxygen dissociation curves of solutions of hæmoglobin obtained from cord blood and from normal adults showed no difference between the oxygen affinities of such solutions[1].

(2) In one report, superimposable oxygen dissociation curves were found for fœtal bloods in which the relative amounts of hæmoglobin F-A varied from 1 : 1 to 4 : 1, providing such curves were constructed at identical *p*H. The proportions of fœtal to adult hæmoglobin were measured in the same blood samples with which the dissociation curves were determined[2].

Results are reported here on the oxygen dissociation curves of hæmoglobin solutions prepared from bloods containing hæmoglobins AA, AF and SS at various *p*H values. They confirm and extend the findings described in sub-heading (1).

It may be argued that in the course of the preparation of hæmoglobin solutions from whole blood the different hæmoglobin types are so altered as to cause elimination of the differences in their oxygen affinities. For this reason we are reporting the oxygen dissociation curve of whole blood obtained from an adult with 69 per cent fœtal and 31 per cent adult hæmoglobin showing this curve to be markedly different from that of cord blood with the same proportion of the two hæmoglobins, and in fact identical with that of normal adults whose bloods contain 100 per cent hæmoglobin A. This patient is the propositus R. M. in a report previously published from this hospital[3] and the details of her medical history can be found in that report.

The adult and fœtal blood used for these investigations was obtained from patients delivered on the obstetrical service of the Johns Hopkins Hospital. Sickle-cell whole blood and blood from the patient R. M. were obtained through courtesy of the Hæma-

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* Henry Strong Denison Scholar, 1960-62.

† Senior Research Scholar, Joseph P. Kennedy, jun., Memorial Foundation.

Table 1. POINTS ON OXYGEN DISSOCIATION CURVES OF HÆMOGLOBIN AA SOLUTIONS

pH	P_{O_2}	S	pH	P_{O_2}	S
6·90	13·87	20·17	7·40	13·69	37·53
,,	16·89	25·53	,,	15·36	50·76
,,	19·16	31·06	,,	15·75	59·47
,,	21·25	32·84	,,	17·37	65·29
,,	22·64	43·50	,,	19·47	73·16
,,	24·83	50·63	7·90	7·41	35·57
,,	25·04	46·29	,,	8·03	51·10
,,	27·55	55·86	,,	8·09	36·78
,,	27·83	63·02	,,	8·12	53·06
,,	30·52	71·90	,,	8·81	45·88
,,	31·41	68·06	,,	8·88	58·09
7·40	8·70	24·11	,,	8·91	39·00
,,	10·76	31·60	,,	9·21	44·43
,,	11·18	27·30	,,	9·64	46·39
,,	12·54	39·05	,,	10·10	61·10
,,	13·07	44·83	,,	10·25	56·12
,,	13·54	48·97	,,	11·25	65·13

Table 2. POINTS ON OXYGEN DISSOCIATION CURVES OF HÆMOGLOBIN FA SOLUTIONS

pH	P_{O_2}	S	pH	P_{O_2}	S
6·90	20·10	37·80	7·40	14·10	53·34
,,	20·17	36·28	,,	14·49	59·56
,,	21·59	46·25	,,	16·06	69·05
,,	23·22	51·65	,,	16·56	65·41
,,	24·22	48·98	,,	17·64	69·58
,,	25·22	51·06	,,	17·99	73·02
,,	26·03	56·93	7·90	6·25	25·19
,,	27·30	56·93	,,	7·27	29·96
,,	28·07	56·07	,,	7·39	35·75
,,	29·58	60·94	,,	8·01	39·72
,,	30·23	59·74	,,	8·02	29·57
,,	31·22	69·26	,,	8·41	43·69
7·40	9·58	23·29	,,	8·95	48·92
,,	10·85	42·22	,,	9·28	53·08
,,	11·28	26·13	,,	9·87	59·55
,,	12·21	46·29	,,	9·91	54·96
,,	12·59	47·20	,,	10·39	63·97
,,	13·57	51·21			

Table 3. POINTS ON OXYGEN DISSOCIATION CURVES OF HÆMOGLOBIN SS SOLUTIONS

pH	P_{O_2}	S	pH	P_{O_2}	S
6·90	19·94	29·80	7·40	14·14	40·11
,,	20·09	33·93	,,	14·17	49·08
,,	21·30	38·06	,,	14·48	61·36
,,	22·16	37·54	,,	14·94	54·32
,,	24·32	51·76	,,	15·11	57·88
,,	24·50	44·51	,,	15·80	64·38
,,	26·13	53·24	,,	15·99	65·33
,,	26·22	60·20	,,	17·56	59·13
,,	27·79	58·72	,,	17·97	73·26
,,	28·64	53·64	7·90	5·83	25·07
,,	28·85	63·81	,,	6·05	23·61
7·40	9·39	22·12	,,	7·05	29·93
,,	11·47	44·69	,,	7·31	25·99
,,	12·35	34·34	,,	7·80	34·06
,,	12·35	51·53	,,	8·48	53·09
,,	12·90	32·08	,,	8·64	48·24
,,	13·14	38·60	,,	9·11	51·56
,,	13·27	50·67	,,	9·81	60·85
,,	13·46	55·28	,,	10·36	67·81

tology Clinic of this Hospital. Construction of oxygen dissociation curves was performed by the technique previously described by Hellegers *et al.*[4]. Hæmoglobin solutions were obtained from whole blood as follows: the blood was centrifuged for 30 min at 3,300 r.p.m. in centrifuge cups previously chilled on dry ice. The plasma was removed and the cells washed 5 or 6 times with an approximately 4-fold volume of cold 0·9 per cent saline. The final packed red cells were then frozen for 30 min on dry ice and allowed to thaw at room temperature. The disrupted cell stroma was centrifuged at 4,500 r.p.m. for 10 min and the supernatant hæmoglobin solution filtered in the cold through activated charcoal (6–14 mesh) previously wet with cold 0·9 per cent saline. The approximate hæmoglobin concentration in the resultant solution was determined either by use of a hand protein refractometer (Hitachi) or colorimetrically. The solutions were then diluted accordingly with physiological saline to a final concentration of 4·4 g per cent.

The hæmoglobin solutions were buffered at pH's of 6·9 and 7·4 with 0·1 M phosphate buffer and at pH 7·90 with *tris*(hydroxymethyl) amino-methane (THAM) buffer. Curves at pH 7·40 were constructed alternatively with phosphate or THAM buffer. The use of either buffer produced identical dissociation curves, even in the one case buffered by a THAM–hydrochloric acid mixture at a 1·1 M concentration. The final hæmoglobin solution–buffer mixture was at a concentration of 4 g per cent.

Blood gas analyses were performed by the technique of Van Slyke and Neill[5], gas analyses by the method of P. F. Scholander[6] and pH measurements on the Beckman model *G*, pH meter. The sickle whole blood values obtained were corrected to 38° C by means of Rosenthal's factor[7]. The pH measurements for the hæmoglobin solution dissociation curves and for the whole blood dissociation curve of the adult R. M. were carried out on a radiometer pH meter, model *pHM 4C*, at a temperature of 38° C.

Tables 1, 2 and 3 contain the P_{O_2}, percentage saturation, and pH of each point of the dissociation curve for adult, cord, and sickle hæmoglobin solutions.

By means of the least-square method, a regression analysis was carried out on these results to obtain the oxygen tensions required to half-saturate the three different hæmoglobin solutions at pH 6·9, 7·4 and 7·9 respectively.

The standard deviations from the regression for the data of hæmoglobin solutions AA, FA and SS were 0·035, 0·034 and 0·033 respectively.

In Table 4 the oxygen tensions required to half-saturate the respective hæmoglobins at the various pH's are given.

It will be noted that the different hæmoglobin solutions have identical oxygen affinities at the three stated pH values. In addition, since these identical affinities hold true at all pH's, the Bohr effects of the three hæmoglobin solutions must be identical within these pH limits.

In Table 5 are given the P_{O_2}, percentage saturation, and pH of an eight-point oxygen dissociation curve constructed with the blood of the adult patient with 69 per cent persistent hæmoglobin F. In the fourth column of the same table the P_{O_2} given denotes that (obtained from the nomogram of Hellegers and Schruefer[8]), which would produce the same percentage saturation at the same pH, if the blood of the patient had contained only AA hæmoglobin. The P_{O_2} given in column 5 denotes that which would have been required to produce the same saturation at the same pH, similarly obtained, if the blood sample were of umbilical cord origin.

It is thus obvious that the oxygen dissociation curve of the particular patient's blood corresponds

Table 4. P_{O_2}/MM MERCURY REQUIRED TO HALF-SATURATE HÆMOGLOBIN SOLUTIONS AA, FA AND SS AT pH 6·90, 7·40 AND 7·90

	pH 38° C 6·90	7·40	7·90
Adult	24·15	14·69	8·95
Cord	23·55	14·29	8·67
Sickle	24·32	14·26	8·38

Table 5. P_{O_2} REQUIRED TO PRODUCE THE PERCENTAGE SATURATION AT THE GIVEN pH IN THE PATIENT R. M. COMPARED WITH ADULT AND CORD WHOLE BLOOD

pH (38° C)	Percentage saturation	P_{O_2} mm Hg. Patient R.M.	P_{O_2} mm Hg. Adult Blood*	P_{O_2} mm Hg. Cord Blood*
7·398	27·2	19·4	18·8	13·3
7·354	34·7	21·0	22·0	16·7
7·370	41·8	23·8	24·2	18·0
7·358	35·9	23·1	22·2	16·8
7·347	55·6	28·1	29·3	23·5
7·358	61·6	30·3	31·9	25·6
7·352	64·1	31·0	34·0	27·0
7·363	63·7	33·2	33·1	26·5

*From nomogram

within the limits of experimental error with that obtained in normal adults, and is significantly different from that obtained with blood of fœtal origin.

In a previous report from this laboratory[9] we have shown that the oxygen dissociation curves constructed with blood from non-pregnant and pregnant patients are identical, providing the curves are constructed at identical pH.

In another report[4] it was shown that any point on the oxygen dissociation curve of whole blood could be obtained by the use of the equation:

$$\log P = K_1 - K_2\,p\mathrm{H} + K_3 \log \frac{S}{100\text{-}S}$$

This equation yields constants K_1, K_2 and K_3 for maternal and fœtal blood respectively. For human maternal blood the three constants are 4·734, 0·45 and 0·351 respectively, and for fœtal blood 5·748, 0·60 and 0·389 respectively. In this equation the constant K_3 describes the slope of the oxygen dissociation curve and is identical with the reciprocal of the constant n in Hill's equation[10]. It is not statistically significantly different for adult and fœtal whole blood.

The constant K_2 reflects the magnitude of the Bohr effect, describing the amount by which the position of oxygen dissociation curves shifts per unit pH change. It is significantly different for adult and fœtal whole blood of humans and also for adult and fœtal blood of goats[4]. Applying the constants in this equation, half saturation of maternal whole blood at a pH of 6·9, 7·4 and 7·9 is produced at a P_{O_2} of 42·6, 26·2 and 15·1 mm. mercury respectively. Comparative P_{O_2}'s calculated for umbilical cord blood would be 40·6, 20·3 and 10·2 respectively.

Numerous other workers have also shown half saturation of maternal and fœtal blood to be produced at P_{O_2}'s of about 26 and 20 mm mercury respectively providing the blood is at pH 7·40[11-14].

It will be seen, therefore, that despite the wide variety of percentages of fœtal and adult hæmoglobin reported to exist in the bloods of fœtuses at birth, the reported oxygen dissociation curves for whole blood of fœtuses are remarkably constant at identical pH's. On a statistical basis it would therefore be unlikely that the percentage of fœtal hæmoglobin contained in the erythrocytes would, by itself, be the sole determinant of the position of the oxygen dissociation curve. Indeed, Allen, Wyman and Smith[1] reported that cord blood and adult blood hæmoglobin solutions showed identical oxygen affinities at pH 7·20. Their points were corrected to this pH on the assumption of a K_2 of 0·60. Thus, even assuming this K_2 to be applicable to hæmoglobin solutions, their results provided no information on the magnitude of the Bohr effect in hæmoglobin solutions. Our data clearly demonstrate that the differences between the affinities of SS, AA and FA hæmoglobin, normally present when these hæmoglobins are in their red cell environment, disappear on their removal from the erythrocytes. In this respect our results therefore confirm those of Allen, Wyman and Smith. The results also show that the difference in the Bohr effect of fœtal and maternal blood disappears when the hæmoglobin is removed from its erythrocyte environment. For the hæmoglobin solutions then constant K_2 does not differ from that applicable to the whole blood of adults, that is, 0·45.

It might be argued that differences between adult and fœtal hæmoglobin should not be equated with differences between other hæmoglobin types, considered pathological, since the presence of fœtal hæmoglobin in cord blood is an invariable natural finding, not seemingly related to a specific genetic mechanism. It was for this reason that we also sought to study hæmoglobin solutions obtained from blood of patients with electrophoretically proved sickle-cell anæmia. This was done in the knowledge that the oxygen dissociation curves of sickle-cell whole blood obtained by the above method are displaced to the right of those of normal adult blood[15], while conflicting data for sickle hæmoglobin solutions exist[16,17]. It will be noted that sickle hæmoglobin solutions have the same oxygen affinity as hæmoglobin solutions AA and FA.

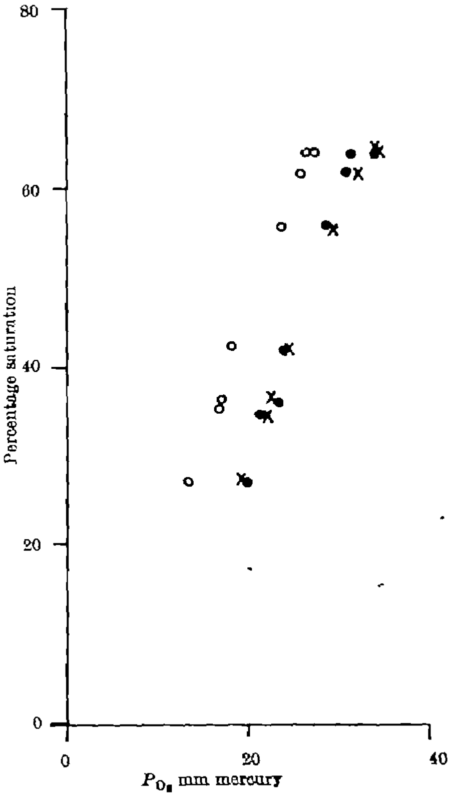

Fig. 1. Oxygen dissociation curve for the blood of patient R. M. (●), normal adult blood (×) and cord blood (○).

Previously it was shown that blood of fœtal origin, containing 69 per cent fœtal hæmoglobin and 31 per cent adult hæmoglobin, gave a whole blood oxygen dissociation curve which was in no way distinguishable from that of fœtal blood with other widely varying proportions of adult and fœtal hæmoglobin. Since a whole blood, obtained from an adult, but containing the same proportion of fœtal to adult hæmoglobin as many cord bloods, yields an oxygen dissociation curve (Fig. 1) identical with that of blood containing 100 per cent adult hæmoglobin—and different from that of cord blood with the identical proportion of the two hæmoglobins—it can only be concluded that the hæmoglobin type, *per se*, is not the determinant of the position of whole blood oxygen dissociation curves.

Some other factor or factors, operating in conjunction with or independently of the hæmoglobin type within the erythrocyte, must therefore be the determinant of the position of whole blood oxygen dissociation curves.

The results from experiments designed to elucidate the role of such other factors will be the subject of a future report.

We thank members of the Division of Hæmatology, Department of Medicine, the Johns Hopkins Hospital, and in particular Dr. C. Lockard Conley, Dr. Samuel Charache, and Dr. David Weatherall, for their co-operation.

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TRICHOBEZOARS (HAIR BALLS) IN *RATTUS ASSIMILIS*

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THE occurrence of trichobezoars in the stomach is well known in certain of the ungulates and carnivores, and even in man. Among rodents hair balls have been reported from *Sigmodon*[1], *Peromyscus*[2] and *Rattus norvegicus*[3]. This article extends these findings to a native Australian rat, *Rattus assimilis*, and gives additional information regarding the cause and prevention of trichobezoars.

The nucleus of the stock of animals here described was live-trapped in the Hawkesbury River area of coastal New South Wales, Australia, in August of 1955, and flown to the United States. Here the stock was divided into two colonies. One colony was maintained at the University of California at Berkeley and the other at Smith College. The animals bred well in both colonies[4], producing a total of several hundred offspring. Routine autopsies disclosed the presence of stomach hair balls in 16 specimens, 15 of these animals being in the California colony and one in the Smith College colony.

These hair balls are shown in Fig. 1, the missing one having been destroyed by moths. In shape the bezoars varied from semi-spherical and ovoid to broadly curved forms resembling the cavity of the stomach. All were dark brown, hard and compact. That they were composed largely of hairs and hair fragments was determined by microscopic examination of bezoar sections. The hairs formed a felt-like mass of similar texture throughout the interior of the hair ball. The surfaces of all the hair balls were fairly smooth, with the smoothness enhanced in some instances by a thin and incomplete lacquer-like coat. The smallest bezoar measured 13 × 8 mm and had a dry weight of 0·16 g; while the largest measured 26 × 13 mm and weighed 1·21 g. Within the stomach some were pyloric in position, some were cardiac and some extended into both areas, the largest hair ball (Fig. 1) filling almost the entire stomach and forming a virtual stomach replica with regard to size and shape.

With the exception of their diet the animals in the two colonies were kept under essentially the same laboratory conditions in that the rats were maintained almost continuously as mated pairs, with at least one cubic foot of space per pair, and with food, water and nesting material available at all times. The California animals were fed on 'Diet I' (ref. 5) with a small bi-weekly supplement of sunflower seeds and greens or apple. 'Diet I' consists of: 67·5 per cent ground whole wheat, 15·0 per cent technical casein, 7·5 per cent skimmed milk powder, 6·75 per cent hydrogenated vegetable oil, 1·5 per cent calcium carbonate, 0·75 per cent iodized sodium chloride, and 1·0 per cent fish oil (vitamin A–D concentrate). The animals in the Smith College colony were kept on a more varied diet. Carrots, apples, pears, grapes, oranges or greens were supplied daily. Wheat germ was provided at bi-weekly intervals, as also were one or more of the following: whole milk, oatmeal, liver meal, sunflower seeds and hemp seeds. 'Purina' laboratory chow was always available but was seldom eaten when other foods were present.

In both colonies the animals reproduced well and attained the adult size characteristic of field-caught animals[6]. The animals in which the hair balls were

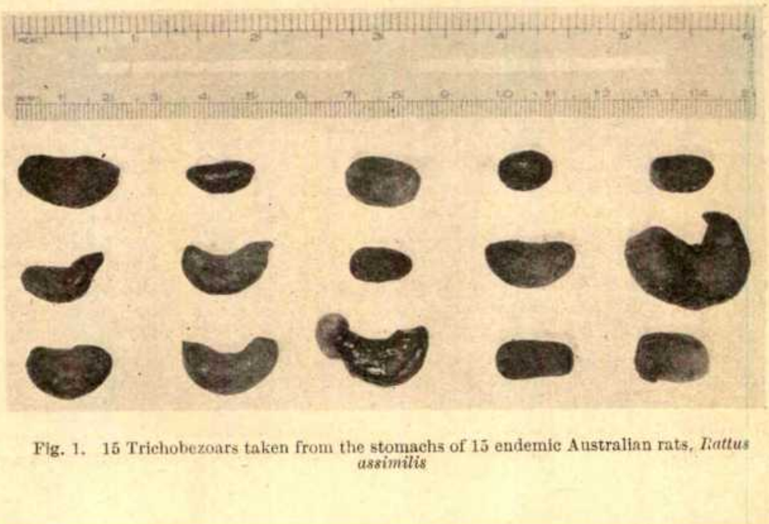

Fig. 1. 15 Trichobezoars taken from the stomachs of 15 endemic Australian rats, *Rattus assimilis*

found showed no convincing malnutrition nor prolonged debility prior to death, and all equalled or exceeded the average life span of 16 months for *Rattus assimilis* born in captivity[6]. Although the hair balls may have been a contributing factor to death, they never filled the entire stomach, even the largest hair ball permitting a small amount of material to pass through the stomach, and there was no evidence of ulceration.

Among the differences in the diets of the two colonies were at least two which may be related to the greater incidence of trichobezoars in the California colony: the somewhat greater percentage of fat and the considerably smaller percentage of roughage in the California diet. Mitchell *et al.*[3], working with white rats (*Rattus norvegicus*), found that animals receiving paste foods of high fat content (25–27 per cent) show a high frequency (90 per cent) of hair ball formation, while those receiving food of low fat content in either a dry diet or a liquid diet show no hair balls. These authors postulate that the paste-like fatty food left in the cage all day accumulates hair on its sticky surface and that when eaten it coats and cements together the additional hairs ingested as a result of grooming activities. 'Diet I', a moist powder fed to the *Rattus assimilis* of the California colony, although lower in fat content than the paste food described by Mitchell *et al.*[3], was higher than their non-bezoar-forming diets. 'Diet I' contained also a higher percentage of fat than did the diet supplied to the Smith College *Rattus assimilis*, only one of which exhibited hair ball formation. Although it is possible that 'Diet I' was conducive to hair ball formation in the California rats, the smaller percentage of roughage in their diet seems far more important.

The work of Howell *et al.*[1] on *Sigmodon hispidus* gives convincing evidence that supplemental rations of cellulose inhibit trichobezoar formation, and the present study supports this finding. The Smith College colony, with a very high percentage of cellulose in its diet, produced only one hair ball; while the comparable colony at California, with a relatively low amount of cellulose in its diet, produced 15 hair balls. Furthermore, once the supplement to 'Diet I' of the California animals was altered, as it eventually was, to include daily, rather than bi-weekly, rations of greens and fruits, no hair balls were encountered in animals raised on the additionally supplemented diet.

Another factor contributing to hair ball formation in the *Rattus assimilis* studied was the extensive grooming associated with sexual behaviour. This aspect of behaviour was well developed in the caged animals, often to the extent that one or both animals of a pair showed conspicuously epilated patches, commonly in the mid-shoulder region[6]. *Rattus norvegicus* reared in the same laboratory under identical dietary conditions groomed one another to a considerably less extent, and there was no evidence of denudation (Taylor, J. M., *in litt.*).

In summary, it appears that in the case of the laboratory-maintained *Rattus assimilis* here described, hair ball formation is facilitated by the ingestion of large amounts of hair in association with a relatively low intake of cellulose-containing food materials. A relatively high fat content in the diet may also be important. To what extent these factors are operative under natural conditions is unknown. However, since *Sigmodon*, *Rattus* and *Peromyscus* are predominantly herbivorous, one would suspect that the large amounts of cellulose normally ingested would amply compensate for the effects of grooming and occasional meals of high fat content. Certainly of the many *Rattus rattus* and *Rattus assimilis*, as well as a wide variety of other rodents examined in the field, no hair balls have been encountered by me; and to the best of my knowledge no such trichobezoars have been reported by other authors.

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INTRA-SUB-SPECIFIC VARIATION IN RETINAL STRUCTURE IN *SEBASTES MARINUS MENTELLA*

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DURING the course of an investigation of retinal structure in fish found in moderately deep waters of the western North Atlantic, a marked variation in the size of the retinal cones was observed in *Sebastes marinus mentella*. This difference is evidently related to the depths from which the specimens were collected.

One specimen of *Sebastes marinus mentella* was collected from a depth of 150 fathoms near the coast of Labrador (54° 42′ N.; 53° 50′ W.) on August 7, 1960. Another specimen belonging to the same sub-species was taken from an adjacent area (54° 47′ N.; 53° 32′ W.) on the same day, but from a much deeper habitat (300 fathoms).

The following description of the retinæ from both the specimens pertains to the region near the central part of the fundus. This is being done in order to minimize the descriptive part of this communication. It is felt that although there are differences among the various regions of the retina, as has been reported in the case of many teleosts[1-3], the basic consideration to be presented here is adequately supported by the evidence from this region alone.

The general features of the retinæ of the two specimens are given in Table 1. Figs. 1*B* and 1*C* show the photomicrographs of cross-sections of these retinæ. As can be seen, they differ in several respects. A striking difference between the two is demonstrated by the visual cell layer. Here, the cone ellipsoids of the specimen from the less-deep waters are much larger, being twice as long and twice as wide as those of the fish from the deeper environment. Their volume is therefore some eight times greater. Accordingly, the outer segments of these two differ considerably in their diameters although they are approximately the same in length. The entire visual cell layer (composed of the cones and the rods) is thinner in the retinæ of the fish from 150 fathoms. This indicates that the rods of the fish from the deeper waters are longer than the rods of the fish from 150 fathoms. It is also observed that the internal nuclear layer and the internal plexiform layer from the retinæ of the fish from 150 fathoms are thicker in general than those of the fish from 300 fathoms. This can be related to the differences between the visual cell layers.

In the vertebrate retina, cones are the photopic elements while the rods are scotopic. In the case of the teleostean retina this is adequately demonstrated by the pronounced photomechanical changes undergone by these receptors[4]. It has also been shown that the cones are larger in size and greater in numbers in a diurnal and surface water species than in a nocturnal one[2]. On the other hand, they are smaller and fewer while the rods are longer, more slender and numerous in the nocturnal and deep sea forms[5]. These differences are interspecific and are related to differences in habitats. This is the first time that intra-specific variation of a similar nature related to habitats has been observed.

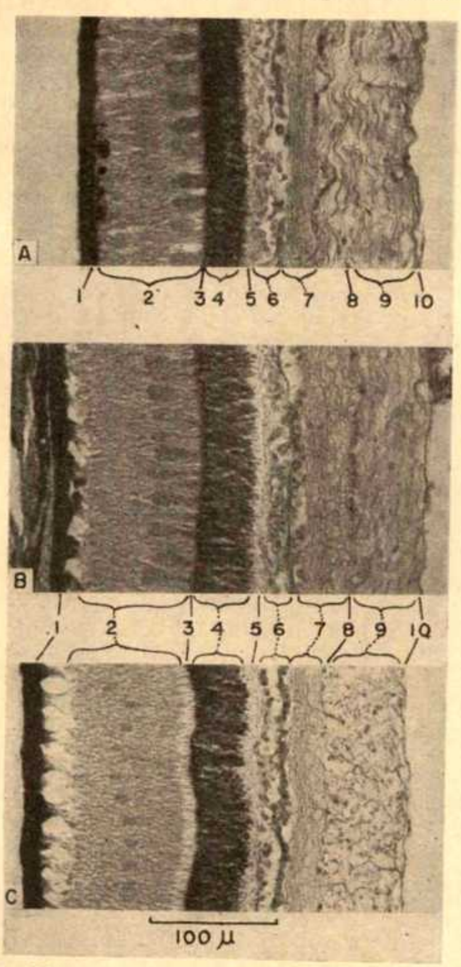

Fig. 1. Photomicrographs of transverse sections of retinæ. *A*, *Sebastes marinus marinus* from 150 fathoms; *B*, *S. m. mentella* from 150 fathoms; *C*, *S. m. mentella* from 300 fathoms. 1, Retinal epithelial pigment; 2, visual cell layer; 3, external limiting membrane; 4, external nuclear layer; 5, external plexiform layer; 6, internal nuclear layer; 7, internal plexiform layer; 8, ganglion cell layer; 9, nerve fibre layer; 10, internal limiting membrane

Table 1. DETAILS OF RETINAL STRUCTURE OF THE TWO SPECIMENS FROM TWO DEPTHS

	S. m. mentella from 150 fathoms	*S. m. mentella* from 300 fathoms
Retina	300–325µ	300–315µ
Retinal epithelial pigment layer	15–18µ. Contracted proximally. Some pigment in the epithelial cell processes	Similar to that of other specimen
Visual cell layer	88–100µ. Cones bulky, about 5–7 per 100µ length of the section. Most are twin. Cone ellipsoids 25µ long, 12–15µ in diameter. Outer segments 10–13µ high. Rods, small and numerous	105–108µ. Tiny cones; almost all twins; cone ellipsoid 10–13µ long, 7–8µ in diameter. Outer segments 10–13µ high. About 5–6µ cones per 100µ length of the section. Rods, small and numerous
External nuclear layer	45–50µ. Well packed. 8–10 rows	45–50µ. Well packed. 8–10 rows
External plexiform layer	5–8µ	5–8µ
Internal nuclear layer	32–38µ. Poorly developed. Much less packed than E.N.L. Nuclei increasing in size from outer to inner region	30–32µ. In other respects similar to that of other specimen
Internal plexiform layer	40–43µ	35–40µ
Ganglion cell layer	1 row of scattered cells. About 5–7 cells per 100µ length of the section	Similar to that of other specimen
Nerve fibre layer	63–75µ	63–75µ

Although some information is available regarding the habits and ecology of this bottom dwelling species[6,7], it is not sufficient to explain the cause or causes for this intra-sub-specific variation. However, it may be suggested that this variation might be caused by the separation of populations. This separation may be expected to bring about, over a period of time, certain differences such as those enumerated here. On the other hand, there may not be genetically fixed populations and the retina of a larva, regardless of the depth of its origin, may develop in such a way as to function best in the conditions obtaining in the depth at which it establishes itself. It is possible that the cones of those fish which establish themselves in deep waters do not attain the proportions attained by those in less deep waters. Similarly, the rods of the deeper water fishes may become longer than those of the others. In either case, we consider light to be the principal factor responsible for bringing about this variation.

It is of interest to compare the features enumerated here with those of *Sebastes marinus marinus*, a very closely related form, taken from the same locality and depth as the *S. m. mentella* from 150 fathoms. The retina of the former (Fig. 1*A*) appears considerably similar to that of the latter (Fig. 1*B*). However, its cones are a little larger, the rods shorter and the external nuclear layer narrower. The last feature indicates fewer rods. As Templeman and Sandeman[8] observe, "While both are deep water forms, *Sebastes marinus mentella* . . . occurs in deeper water than *Sebastes marinus marinus*. *Mentella*-type redfish are to be found in small numbers deeper than 400 fathoms (730 m) and they often have their centre of abundance between 200 and 300 fathoms (370–550 m), whereas the *marinus*-type usually does not occur in abundance deeper than about 160–180 fathoms (290–330 m)". The variation in retinal features enumerated here is supported by this information.

Another feature which warrants mention is the position of the cones in relation to the contracted state of the retinal epithelial pigment. The latter being in a contracted state would seem to indicate that the retina is in a dark-adapted state. However, the position of the cones, at least in the retinæ of the fish from 150 fathoms, indicates a light-adapted state. The cones of the fish from the deeper habitat appear to be in a semi-adapted state. However, since the function of the retinal epithelial pigment in the teleostean eye is to control the amount of light impinging on the cones[9] it may be argued that in these deep water forms which live in areas where light is scarce the ability of the retinal epithelial pigment to undergo photomechanical changes may be considerably reduced or even lost altogether. Or, it may be that since the ventral part of the retina is directly exposed to light, at least in the fish from 150 fathoms, it may retain somewhat the ability to undergo photomechanical changes. This is supported by the fact that, in the fish from 150 fathoms, the ventral part of the retinal epithelial pigment is expanded, at least sufficiently to cover the rod outer segments.

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REACTIVATION OF POXVIRUSES: FATE OF REACTIVABLE VIRUS WITHIN THE CELL

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MILD treatment with heat or urea converts poxvirus particles into a state in which they can only multiply in cells which are in addition infected with infectious poxvirus[1-4]. Poxvirus behaving in this manner is said to be in the reactivable state[2]. More extensive denaturation or interference with the structure of the reactivable particle leads to loss of reactivability[5].

The progeny of reactivable virus is indistinguishable in all respects from the original virus[6]. Reactivable virus therefore contains intact DNA. This DNA, like that of infectious particles, is not accessible to the action of DNase[5].

The reactivation phenomenon extends throughout the whole poxvirus group. Any poxvirus strain can be converted to the reactivable state, and is then capable of being reactivated by any other poxvirus. However, no virus outside this group is capable of acting as the reactivating virus[6]. Infection with reactivable virus and reactivating virus need not be simultaneous: reactivable virus can infect as much as 72 h before the other, and is then still capable of being reactivated. Similarly reactivating virus may precede by at least 12 h[2].

Mild denaturation of the virus particle therefore does not prevent the uptake into, or at least the adsorption to, the cell. Yet the particles are altered sufficiently to render viral DNA incapable of exercising its executive function within the cell. It is clear that the presence of reactivating virus in some way restores this ability, so that the genome of reactivable virus can then replicate and organize the synthesis of such proteins as are required for mature virus to be produced.

The lesion in the reactivable particle appears to be the denaturation of an essential protein. This hypothesis was strengthened when it was found that it was not necessary for reactivating virus to contain functionally intact DNA, but rather functionally active protein: virus completely inactivated with nitrogen mustard (which binds preferentially to DNA) is capable of reactivating virus completely inactivated by heat or urea, and the genotype of the progeny is then exclusively that of the latter[7].

The present model to explain the phenomenon of poxvirus reactivation is then that mild treatment with either heat or urea denatures a protein which is essential if the viral genome is to express itself within the host cell. This protein can be carried into the cell by any other poxvirus particle, which may itself be damaged in the genome. It follows that this protein is a common component of all poxviruses, or that all poxviruses contain a protein component with the same biological function.

Nothing is known concerning the interaction between reactivable poxvirus particles and the cell. Information on this point could well be valuable in defining at the molecular level the component inactivated during denaturation.

We have recently developed a method[8] for preparing very highly purified poxvirus labelled with phosphorus-32 or leucine-1-^{14}C and have used such virus preparations to determine the fate within the infected cell of viral DNA, phospholipid and protein[9]. It has thus become possible to test directly how the fate of reactivable virus differs from that of infectious virus. It is clear, however, that this direct biochemical approach would only be successful if a sizeable proportion of virus particles in a given preparation are in fact in the reactivable state. Early estimates of this proportion gave values of about 1 in 1,000 (1 per cent of the infectious particle titre)[5]. Recently, however, we have obtained evidence that this is a gross underestimate and that in fact well over one-tenth, and possibly more than one-half, of the particles may be in the reactivable state after suitable treatment (Joklik, W. K., to be published). The contemplated approach is thus feasible.

The fate of viral DNA, phospholipid and protein was thus determined for infectious and reactivable radioactively labelled rabbitpox virus. The procedure was to infect cells for 15 min, remove unadsorbed virus and then sample the infected cells at intervals. The following results were obtained:

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(*a*) *DNA*. When rabbitpox virus labelled with phosphorus-32 (20 atoms of phosphorus-32 per particle, 70 per cent in DNA and most of the rest in phospholipid) was used as the infecting agent, its DNA gradually became accessible to the action of DNase (tested after breaking open the cells). As shown in Fig. 1, the fraction of DNA accessible to DNase rose from a background-level of about 3 per cent to some 50–60 per cent 6 h after infection. Certain features of this phenomenon have already been discussed[8], and a full report will follow elsewhere. For the present purpose, the significant feature is that within a period of some 6 h 50–60 per cent of the infecting virus particles are altered in such a way that their DNA becomes accessible to the action of DNase, that is, they are uncoated. When reactivable virus, similarly labelled, is subjected to the same experiment, it is found that none of the DNA is uncoated.

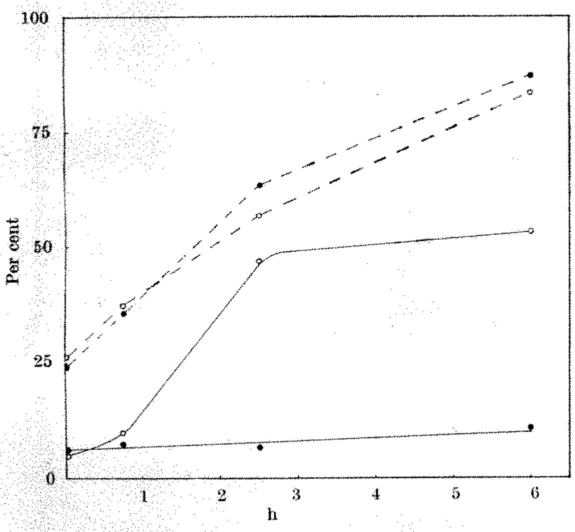

Fig. 1. Breakdown of infectious and reactivable rabbitpox virus, labelled with phosphorus-32, following adsorption to HeLa cells. Open circles: infectious virus; filled circles; reactivable virus; solid curves; viral DNA accessible to DNase; broken curves; viral phospholipid rendered acid-soluble. Multiplicity of infectious virus, 25; multiplicity of reactivable virus, 45. Reactivable virus was prepared by heating infectious virus suspended in sodium chloride (0·15 M)–sodium citrate (0·015 M) at 55° for 2 h. No detectable infectious virus was then present

(*b*) *Phospholipid*. In similar experiments some 50 per cent of the phospholipid of infectious virus is rendered acid-soluble within the first 2 h after infection, and up to 85 per cent within 6 h (Fig. 1). This is also the case when labelled reactivable virus is the infecting agent; the degradation of the phospholipid of both infectious and reactivable virus proceeds *pari passu*.

(*c*) *Protein*. Information concerning the fate of the protein moiety of reactivable virus has been obtained by the use of virus labelled with leucine-1-^{14}C (Table 1). At the end of the infection period virtually all label is associated with the cells and is, after breaking them open, depositable in centrifugal fields sufficient to deposit more than 95 per cent of intact virus (20,000*g* for 20 min). Label is gradually lost from this fraction and appears as (*a*) cell-associated label no longer depositable under these conditions, but still acid-insoluble ('sub-units'); (*b*) similar material in the medium; and (*c*) acid-soluble material in the medium. When reactivable virus is used in this way the overall pattern is virtually the same.

Table 1. FATE OF ADSORBED RABBITPOX VIRUS LABELLED WITH LEUCINE-1-^{14}C*

Time (h)	Cell-associated Depositable fraction	 Sub-unit fraction	Medium Sub-unit fraction	 Acid-soluble fraction
0	96	2·5	< 1	< 1
1	88	7	4	< 1
2	75	12	7	5
3	65	15	11	8
4	57	17	16	10
5	50	18	19	11

* The multiplicity was 66. The figures represent the percentage of label recovered in the various fractions.

The conclusions are: the DNA of reactivable virus is not uncoated. However, the phospholipid (the major part of which is probably located near the surface of the particle) of reactivable virus is treated in the normal manner, as is at least a large part of the protein. It should, however, be stated that the experimental technique was not sensitive enough to detect the possible presence of a minor protein fraction, more or less closely associated with the DNA, and perhaps structurally a component of the nucleoid, which is not broken down when reactivable virus is the infecting agent.

The failure of the DNA of the reactivable particle to express itself is thus most probably due to the fact that it is not uncoated. It would therefore be expected that reactivation would result in the DNA of the reactivable particle being uncoated. This has been tested. Cells were infected with unlabelled infectious rabbitpox virus for 5 h and then superinfected with reactivable virus labelled with phosphorus-32. A significant portion of the DNA was rendered accessible to DNase (Fig. 2). This fraction did not reach the 50–60 per cent attained in the case of infectious virus: the reason for this is most probably that only a certain proportion of virus particles were in the reactivable state, the rest probably being denatured beyond the point at which they could still be reactivated. However that may be, it is clear that an appreciable fraction of reactivable particles is

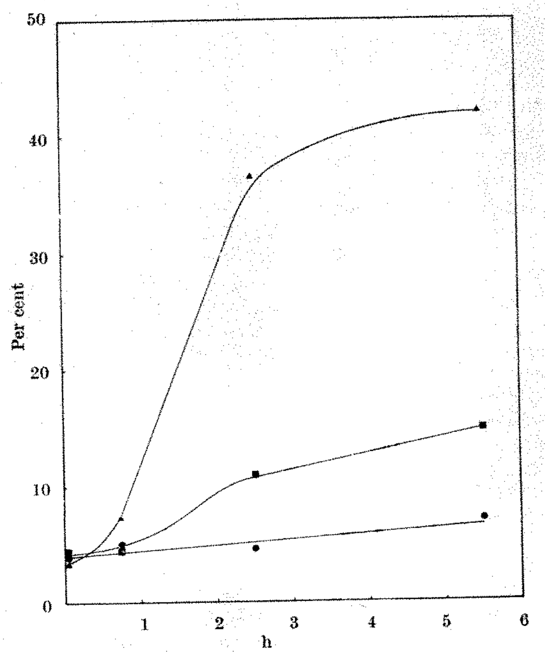

Fig. 2. Partial uncoating of reactivable virus in pre-infected cells. Triangles, infectious virus, multiplicity, 28; circles, reactivable virus, multiplicity 35, no pre-infection; squares, reactivable virus, multiplicity 31, in cells pre-infected with unlabelled rabbitpox virus for 5 h. The ordinate is expressed as the percentage of viral DNA accessible to DNase

uncoated in cells simultaneously infected with infectious poxvirus.

The manner in which the reactivating virus causes the DNA of reactivable virus particles to be uncoated is at present under investigation.

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RELATIONSHIP BETWEEN SPLENOMEGALY, RUNTING AND TOLERANCE TO SKIN HOMOGRAFTS IN CHICKEN

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WHEN immunologically competent chicken cells are transferred to the chick embryo an early symptom of their immunological reaction to the host antigens is an increase in spleen weight[1]. This splenomegaly consists of proliferation of both donor and host cells[2] but the extent of splenomegaly under given conditions is proportional to the dose of donor cells[3,4]. It was noted that in the experiments of Terasaki[3,5] that the doses of whole blood which were required to produce splenomegaly in the chick embryo were similar to those required to produce tolerance to skin homografts from the blood donor. This suggested a causal relationship between splenomegaly and the degree of tolerance to subsequent adult skin homografts. As the tolerant state requires the persistence of antigen[6] (in this case, the donor cells) the survival of skin homografts may provide information about the life-span of the donor cells which were initially active in producing splenomegaly.

Citrated whole blood (0·1 ml.) or dilutions of blood (from 1/80–1/2) from adult Rhode Island Red (RIR) hens was injected intravenously into White Leghorn (WL) or Light Sussex (LS) embryos at 15 days of incubation. The embryos were injected at this age, first to avoid the tremendous mortality resulting from injection at an earlier age, and secondly, 15 days is near the optimum time for the production of splenomegaly because the embryo is still apparently immunologically unreactive at this age[7–9]. Five days after injection half the embryos (about 20) in each group were killed and their spleens weighed. The remainder were hatched and then skin-grafted 2–3 days later. The technique of Cannon and Longmire[10] for skin grafting was followed except that adhesive dressings were used to protect the grafts during the first week. Tolerance was measured by the percentage of homografts surviving at 30 days after grafting[11]. In embryos injected with Ringer solution no homografts survived at 30 days. In the experiments described 282 embryos were used for spleen weight assay and 142 chickens were used in the analysis of homograft survival.

Szenberg and Warner[12] have suggested that the large lymphocyte is responsible for these transplantation reactions in chicken. The dose range in these experiments of 0·1/80–0·1/2 ml. whole blood corresponds to an average dose-range of about 5×10^2 to 2×10^4 large lymphocytes. This narrow dose-range includes the threshold dose for splenomegaly and the induction of tolerance and the dose which produces severe runting with 60–80 per cent mortality.

When 15-day-old LS embryos were injected with adult whole blood (0·1/60–0·1/2 ml.) there was a close correlation between the log of the host mean spleen weight five days after injection and the subsequent tolerance to skin homografts in each group (Fig. 1). When WL embryos were similarly injected the degree of tolerance was directly related to the mean spleen weight only for doses from 0·1/80 to 0·1/10 ml. RIR blood. This relationship broke down completely with larger doses of RIR blood. When 0·1/7 ml. RIR blood was injected the spleen assay on half the group gave a mean weight of 90 mg; three weeks after hatching there was an 83 per cent reduction in mean body-weight and only 26 per cent of the birds were tolerant at 30 days after hatching. Larger doses of blood (0·1/2 ml.) produced greater mean spleen weights (140–190 mg), more severe runting (60–70 per cent reduction of body-weight) and virtually complete breakdown of tolerance to skin homografts (Fig. 2, Table 1). At these doses of blood from 0·1/10 to 0·1/2 ml. there is considerable mortality both just before and after hatching (Table 1). However, such deaths do not apparently result in selection as the highest mortality occurred in two experiments one with the greatest and the other with the least degree of tolerance (Table 1). It appears

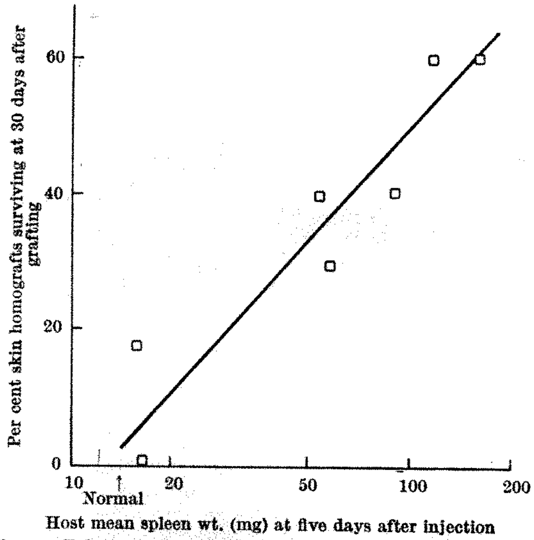

Fig. 1. Tolerance proportional to mean spleen-weight of Light Sussex recipients after injection of adult blood

Table 1. INVERSE RELATIONSHIP BETWEEN THE DEGREE OF TOLERANCE AND THE SEVERITY OF RUNTING AND SPLENOMEGALY

Dose of RIR blood (vol. (ml.)/ dilution)	Per cent survival from injection to 3 weeks of age	Per cent reduction in body-wt.	Per cent tolerant after 30 days	Mean spleen wt. (mg) 5 days after injection
0·1/10	17	100	66	67
0·1/7	50	83	26	90
0·1/3	18	60	16	193
0·2/5	35	71	0	138
0·1/2	15	64	0	167

that runting is associated with a breakdown of tolerance in WL. In one experiment with LS recipients in which slight runting occurred there was no breakdown of tolerance, but there are, at present, insufficient data from LS recipients to determine whether the degree of runting is inversely proportional to the degree of tolerance. This result with WL is contrary to expectation as larger doses of blood would be expected to provide a larger antigenic dose and therefore a greater degree of tolerance to skin homografts would be anticipated. The degree of tolerance in WL chicken at all dose-levels appears to be associated with the percentage incidence of host spleen weights in the 25–100 mg range at 5 days after injection, despite the occurrence of runting in some of these experiments (Fig. 3). The proportion of host spleen weights within the 25–100 mg range at five days after injection determines the subsequent tolerance to skin homografts. As splenomegaly is proportional to the blood dose and at high dose-levels (> 0·1/10 ml.), when runting (and breakdown of tolerance) occurs, there are few small spleens at five days after injection. If there is a low incidence (below 50 per cent) of spleen-weights within this range at five days after injection of the donor blood then only slight tolerance to subsequent skin grafts can be predicted (about 20 per cent grafts surviving at 30 days). On the other hand, the greatest tolerance (66 per cent homografts surviving at 30 days) was obtained in WL when 85 per cent of the sample of host spleen-weights at five days after injection were within 25–100 mg. (Fig. 3).

Blood doses of 0·1/80–0·1/10 ml. do not produce runting in WL, and within this range the degree of initial splenomegaly is proportional to the subsequent extent of tolerance to skin homografts. This relationship appears to hold over a wider dose-range in LS recipients (0·1/60–0·1/2 ml. blood) because there is little runting. However, in WL recipients runting (and mortality) progressively increases with increasing blood doses from 0·1/7 to 0·1/2 ml. and there is also a progressive breakdown of tolerance to skin homografts (Table 1). It therefore appears that at these higher blood doses the donor cells, while producing greater splenomegaly, also attack the organs concerned with growth. In this process the donor cells in the spleen probably suffer an accelerated death so that insufficient donor cells remain in the spleen to maintain the antigenic stimulus necessary for tolerance to continue. This effect may be similar to the 'allergic death' of immunologically competent cells postulated by Gorer and Boyse[13].

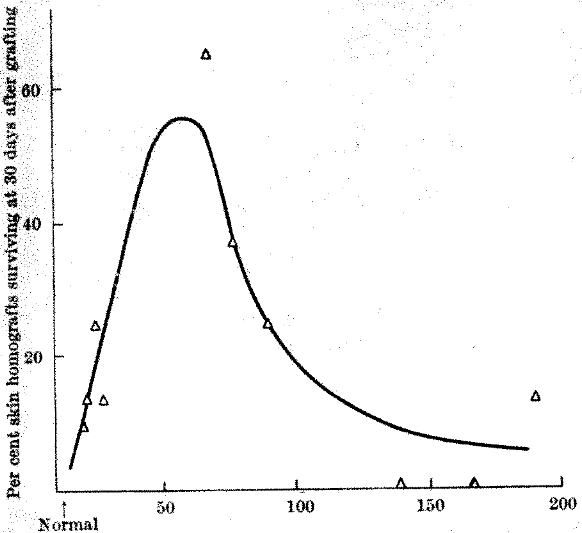

Fig. 2. Tolerance and spleen-weight of White Leghorn recipients after injection of adult blood

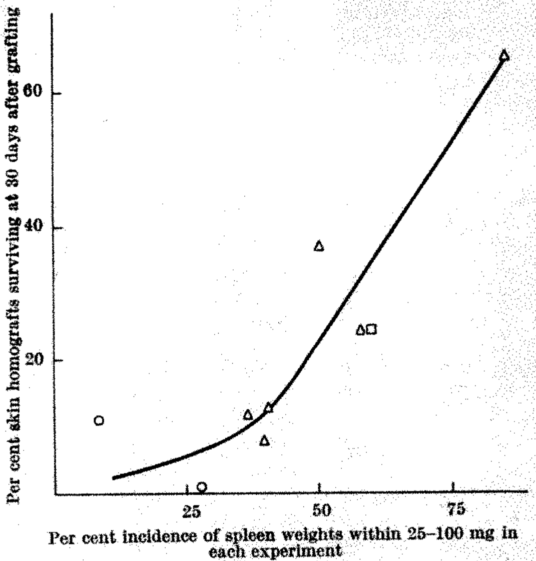

Fig. 3. Tolerance proportional to the incidence of low spleen weights in White Leghorns. △, no reduction; □, 83 per cent reduction ; ○, 60–70 per cent reduction in mean body-weight

Other workers have obtained similar results. Closer examination of Terasaki's results[5] revealed that, whereas 0·1/9 ml. blood produced a relatively high degree of tolerance, 0·1/3 ml. gave much weaker tolerance. Runting has been observed in mice which were not tolerant[14,15], particularly when thymocytes were injected[14]. Rats which had recovered from runt disease were not tolerant when afterwards skin grafted[16]. Trentin and Session[17] found that the degree and duration of tolerance to skin grafts in mice was inversely proportional to the severity of runting.

The close relationship between the dosage of donor cells required to produce tolerance and that required to produce splenomegaly that was inferred from separate experiments in Terasaki's work[3,5] has now been established when LS were used as recipients and when only slight splenomegaly and no runting was produced in WL embryos. Recently, Hasek and Puza[18] showed that some proliferation of the inoculate of donor cells is necessary to establish tolerance in ducklings. Billingham and Silvers[19] have suggested that the proliferation of donor cells injected into mouse neonates of a different strain increases the antigenic dose of donor cells by graft against the host reaction and thus produces a high degree of tolerance. In this way only small doses of adult lymphocytes would be necessary to establish large colonies of donor cells in the spleen at the critical time when the host's lymphocytes are appearing in the spleen and are thus able to accept the donor cells as 'self'. Splenomegaly is apparently an essential initial process in the induction of tolerance to skin homografts by adult lymphocytes. Owing to the rapid growth of the spleen immediately after hatching, splenomegaly cannot be detected after about 10 days. However, as the survival of skin homografts suggests, donor cells are known to survive in the spleen of tolerant birds[20].

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A RELATIONSHIP BETWEEN SKIN TEMPERATURE AND AMOUNT OF WOOL GROWN ON VARIOUS POSITIONS ON THE NEW ZEALAND ROMNEY MARSH SHEEP

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HENDERSON[1], in an investigation of fleece development in Romney Marsh lambs, found gradients in the amount of wool grown on different positions of the body. He suggested that differences in blood supply to these positions might account for the differences in the amount of wool grown on them. Ryder[2] was unable to find evidence that the patterns of the blood vessels in the skin of the sheep could account for the gradients in the amount of wool grown, which were found by Henderson. However, Cockrem and Wickham[3] pointed out that the actual blood supply will depend on factors such as the size of the vessels and their distance from the heart as well as their distribution. These factors were not considered by Ryder and, as Ferguson[4] had demonstrated an effect of blood supply on wool growth, further investigation seemed desirable.

An indirect method of estimating blood supply is by measurement of skin temperature. This measurement will depend on the volume of blood-flow, the deep body temperature, the ambient temperature and the insulation of the skin. However, if a series of temperatures of different positions are measured on the one animal within a short period of time on one day, then those effects other than blood flow will be reasonably constant. In this case, any differences found in temperature can largely be attributed to differences in blood supply.

In the present experiments two groups, each of nine New Zealand Romney Marsh yearling ewes, were used for measurements of skin temperature and wool growth in various positions.

The first group (*A*) averaged 12 months of age and had eight months fleece growth. The second group (*B*) averaged 14 months of age and, for this group, skin temperatures were measured immediately after shearing and again 14 days later when wool growth samples were also taken. The means of these two series of skin temperatures were used for analysis. This second group had had a unilateral cervical sympathectomy at the age of 10 weeks in order to increase the blood flow to the right side of the face (position *B*).

The positions measured for each group are shown in Table 1. Those main fleece positions used were similar to those sampled by Henderson[1]. Skin temperatures were measured with a 'Thermophil' (trademark of Ultrakust, Ltd., West Germany; a direct reading thermistor instrument) and the methods used and their accuracy and interpretation have been described[3,5].

Table 1. MEAN SKIN TEMPERATURES (°F) AND WOOL GROWTH (MG/CM²/14 DAYS) FOR EACH POSITION OF EACH GROUP OF NINE SHEEP

	*A**	*B*	*L*	*K*	*C*	*F*	*H*	*G*
Group *A*								
Wool growth	2·76		—	—	18·8	19·7	—	25·2
Skin temperature	90·2		—	—	95·4	96·2	—	96·6
Group *B*								
Wool growth	2·01	2·69	10·4	12·1	16·2	23·5	22·3	23·7
Skin temperature	86·8	89·7	90·6	90·9	89·2	90·0	90·9	91·3

* Positions *A*, *B*, left and right side of nose; *L*, cheek left side; *K*, ventral, below *C* (belly) — Extremities
C, mid-side; *F*, thigh, posterior to *H*; *G*, upper shoulder; *H*, back dorsal to *C* — Main fleece area left side

For the present experiment two readings were made on each body position, in random order, on each of three days. The sheep were allotted to three groups at random and each group was measured at a different time each day. The effects of the day, time of day and group were allowed for in a simple 3 × 3 Latin square design. The purpose of this design was to minimize effects from diurnal trends in temperatures as it required about 15 min to measure each sheep.

Wool growth was measured by clipping a measured area and the resulting wool was scoured and weighed at a constant humidity and temperature. Wool growth was expressed as mg/cm^2/14 days.

Initial plotting of the data indicated that the relationship of wool growth to skin temperature was curvilinear. Hence the data were analysed as the regressions of $\log_e$ wool-weight on mean skin temperature (°F) from the three days' readings. Groups *A* and *B* were analysed separately. For Group *A* only, the mean of positions *A* and *B* was used. For Group *B* regression coefficients were estimated for the main fleece position (*CFGH*), the extremities (*ABLK*) as well as for all the positions together. All the regression coefficients were estimated as the pooled regression coefficients within sheep, that is, these are the average relationships of the individual sheep in a group.

The results of the analyses are shown in Table 2 and the mean temperature and amount of wool grown (arithmetic scale) for each position are shown in Table 1.

The results in Table 2 indicate that, on average, a significant amount of the variation of wool growth over the body of the individual sheep can be attributed to differences in skin temperature. In so far as skin temperature differences reflect blood supply differences over the body of the individual sheep under the conditions of this experiment, then the gradients of wool growth in part arose from differences in blood supply to the different positions.

The regression coefficients over all positions were very similar for Groups *A* and *B* although the periods over which the wool was grown were very different. The differences between the amount of wool grown at a given skin temperature (Table 1) for Groups *A* and *B* probably reflect differences in deep body temperature resulting from the shearing of the latter group. Group *A* had higher rectal temperatures, which could mean that the higher skin temperatures reflected similar relative blood-flows to that of Group *B*, which had lower rectal and skin temperatures, in that Bligh[6] has shown that there is a reasonable correspondence between rectal and deep body temperatures under uniform conditions (for the calf). If relative blood-flows were similar, then the result suggests that the substrate made available by the blood supply is of greater importance than an actual temperature effect on reactions within the follicle bulb.

Table 2. THE POOLED REGRESSION COEFFICIENTS WITHIN SHEEP OF LOG_e WOOL WEIGHT (MG/CM^2/14 DAYS) ON MEAN SKIN TEMPERATURE (°F) OF VARIOUS POSITIONS

Group	Positions	Regression coefficient	Standard error	d.f.	Per cent sums of square due to regression
A	*ABCFG*	+0·29	0·025	26	84
B	*CFGH*	+0·13	0·036	26	33
	ABLK	+0·34	0·063	26	53
	All	+0·30	0·040	62	48

For Group *B* the regression coefficient is lower for the main fleece areas than for the extremities. In addition, it can be seen from Table 1 that a particular skin temperature on the extremities leads to less wool growth than on the main fleece area. In particular, sympathectomy, while markedly increasing skin temperature, did not increase wool growth proportionally. These results considered together suggest that an inherent factor associated with the skin or with its follicle population is also involved in the determination of the amount of wool grown on a particular position.

Overall these results suggest that about 50 per cent of the variation in wool growth over the body of the New Zealand Romney Marsh sheep is the result of variation in substrate available to the follicle. The remainder of the variation is likely to be the result of inherent differences in the follicle populations themselves. That is, in follicle size, density of follicles or in the ratio of secondary to primary follicles.

I thank Mr. J. C. Newhook, who performed the sympathectomies, and Messrs. B. Thatcher, D. G. F. Orwin and D. A. Evans, who assisted with the measurements, and the New Zealand Wool Board for finance.

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INSTABILITY OF PULSE ACTIVITY IN A NET WITH THRESHOLD

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FOR half a century, threshold has been known to be important in the activities of nerve cell and synapse, but little is known of the general properties that so ubiquitous a feature must impose on the system's behaviour in the large. Beurle[1], in his investigations of the transmission of waves of activity over a conducting net, noticed that a net with threshold would have a marked tendency to be unstable, but his assumptions were complex, and the origin of the instability is not easily identified. Here we propose to show that an instability, similar to his, can readily be traced from a simpler origin.

The instability we refer to would soon show itself if one made an artificial nerve-net with unspecified, rich connectivity, with threshold at the junctions, and then attempted, as the net transmitted pulses, to keep the net's activity at a moderate value. The net would tend to run either down to a state of total inactivity, from which one could scarcely get it to stir, or up to a state of total activity, which would lessen only at the onset of exhaustion or some other extraneous factor.

The origin of this instability can be traced as follows. Suppose the whole net is composed of a large number of interconnected units which handle information represented everywhere by identical pulses of some physical activity. Each of these units has n identifiable inputs. A unit 'fires' in time interval t to $(t + \Delta t)$—emits a pulse of duration $\delta t \ll \Delta t$—if and only if at least θ of the inputs have received a pulse within the preceding time interval $(t - \Delta t)$ to t. The following argument is applicable both to nets with essentially one-directional information flow (from a network-input to a network-output) and also to nets with rich internal cross-connexions (provided none of the feed-back loops is short).

We shall describe the activity on the inputs of a particular unit by first associating a probability p with the occurrence of a pulse in a particular time interval Δt on a specified input. We define:

$$p = \lim_{t \to \infty} \left(\frac{N}{L} \cdot \frac{\Delta t}{t} \right) \qquad (1)$$

where N is the number of pulses counted in time t which have passed through a sufficiently large bundle of L randomly selected inputs. From this we have the probability p_i that precisely i inputs on a particular unit receive a pulse in the time interval Δt:

$$p_i = \binom{n}{i} p^i (1 - p)^{n - i} \qquad (2)$$

Consequently, the probability p that at least θ inputs are active in this time interval, that is, that the unit fires, is given by the cumulative binomial probability function[2]:

$$p' = \sum_{i=\theta}^{n} \binom{n}{i} p^i (1 - p)^{n - i} = \frac{n!}{(\theta - 1)!\,(n - \theta)!} \int_0^p x^{\theta - 1} (1 - x)^{n - \theta}\, \mathrm{d}x \qquad (3)$$

The instability arises when we allow the resulting pulse frequency $f' = p'/\Delta t$ to be itself the generator of a further frequency $f'' = p''/\Delta t$, and so on. Under these conditions we ask what will happen in the net as time goes on. Equilibrium is reached if the pulse activity on each input equals the pulse activity on each output, that is, if:

$$p' = p = p^* \qquad (4)$$

The equilibrium will be stable at the activity p^* if an increased activity at the input leads to a lesser activity at the output; that is, if:

$$\left| \left(\frac{\partial p'}{\partial p} \right)_{p^*} \right| < 1 \qquad (5)$$

However, differentiating expression (3) once and twice with respect to p shows that for all cases where solutions for (4) with $0 < p^* < 1$, and $n > 1$, exist, $p'(p)$ starts at the origin with zero slope and exhibits a single inflexion at the point:

$$p = \frac{\theta - 1}{n - 1} \qquad (6)$$

Thus the stability criterion (5) is satisfied only where $p^* = 0$ or $p^* = 1$.

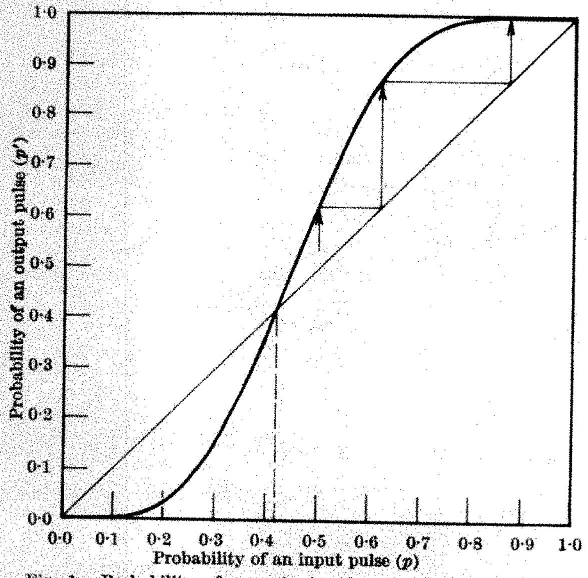

Fig. 1. Probability of an output pulse, as a function of the probability of an input pulse (threshold 5, 10 inputs)

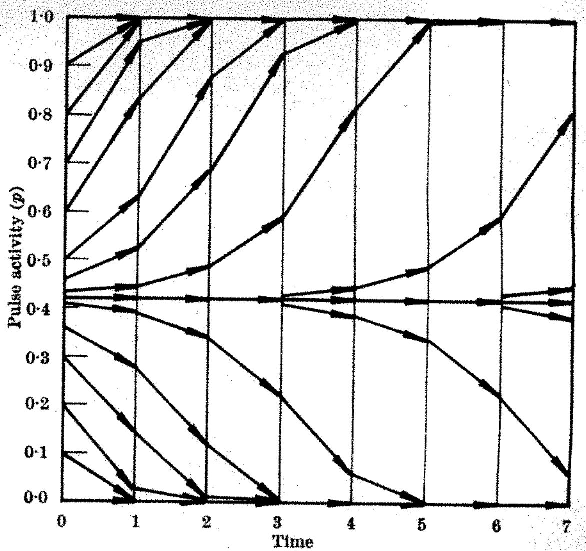

Fig. 2. How pulse activity changes with time, from various initial activities (threshold 5, 10 inputs)

Fig. 1 illustrates the situation in a simple case where p' is plotted against p for $n = 10$ and $\theta = 5$, using (3). The equilibria p^* are the three points where $p'(p)$ intersects $p' = p$: 0, 0·42 and 1. Those at 0 and 1 are stable; that at 0·42 is unstable in the sense that the slightest perturbation from this value is followed by the system going to one of its extreme values. (In Fig. 1 the change in p from an initial value of 0·5 is indicated by the stairway.) Fig. 2 shows how various perturbations provoke a runaway to an extreme value.

It seems, therefore, that the more richly organized regions of the brain offer us something of a paradox. They use threshold intensively, but usually transmit impulses at some moderate frequency, seldom passing in physiological conditions into total inactivity or maximal excitation. Evidently there must exist factors or mechanisms for stability which do not rely on fixed threshold alone.

One such mechanism that offers itself readily is the non-linear dependency of threshold on output that is sometimes referred to as 'inhibition by depletion'. If this dependency is of the form:

$$\theta = \mu^{-1/\lambda} (p')^m$$

with:

$$1 < \frac{1}{\lambda} \leqslant m$$

(λ, μ and m being constants), stability criterion (5) is fulfilled if:

$$\left(\frac{\partial p'}{\partial p} \right)_{p^*} \leqslant \lambda < 1$$

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LETTERS TO THE EDITOR

RADIO ASTRONOMY

Measurement of Magnetic Fields in Interstellar Clouds

PREVIOUS attempts[1-3] with the 250-ft. radio telescope at Jodrell Bank to measure magnetic fields in interstellar clouds in the Milky Way by observing the Zeeman splitting of their neutral hydrogen line profiles failed to show any clear evidence for fields greater than 10^{-5} gauss. The observations of February 1962[3] indicated a possible Zeeman splitting in some clouds, but with the sensitivity of the receiver and the band-width of 5 kc/s used in that work the result could not be verified in a significant manner. This communication describes a new and successful attempt to measure the magnetic field.

These observations were made in September and October, 1962, with the 250-ft. telescope using a parametric amplifier (*AIL* Type 1930) and a radio frequency switch incorporating varactor diodes as switching elements which gave an overall system noise temperature of about 200° K. Crystal filters having a square bandpass response 3 kc/s wide at the 6 db. points were used for giving the frequency resolution required. Six of these channels simultaneously monitored adjacent 3 kc/s bands and were used for measuring both the hydrogen line intensity and the difference between right and left hand circular polarization. Two methods of observation were used. In the first the channels were fixed in frequency, connected to integrators and set alternately on the hydrogen line profile and off the profile. Only narrow absorption features were studied in this way. In the other method the channels were swept in frequency through the line profile, and the output was fed on to punched tape. Two of these channels were monitored on a pen recorder.

The strongest indication of Zeeman splitting was shown by the narrow deep feature of Taurus *A*. The normal line profile is shown as *C* in Fig. 1*a*, where the optical depth τ is plotted as a function of frequency difference from the frequency of the line centre. With the 3 kc/s band-width used in the present work this feature has been shown to be double and has been resolved into two absorbing clouds with profiles given by *A* and *B* in Fig. 1*a*. In Fig. 1*b* the difference between right and left hand polarization is plotted as a percentage of the profile depth observed in one polarization. These results were obtained by the first method (the integration technique) described here. Preliminary analysis of the records using the second method (frequency sweep technique) confirms the form of Fig. 1*b* but the full punched-tape data have not yet been analysed.

The observed Zeeman splitting curve of Fig. 1*b* cannot be interpreted as the result of a uniform field in the two clouds, but it is compatible with a field directed away from the observer of $2 \cdot 5 \times 10^{-5}$ gauss in the cloud giving the profile *B*. The expected Zeeman profile is shown as a dotted line in Fig. 1*b*. The field in the cloud giving the profile *A* appears to be less than 5×10^{-6} gauss. In retrospect it is clear that this Zeeman curve was visible in data from previous observing programmes, but because its shape was not that to be expected from a uniform field through the whole feature it was suspected to be caused by some systematic effect in the equipment.

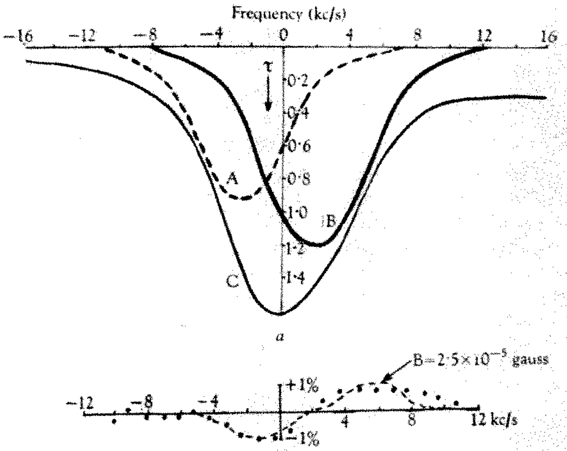

Fig. 1. *a*, The absorption profile (*C*) of the deep narrow feature in the Taurus *A* spectrum, as observed with a 3-kc/s band-width. It is plotted in optical depth (τ) as a function of frequency, and the diagram also shows the profile resolved into two components *A* and *B*
b, The Zeeman profile plotted as right hand minus left hand circular polarization amplitude. The ordinate is expressed as a percentage of the profile depth as it would be observed on one polarization. The dots are the observed points and broken curve is the Zeeman profile expected for a field of $2 \cdot 5 \times 10^{-5}$ gauss directed away from the observer in component *B*

Similar but weaker Zeeman features have been found in the absorption spectra of Cassiopeia *A* and Cygnus *A*. The interpretation of these data and of the emission data awaits the complete analysis of all the 6 channel Zeeman and hydrogen line observations. Nevertheless several important conclusions can be drawn. The absorption and emission profiles seen with the 12 min of arc beam of the 250-ft. radio telescope and with a 3 kc/s square bandpass are much more complex than previously suspected. For example, the narrow feature in the Cygnus *A* spectrum at about + 400 kc/s has three main components. The data already analysed suggest that each component may have a different magnetic field component in the line of sight. The maximum components of the magnetic field seen in the clouds of the Cassiopeia *A* and Cygnus *A* directions are about 5×10^{-6} gauss. In other clouds the field is below the level of detection and is significantly less than 5×10^{-6} gauss. These preliminary results suggest that the general galactic magnetic field is of the order of 5×10^{-6} gauss.

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PHYSICS

Growth of Positive Channels in Non-uniform Fields during Impulse Breakdown

In a rod–plane gap subjected to 1/50-µsec impulse voltages (rod positive–plane negative) the breakdown is initiated by a corona discharge at the rod which is then followed by a positive channel crossing the gap. The main discharge of the breakdown occurs after the bridging of the gap by the channel. Investigations concerning this phenomenon are reported by Allibone[1] and recently by other workers[2-9].

This communication concerns an investigation of the positive channel propagation in a plane to sphere gap from 10 to 20 cm in length and is aimed at a physical approach of this phenomenon. The plane is charged negatively from an impulse generator, and the positive channels starting from the earthed electrode (5 mm diameter) were investigated by chopped impulse voltages so they do not reach the high-tension negative electrode. Photographs of the discharge and simultaneous current recordings by means of a double-beam oscillograph are used for investigating the propagation of the channel. The upper beam of the oscillograph is used for recording the whole current variation up to the end of the chopping, while the lower one registers, at a higher sensitivity and at a faster sweep, only a part of the upper current trace. This is done by using, as described here, a model 555 'Tektronix' oscillograph. Figs. 1 and 2 are examples of channel photographs and of the corresponding current recordings. The lower trace of the oscillograms gives the fine structure of that part of the upper record, which appears brighter, this being a special feature of the oscillograph used.

In cases where a better discrimination of the current steps is desired in order to facilitate the correlation of the channel photographs with the detailed oscillogram, a mixed divider consisting of resistances and a small inductance can be connected at the input of the lower beam. This inductance introduces to the voltage recorded by this beam a component proportional to di/dt, so that current variations are reproduced on the oscillogram more pronounced. Obviously this method introduces a considerable distortion in the current trace and can be applied only in those cases where a faithful record of the current is not important.

Well-known investigations on long gaps, of the order of more than 1 m, have shown that leader channels are stepped and that this discontinuity of propagation is influenced by the series resistance of the gap circuit. During the investigation reported here the gap is connected directly to the front capacitor of the impulse generator so that, practically, the voltage across the gap is not changed by drops in voltage in the circuit caused by current variations. In spite of this fact and of the small dimensions of the gap, the form of the channel on Fig. 1, for example, correlated to the corresponding current oscillograms of Fig. 2 justifies the suggestion that the channel propagation is in this case also in a way stepped. It also seems that each step is preceded by a corona discharge paving the way for the next step. This stepped mechanism should rather be attributed to the nature of the breakdown phenomenon in air than to time constants of the circuit. It is hoped that the comparison of a great number of channel photographs with simultaneous current records will provide information for a better approach to the physics of the phenomenon.

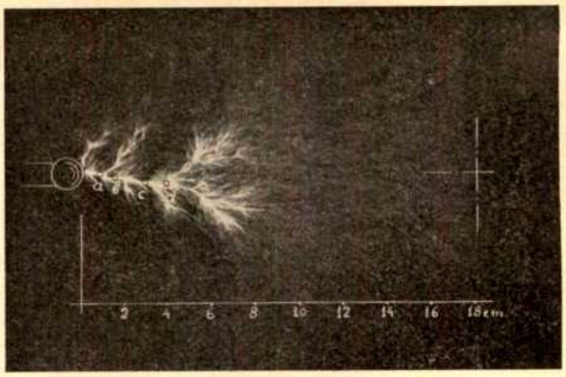

Fig. 1. Photograph of a chopped positive channel. Correlation with the oscillograms of Fig. 2 is reliable up to the branching point *d*

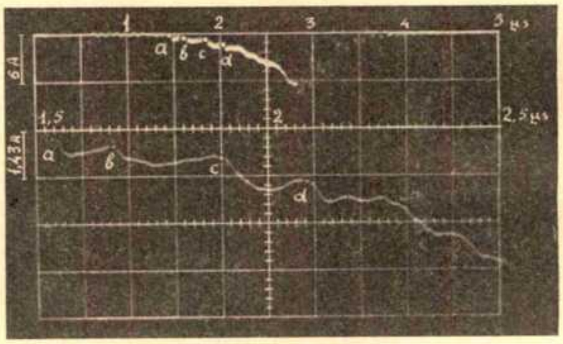

Fig. 2. Oscillograms of the current through the gap. Points *a*, *b*, *c*, *d* correspond to those of Fig. 1

By correlating photographs of the channels to current records obtained so far, the propagation velocity of the channels could be determined as being of the order of 10 cm/µsec, which is in accordance with values found by other methods. Since the channels are very often branched, the correlation is useful in those cases only where branching is restricted to a minimum.

The impulse generator used, of a single 200-kV stage, has a front capacity of 4,140 pF. The gap consists of a plane disk, 30 cm in diameter, directly connected to the output of the generator, and a small sphere, 5 mm in diameter, fixed at one end of a 75-ohm co-axial cable. At the other end of the cable, a 75/7-ohm attenuator is connected to the input terminals of the C.R.O. pre-amplifiers. The chopping device consists of two 25-cm spheres separated by a plane disk to form a three-electrode arrangement. This device is connected in series with a 100-ohm resistor across the front capacitor of the impulse generator and allows interruption of the positive channel development at any point in the gap investigated.

The oscillograph used has a rise time of 12 nsec, two beams with *L*-type pre-amplifiers and two separate time-bases. A special interconnexion of the time-base devices permits use of the upper beam to record the whole variation of a phenomenon, while a pre-selected part of the upper trace can be magnified and registered by the lower beam. The delay-time between the starting moments of the two sweeps can be adjusted at will.

The 75/7-ohm attenuator is designed in such a way that its time constant, estimated to be less than 10^{-8} sec, is so low that practically no distortion of the current form occurs.

The gap is installed in a 90 × 90 × 90 cm^3 box of hardboard, at one side of which an $f/1{\cdot}5$ Leica camera is fixed, allowing the channels to be photographed from a distance of 45 cm.

This investigation is financially supported by the Royal Hellenic Research Foundation and is being continued. I thank Prof. G. M. Petropoulos for his advice.

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Hot Resistivity of Tungsten Wires

WHILE a number of more recent papers have dealt with the variation of electrical resistivity of tungsten with temperature, it remains a not uncommon practice to refer to the tables compiled by Langmuir and Jones[1] for estimating the temperature of a heated filament. These tables, in turn, were taken from earlier work by Zwikker, Worthing and others.

In an investigation into the progress of changes to the electrical parameters of tungsten filaments in thermionic devices, which will be published later, it was soon found that the Langmuir values can lead to errors. For example, at the operating temperature of valve heaters (about 1,100° C), errors of the order of 100° C were common. Furthermore, these errors were not consistent from heater to heater so that, in experiments where heater temperatures were critical, many results could be invalidated by undetermined differences.

Apart from the conventional precautions in measuring resistances, the determination of the resistance of tungsten wires imposes the need to maintain a rigidly controlled temperature, and we have found it necessary to use an a.c. bridge with heating currents not exceeding 1 m.amp on fine wires.

Many expressions have been proposed for relating resistivity with temperature. However, most of these are applicable over but limited temperature ranges, and in our experience, for tungsten, the most useful remains the polynomial of the form:

$$R_T = R_0[1 + \alpha T + \beta T^2 + \gamma T^3]$$

For temperatures up to about 100° C, terms above α can be neglected and γ can be neglected below about 1,200° C, for a precision of about 1 per cent. If Jones and Langmuir's values are plotted, they are found to follow not a smooth curve, but a sequence of short lines, indicating perhaps that a number of the values are interpolated estimations. The 'constants' from their results are roughly:

$$\alpha = 4{\cdot}8 \times 10^{-3};\ \beta = 1 \times 10^{-6};\ \gamma = 0{\cdot}5 \times 10^{-9}$$

Examining a large number of samples of wire and wire products from a wide range of sources, we have found the values of these parameters to vary: especially α and β seem sensitive to the influential factors of purity and work hardening and α has been found to lie between 3·9 and $4{\cdot}7 \times 10^{-3}$ and β between 0·5 and $2{\cdot}0 \times 10^{-6}$: γ has averaged near $-0{\cdot}5 \times 10^{-9}$. The room-temperature resistivity, correspondingly, is higher than Langmuir's by about 10 per cent.

This makes it necessary, for really precise work, to determine the characteristics of each sample. But for temperatures up to 1,200° C if a precision close to 1 per cent is adequate, a simplified procedure can be adopted. The resistance at 0° C and 100° C is determined and an average value of $1{\cdot}2 \times 10^{-6}$ is taken for β. From this α can be derived and a good approximation up to about 1,200° C can be obtained. Inclusion of the value of γ above permits extension of this range by several hundreds of degrees. It is usually not difficult to determine R_0 and R_{100} even on a completed device, and if there is reason to believe that the characteristics may vary throughout the experiment, rechecks can be made at appropriate intervals. These serve to track changes in state of the wire—for example, by annealing, or contamination—but also to discriminate between such changes and dimensional alterations.

On no samples of fine wire have α's been found as high as Langmuir's $4{\cdot}8 \times 10^{-3}$: more commonly α has fallen between 4·1 and 4·3, and coils tend to have lower values than straight wire.

This lowering of the temperature coefficient is partly due to the consequences of working. While considerable recovery is effected by relatively low-temperature annealing, progressive changes have been observed to continue up to the highest temperatures studied (2,600° C), so that it is not safe to assume that a sample treated, say, at 1,800° C, has been stabilized.

Purity also plays a part: the deliberate addition of trace impurities made to most commercial wires influences the electrical characteristics[2], but the pick up of trace impurities inevitable in the working of materials from ingot to wire is demonstrated in a progressive fall in α (0–100) from the unworked metal (about $4{\cdot}7 \times 10^{-3}$) to fine wire (about $4{\cdot}4 \times 10^{-3}$ fully annealed). As may be expected, some increase in α is obtained if wire is heavily etched before annealing, illustrating the superficial nature of the contamination.

It is the interplay of these factors which makes imperative the actual measurement of the electrical characteristics of tungsten wire components for critical work, and this applies especially to temperature determinations.

I thank the Directors of the Mullard Radio Valve Co., Ltd., and Dr. van Moll, head of the Material Research Laboratory, for permission to publish this communication.

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METALLURGY

Aluminium Nitride in a Mild Steel

The presence of aluminium nitride in mild steels de-oxidized with aluminium has been suspected for many years. Various investigators have reported indirect evidence for its presence and several have attempted to identify it.

Hsiao[1] took reflexion diffraction patterns from an etched section of a mild steel, containing 0·035 per cent aluminium, that had been decarburized, nitrided, and homogenized at 675° C *in vacuo*. The analysis of the ring pattern indicated the presence of aluminium nitride and α-iron. Particles were extracted from the steel surface on a 'Formvar' replica, and it was assumed that these were aluminium nitride.

Booker and Norbury[2] used a 1 per cent aluminium steel, nitrided at 640° C, then furnace cooled. They studied extraction replicas by transmission electron diffraction with an *EM*3 electron microscope and were able to correlate their ring pattern directly with the diffracting precipitate, which was identified as aluminium nitride.

Dahl *et al.*[3] in more recent work claimed to have identified aluminium nitride from single crystal patterns but give no details of '*d*' values or lattice constants. Wright and Quarrell[4] have recently published work identifying plate-like and dendritic precipitates in steel castings as aluminium nitride. They attribute the variance in morphology compared with the better-known 'needle-shaped precipitates' to the fact that their dendritic precipitates have been extracted from fracture surfaces. It must also be added that their '*d*' values for these dendrites differ from those in the *A.S.T.M.* index by more than 5 per cent.

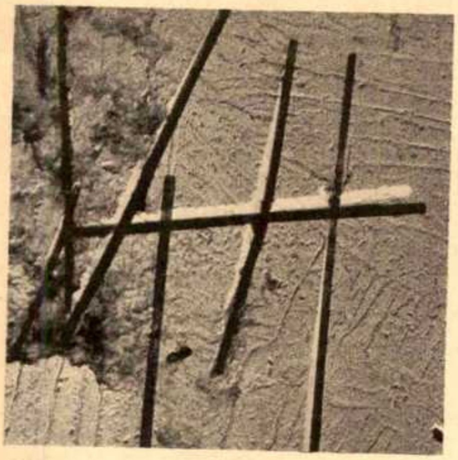

Fig. 1. Carbon extraction replica. (× 10,000)

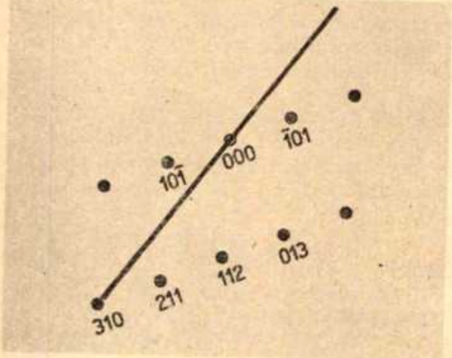

Fig. 2. Reciprocal lattice-$1\bar{3}1$ plane

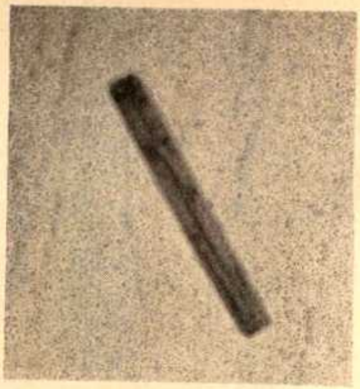

Fig. 3. Carbon extraction replica (× 20,000)

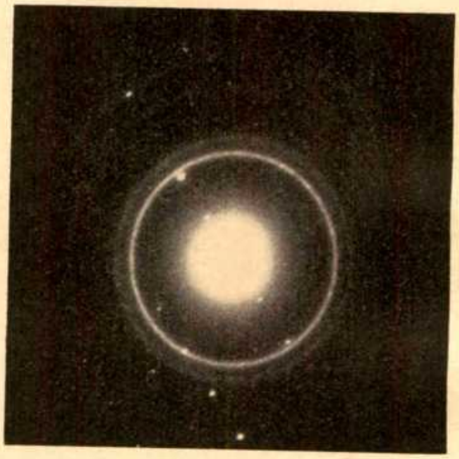

Fig. 4. Electron diffraction pattern of needle shown in Fig. 3 with superimposed gold standard

The investigation reported here was carried out to try to obtain a positive identification of aluminium nitride precipitates in a synthetic steel of the following composition (per cent): carbon 0·19, manganese 0·80, aluminium (insoluble) 0·001, aluminium (soluble) 0·08, nitrogen 0·0078, oxygen 0·0008, sulphur 0·01, phosphorus 0·001, silicon 0·01.

This steel was kindly provided by the British Iron and Steel Research Association, and details of melting, casting and heat treatment are given by Sage *et al.*[5]. This particular specimen was finally water-quenched from 900° C.

Carbon replicas were taken from polished sections by electrolytically etching in 20 per cent perchloric–alcohol solution for 1–2 min and were then examined in the Siemens Elmiskop I and Metropolitan Vickers *EM*3*A* type electron microscopes.

A large number of electron micrographs of needles (Fig. 1) with their corresponding single crystal diffraction patterns have been obtained. The most frequently occurring diffraction patterns were indexed as the (010) and (110) reciprocal lattice planes. Fig. 3 shows a typical needle and Fig. 4 the corresponding

Table 1

	a	*c*	*c*/*a*
(110)	3·084 ± 0·035	4·98 ± 0·035	1·61 ± 0·01
(010)	3·114 ± 0·03	5·00 ± 0·05	1·60$_5$ ± 0·01
A.S.T.M.	3·114	4·986	1·61

diffraction pattern. The (010) and (110) planes give the two patterns expected from a hexagonal structure, both being perpendicular to the *c* axis. The lattice constants were calculated from these patterns.

Taking into account lens rotation when changing from image to diffraction pattern, it was found in both patterns that the needle axis was parallel to the [001] direction. To support this fact, a third pattern, indexed as $(1\bar{3}1)$, not containing the (001) spot in its plane was used. The intercept common to both (001) and $(1\bar{3}1)$ is in the [310] direction. Allowing for the same lens rotation, the needle axis was again perpendicular to the intercept shown in Fig. 2.

To summarize, in aluminium-killed steels, needle- or lath-shaped precipitates have been found which give single crystal diffraction patterns. Lattice constants calculated from the indexed patterns correspond within 1 per cent with the A.S.T.M. index for aluminium nitride, while the *c*/*a* ratio, which is independent of calibration, has an agreement better than 1 per cent. The patterns were consistent with a hexagonal lattice system having its *c* axis parallel to the length of the needle.

I thank the British Iron and Steel Research Association for providing the steel and the Chairman of Tube Investments, Ltd., for permission to publish this communication.

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Dendritic Structure in the Nedagolla Ataxite

Present versions of the Rose-Brezina system of meteorite classification include a Nedagolla group (D_n) of nickel poor ataxites which show a granulated structure[1]. The Nedagolla mass was observed to fall in 1870 and the main mass is preserved in the British Museum[2]. The nickel content is reliably established as 6·2–6·1 per cent[3,4], which is exceptionally low in relation to the other known members of Brown's gallium–germanium group IV (ref. 5). The presence of small amounts of silicate have been suspected[3] and Nedagolla shares with Tucson, the other silicate-containing member of gallium–germanium group IV, an exceptionally high chromium content[4,5].

I have made a metallographic examination of a 15-g slice taken perpendicular to the surface of the British Museum specimen *B.M.* 51184. A photograph of this specimen after etching in 4 per cent aqueous nitric acid is shown in Fig. 1, where a distinct pattern of slender dendrite skeletons may be seen in the heat-altered zone. The dendrite skeletons in the heat-altered zone are easily revealed by both aqueous and alcoholic nitric acid etchants and are seen to be least distinct at the ablating surface. Thus the dendrite structure in the heat-altered zone is being destroyed by the inflow of heat from the ablating surface of the meteorite, and we must conclude that the macrostructure of the unaltered mass of Nedagolla is dendritic. It is not easy to obtain an etching treatment which simultaneously reveals the dendritic structure in both heat-altered and -unaltered regions, but there is an area (opposite to the least-curved edge of Fig. 1) in which the dendrite skeletons of the heat-altered zone may be seen to merge into the more corpulent dendrites of the unaltered region.

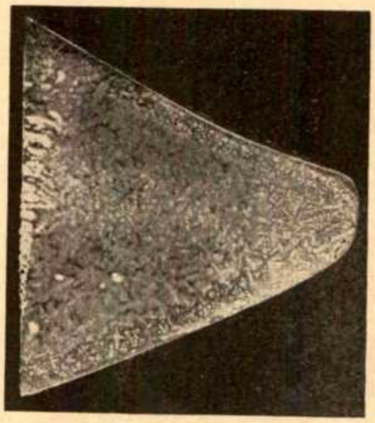

Fig. 1. A macro-etched fragment of the Nedagolla meteorite. The true length of the straight saw-cut edge is 3·0 cm. Curved edges are ablation surfaces below which a heat-altered zone is visible

Microscopic examination of the surface when reprepared and etched in 2 per cent 'Nital' revealed a number of minute globules of silicate but no identifiable phosphide. The unaltered region shows a dendritic pattern of clear kamacite areas in a duplex ground mass. The clear kamacite areas correspond to the iron rich cores whereas the duplex areas arise by precipitation from the alloy enriched outer regions of heavily cored dendrites produced during the solidification of an iron–nickel melt containing 6·1 per cent nickel. It is noteworthy that the γ–δ peritectic of the iron–nickel system is likely to be involved in the solidification process.

Progressing from the exterior inwards, the heat-altered zone shows a gradation of structures from the completely martensitic at the very edge; through a broad region of low nickel dendrite spines in a matrix of martensite and a transition zone of dendrites in a matric of non-acicular alteration product; to the unaltered structure of dendrite spines in a duplex groundmass. This sequence is consistent with the pattern of phase resolution and α–γ transformation events to be expected of cast iron–nickel material when it is reheated in a temperature gradient.

Thus Nedagolla appears to be an extreme example of a nickel-poor ataxite which has been produced by the complete melting and resolidification of material which is normally encountered as hexahedrites. It would be valuable to have gas content and radio-dating studies of this material. Similarly the metallographic examination suggests an appreciable carbon content and it would be valuable to have an analytical value for this element.

I thank Drs. Max Hey and A. A. Moss for making the specimen available and for helpful discussion.

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METEOROLOGY

Pseudo-whiskers of Ice, grown from Clouds of Supercooled Water in an Electric Field

INTERESTING effects of ice formation in an electric field and from a supercooled water fog have been observed by Schaefer[1] and later confirmed by Marshall and Gunn[2], who pointed out at the same time that electrical charging of a suitable support which is immersed in a supercooled cloud greatly accelerates growth of supercooled droplets (and later crystals) on the sustaining medium. Schaefer's report mentions, in fact, very rapid growth of whisker-like aggregates from the tips of which, at high electrical gradients, supercooled water appears to be projected outwards.

Fragile 'ice-whiskers' could, if formed in a cloud and in an electric field, become, after subsequent mechanical fragmentation, the source of relevant quantities of ice nuclei, and information towards their growth and structure thus appeared to be of interest. Since, furthermore, the possible role of electrical effects in ice-nucleation has again recently been stressed[3], it was decided to carry out an experiment similar to those quoted in refs. 1 and 2, but in conditions of dense fogs artificially produced for short periods of time. Relatively intense electrical fields were used in this experiment and the potential was applied on copper wires which were insulated with plastic, in order to reduce the possibility of fragmentation of 'whiskers' by electrostatic wind and corona.

'Whisker' growth experiments were carried out in a plastic, tubular container, 40 cm in diameter and 50 cm high, the bottom part of which was closed. A similar container, but of 30 cm in diameter and made from a black mat carton, was introduced concentrically into the plastic tube, and the side interspace filled with 'dry ice' up to about 30 cm. A standard 1-l. beaker, containing about 600 c.c. of distilled water at 25° C, was placed centrally on the bottom of the assembly; the water was stirred using a magnetic stirrer drive which was situated under the plastic container. Two concentric rings of 6 and 17 cm in diameter, respectively, and made in standard, vinyl-insulated copper wire 2 mm thick, were suspended horizontally about 5 cm above the beaker, and brought to a potential difference of 6 kV using a stabilized d.c. supply. A large amount of supercooled fog was then produced in the apparatus by fast introduction of about 200 c.c. of liquid nitrogen into the beaker. Temperatures at the level of the electrodes usually fluctuated between −6° and −17° C during experimentation. Observation of the dense, thin 'whiskers' was carried out after the fog had subsided. Invariably, large amounts of hair-like ice-particulates were found to extend between electrodes (Fig. 1).

In contrast to the structure of 'normal' metal or salt whiskers, which appear to be of a somewhat unusual but none the less definitely developed crystalline form (see, for example, a recent contribution by L. D. Calvert, A. F. Sirianni and I. E. Puddington[4]), the ice particles obtained in this experiment consist of very thin microcrystalline dendritic aggregates, the agglomeration of which is oriented in the direction of the electric field (Fig. 2).

It can be seen that the 'fibres' consist of particulates more than about 20μ thick: closer examination reveals the existence of minute, frozen ice spherules, which are intermingled with the microscopic dendrites. The photographs were taken using a suitable, microscopic covered cold stage and transmitted light: no particular effects could be detected by use of polarized illumination.

The growth of dendrites is not surprising here, considering the order of magnitude of the temperature in

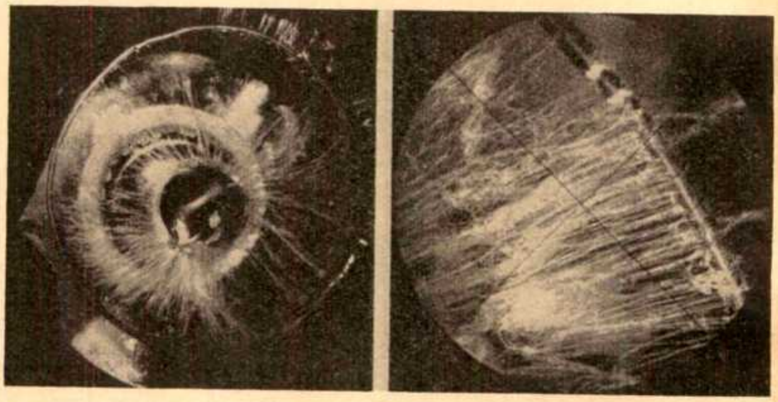

Fig. 1. Photographs of 'ice whiskers' grown between charged electrode wires. One picture was taken directly, while the other was taken through a cathetometer telescope

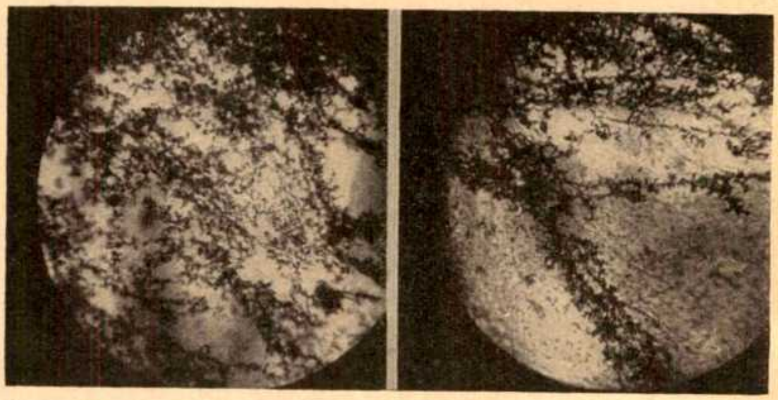

Fig. 2. Two different photomicrographs of dendritic 'ice-whisker' material. Visual field is 0·78 mm in diameter

e region of the electrodes[5]. The speed of growth of e agglomerates in the direction of the electric field, well as their thinness and fragility, are, however, of onsiderable importance. If, in line with Schaefer's bservations, the presence of an ice-forming nucleus s required to initiate 'ice-whisker' growth, and if such rowth is afterwards favoured by a local strong tmospheric electric field, and occurs in a dense under-ooled cloud, then it may easily be expected that the delicate material formed will soon be broken up by atmospheric turbulence into many minute fragments of ice, which will in turn act as individual nuclei. It is therefore thought that a process of a chain-multiplication of ice nuclei, such as that suggested here, could, if occurring in Nature in conditions otherwise suitable, have a bearing on some aspects of precipitation phenomena from congested clouds.

We thank Prof. V. Caglioti for his interest in this work, and the Cassa per il Mezzogiorno and the Società Generale Elettrica della Sicilia for financial assistance.

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GEOLOGY

Possible Relation of Tuff in the Jackson Group (Eocene) to Bediasites

King and Rodda[1] have described rhyolitic tuffs in the Jackson Group; the first report of rhyolitic tuff from the Texas Gulf Coastal Plain. Analysis of tuff from the Jackson Group (Table 1) differs from analyses of unaltered tuffs from the Gueydan Formation (Miocene) in South Texas[2].

Table 1. Analyses of Tuff and Tuffaceous Siltstone from the Manning Formation of the Jackson Group, Walker County, Texas

This locality is known locally as the 'Chalk Pit' (from King and Rodda (ref. 1))

	Upper tuff	Middle tuffaceous siltstone
SiO_2	73·13	73·08
Al_2O_3	13 26	15·23
Fe_2O_3	0·57	3·16
MgO	0·39	0·53
CaO	0 65	1·08
Na_2O	1·33	1 89
K_2O	6·02	2·68
TiO_2	0·19	0·59
P_2O_5	0 02	0·12
MnO	0·06	0·04
Ignition loss	3·62	1·52
H_2O	0·79	0·37
	100 08	100·22

Most bediasites have been found on rocks of the Jackson Group, and analyses of Jackson tuff and tuffaceous rock (Table 1) are similar to some published analyses of bediasites[3,4], especially when water and ignition loss are removed and percentages recalculated to 100 per cent.

Tuff, tuffaceous rocks and bentonite (altered tuff) are abundant in the Jackson Group[5] and are present at or near many bediasite localities. The Purswell Lease of the Baroid Division, National Lead Co., is a recently discovered example.

The Purswell property, situated approximately 5 miles south-east of Waelder, Gonzales County, Texas, contains 2 large pits from which bentonite has been commercially recovered. The old pit, to the west, is abandoned. The new pit, approximately 100 yards east of the old pit, is being worked for bentonite. Mr. E. S. Dorsey, of Flatonia, Texas, mine superintendent of the Purswell Lease, found a bediasite on top of the waste pile immediately north of the old pit. This specimen is now in the possession of Mr. R. E. Offeman, of Houston, Texas, who furnished a fragment to me for examination. Mr. Dorsey states that material in the waste pile on which he found the bediasite came from well below the ground-surface, approximately 6–10 ft., and that this waste material was loaded from the bottom of the pit, hauled to its present location and dumped there. There seems to be only a slight possibility of mixing with surface gravel. The waste pile contains abundant gravel, and gravel is in place in the rock exposed in the east wall of the old pit, although most of the old pit is now flooded. Mr. Dorsey also noted that almost all the gravel in the waste pile on which he found the bediasite occurred in lenses very close to the top of the 6–8-ft.-thick strata of bentonite which was mined. At least 2 other bediasites have been found on waste piles to the north and west of the old pit.

It is probable that the 3 bediasites from this locality came from gravel lenses overlying the bentonite. This locality supports the conclusion that there is a geographical association, as well as a probable stratigraphic association, of bentonite (altered tuff) and bediasites. The stratigraphic position of this locality within the Jackson Group is unknown. There are several faults in the area, outcrops are scarce, and dips within the pit are 4°–6° south-west instead of the 1°–2° south-east which is the regional trend.

Two experimental dry fusions of tuff from the 'Chalk Pit' locality, Walker County, were performed in a Hevi Duty globe-type furnace at one atmosphere pressure in covered Denver fire clay crucibles.

Fusion 1 was heated to 1,420° C over a period of 4 h, cooled to 1,200° C, held at that temperature for 72 h, cooled to 850° C over a period of 6 h and removed from the furnace. Fusion 2 was heated to 1,450° C over a period of 5 h, held at that temperature for 30 min, then was removed from the furnace and immediately quenched in room temperature tap water. The glass resulting from both of these fusions is identical. The glass is transparent, vesicular, greenish yellow-brown, devoid of microlites, and has an index of refraction of 1·490 ± 0·001. The specific gravity of this glass could not be determined accurately because of the numerous vesicles, but because most of the vesicles are spherical or nearly spherical a fair approximation of the specific gravity can be made allowing for the total volume of the vesicles. One such determination was made on a small fragment of glass from fusion 2. The vesicles in the specimen were counted and measured, and their approximate volume calculated. The corrected specific gravity of this glass is 2·351 at 74° F. These properties are very similar to those of some bediasites[3].

Preliminary comparison of the composition of tuff samples from the 'Chalk Pit' with bediasites by X-ray fluorescence analysis does not indicate any marked qualitative differences. However, this remains to be investigated in detail.

Pinson and Schnetzler[4] found 'marked similarity' in rubidium/strontium ratios of bediasites and a sample of 'clayey sand' from the Wellborn Formation of the Jackson Group. The strontium-87/strontium-86 ratios were found to be identical within experimental error, but the chemical composition of the 'clayey sand' that Pinson and Schnetzler analysed did not resemble bediasites. Isotope investigation of the tuffs and tuffaceous rocks in the Jackson Group seems desirable because of their compositional similarity to bediasites.

If bediasites are of terrestrial origin, it is probable that tuffs or tuffaceous rocks in the Jackson Group are the parent material of, or are genetically related to, bediasites.

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GEOCHEMISTRY

Trace Elements in Tanganyika Carbonatites

Von Knorring and du Bois[1] have given analyses for a vesicular carbonate lava from Fort Portal, Uganda, showing close geochemical affinities with intrusive carbonatites. Only two other occurrences of extrusive equivalents of carbonatites have been reported, namely, Kerimasi[2] and Oldoinyo Lengai[3]. Dawson[3] has described both *pahoehoe* and *aa* carbonate lava from Lengai which were extruded from an active vent on to the floor of the northern crater. Analyses of samples of the lava have been completed at the Tanganyika Geological Survey Laboratories together with the trace elements. At the same time, samples of Kerimasi sövites and Oldoinyo Dili sövites were analysed to compare the trace element data of both extrusive and intrusive carbonatitic occurrences. These values, obtained by a semi-quantitative spectrographic method using anode excitation and by comparison with synthetic standards, are listed in Table 1.

Kerimasi, Oldoinyo Dili and Oldoinyo Lengai[3] are situated in an area which extends from the Serengeti Plains in the west to Kilimanjaro in the east, and from the Kenya border in the north to Lake Manyara the south. Kerimasi and Oldoinyo Lengai associated with volcanoes of a highly explosive ty in which lavas are subordinate to pyroclastics, wh Oldoinyo Dili represents an eroded eruptive centre.

Table 1

	Sensitivity	Oldoinyo Lengai		Kerimasi		Oldoinyo Dili	
		BD.118	*BD*.114	*JG*.1523	*JG*.1266	*JG*.2349	*JG*.2536
Li	3	100	100	*	*	5	3
Ti	25	25	25	25	600	3,000	1,500
V	5	50	70	100	100	70	70
Cr	1	*	*	1	*	1	*
Co	1	*	*	10	15	10	3
Ni	3	*	*	3	5	*	*
Ga	3	*	*	3	*	3	3
Rb	50	200	400	*	*	*	*
Sr	25	†	‡	3,500	2,500	2,500	2,500
Y	5	*	*	100	100	40	30
Zr	5	*	*	50	30	200	450
Nb	10	15	*	30	40	800	225
Ba	25	†	‡	2,000	1,500	3,500	3,500
La	50	400	400	250	200	700	500

All values in parts per million.

* Below sensitivity; precision ± 40 per cent.

† Values for SrO = 1·20 and BaO = 1·05 taken from Dawson (ref. 3, Table 1).

‡ Values for SrO = 1·24 and BaO = 0·95 taken from Dawson (ref. 3, Table 1).

BD.118 and 114, carbonate lavas collected by J. B. Dawson. *JG*.1523, 1266, 2349 and 2356, sovites collected by N. J. Guest.

The only characteristic trace element occurring i all three localities is lanthanum, with the values f Oldoinyo Lengai and Oldoinyo Dili approaching th for the vesicular carbonatitic lava from Fort Port (600 p.p.m.). Other rare-earth elements have not ye been sought, but yttrium with geochemical properties similar to the rare-earth group, particularly ytterbium, is only found in appreciable quantity at Oldoinyo Dili. The concentration of strontium i higher than the concentration of barium in the two extrusives Lengai and Kerimasi, a characteristic property of carbonatites noted by Higazy[4] and observed by von Knorring and du Bois[1]. However, in contrast, barium is more concentrated than strontium at Oldoinyo Dili. Niobium, an element often thought to indicate the presence of carbonatite, is notably low for Lengai and Kerimasi but reaches 800 p.p.m. at Oldoinyo Dili. Significantly, the carbonate lavas from Lengai contain up to 400 p.p.m. rubidium and 100 p.p.m. lithium, a marked indication of the alkalinity. Wyllie and Tuttle[5] have commented that if alkali-bearing carbonatitic magmas can persist down to pressures of 1 bar, then the eruption of vesicular carbonate lava is possible. The specimens from Oldoinyo Lengai may thus approach the composition of a carbonatite magma.

It may be tentatively concluded that lanthanum i a characteristic trace element in Tanganyikan carbc atites. Further evidence of this is indicated by recent analysis of a brecciated fenite from Wigu Hi some 300 miles south-south-east of the three north localities, having a concentration of more than 5,0 p.p.m. lanthanum. The presence of lithium a rubidium confirms the alkaline nature of carbona lava from Oldoinyo Lengai.

I thank Mr. J. W. Pallister, commissioner for Geological Survey, Tanganyika, for criticism and permission to publish this short communication.

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CRYSTALLOGRAPHY

Crystal Structure of Peroxochromates, $CrO_5.C_5H_5N$

WORK on the crystal structures of peroxochromates has been going on in this laboratory for some years. The addition compound $CrO_5.C_5H_5N$ was prepared according to Hofmann and Hiendlmaier[1] and was recrystallized in ether, which yielded well-developed needle-like crystals. The compound decomposed in one or two days. $CrO_5.C_5H_5N$ was investigated by the usual X-ray methods. It was found to have monoclinic symmetry, corresponding to the non-centrosymmetric space group $P2_1$ (No. 4).

The dimensions of the elementary cell are: $a = 5{\cdot}107$ Å; $b = 16{\cdot}31$ Å; $c = 11{\cdot}29$ Å; $\beta = 128{\cdot}35°$. The cell contains four formula units. Parameter values for all the atoms have been determined by three-dimen-

Table 1. PARAMETER VALUES FOR $CrO_5.C_5H_5N$. SPACE GROUP $P\,2_1$

om	x	y	z	Atom	x	y	z
Cr_1	0·141	0·996	0·995	N_1	0·212	0·122	0·003
Cr_2	0·857	0·147	0·507	N_2	0·714	0·520	0·503
O_1	0·965	0·503	0·190	C_1	0·291	0·158	0·920
O_2	0·065	0·923	0·843	C_2	0·358	0·244	0·931
O_3	0·465	0·501	0·817	C_3	0·330	0·287	0·016
O_4	0·445	0·920	0·140	C_4	0·233	0·248	0·095
O_5	0·168	0·497	0·986	C_5	0·185	0·166	0·091
O_6	0·518	0·644	0·308	C_6	0·783	0·488	0·422
O_7	0·531	0·723	0·347	C_7	0·845	0·401	0·436
O_8	0·028	0·644	0·685	C_8	0·809	0·356	0·525
O_9	0·914	0·723	0·636	C_9	0·718	0·393	0·602
O_{10}	0·667	0·142	0·486	C_{10}	0·659	0·481	0·590

sional Fourier summation (Table 1). The reliability index R is calculated to be as follows: $R_{hk0}=0{\cdot}15$, $R_{h0l}=0{\cdot}21$, $R_{0kl}=0{\cdot}19$ and $R_{hkl}=0{\cdot}21$.

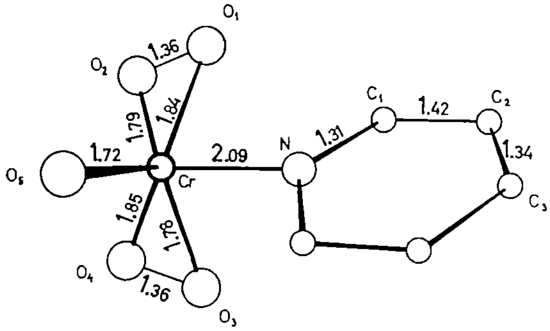

Fig. 1

The constitution of the peroxo complex can be seen om Fig. 1. Thus the chromium atom is 6-co-inated, and the geometrical configuration is that of omewhat distorted pentagonal pyramid with the le oxygen at the top of the pyramid. The bond-ances have been taken as the mean values of the responding distances found in the two non-sym-try-related molecules. It is estimated that the romium to light atom distances are probably eliable to ± 0·05 Å and the light atom to light atom istances reliable to ± 0·10 Å. The distances between the oxygen atoms in the O_2-groups are remarkably short.

A least-square refinement of the structure has been undertaken, and a complete report of the structure nvestigation will be published elsewhere.

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CHEMISTRY

Resolution in Gas Chromatography

THE comparison of columns used for gas chromatographic separation is almost invariably based on the experimentally determined number of theoretical plates (n). A major practical limitation of this concept is that it can only be calculated for isothermal operation; in addition, its use for comparing the performances of different columns is misleading. Although capillary columns have been developed with several hundred thousand theoretical plates, as opposed to the several thousand plates of ¼-in. diameter packed columns, the performance of the apillary columns (in terms of ability to separate omponents) is only a few times better than that of the acked columns. The comparison between distillation and gas chromatographic efficiencies is similarly misleading[1].

Peak resolution has been suggested[2] for assessing the performance of a column in separating any pair of components. This term can be used irrespective of the way in which the column is operated; but it does not provide a clear overall picture of the separating capabilities of the column, nor does it afford a means of comparing column performances.

We therefore propose the concept of the 'effective peak number' (EPN).

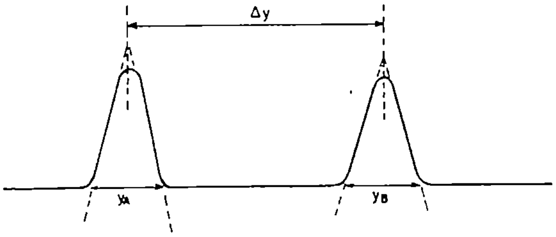

Fig. 1

Fig. 1 represents a chromatogram of two consecutive members of a homologous series. The nomenclature is that recommended by Ambrose, Keulemans *et al.*[2]. We define effective peak number as:

$$\text{EPN} = \frac{2\Delta y - (y_A + y_B)}{(y_A + y_B)}$$

This is obviously related to peak resolution (r), which is given by:

$$r = \frac{2\Delta y}{(y_A + y_B)}$$

$$\text{that is, EPN} = r - 1$$

We emphasize that this holds only where r is calculated for consecutive homologues.

We suggest that the two homologues, by which effective peak number is defined, are specified. For example, if n-octane/n-nonane be the pair, then the appropriate value would be EPN_{nC_8/nC_9}, similarly $\text{EPN}_{\text{bz/tol}}$ for the benzene/toluene pair.

The value of effective peak number lies in the fact that it is the maximum possible number of peaks which can be completely resolved between two homologues with a given set of operating conditions. The effective peak number can, of course, be calculated for any type of column, operated either isothermally or with any form of programming. By the same token,

Table 1. VALUES OF THE EFFECTIVE PEAK NUMBER AND NUMBER OF THEORETICAL PLATES FOR SOME REPRESENTATIVE COLUMNS

Stationary phase, dimensions and mode of operation of column	Component	EPN	No. of theoretical plates
Silicone gum, 2′ × ¼″, programmed temp., 60°–300° C	nC_{23}	1·0	400 (isothermal)
	nC_{24}		
'Apiezon *L*', 30′ × ¼″ isothermal (125° C)	benzene	8·4	10,240
	toluene	9·1	—
	ethylbenzene		—
programmed, 60°–220° C	toluene	9·2	
	ethylbenzene		
2,5-Hexanedione, 45′ × 1/8″ isothermal (0° C)	C_3	6·5	26,400
	nC_4	14·4	23,410
	nC_5		20,770
'Apiezon *L*', 60′ × 1/8″ isothermal to nC_6, then programmed	nC_5	16·6	30,000
	nC_6	23·1	—
	nC_7	25·3	
	nC_8	20·3	
	nC_9		
Squalane, 120′ × 0·01″ isothermal (70° C.)	nC_{18}	26·6	160,500
	nC_{19}		—

The effective peak number of the best of the above columns (the efficiency of which is good by any standards) puts into perspective the limit of separation (for example, of isomers) obtainable by gas chromatography. It appears that EPN values above say, 40, are unlikely to be obtained easily so that, should a mixture contain more than this number of components between any pair of homologues, they could not be completely resolved, however ideal the stationary phase.

it can be used for comparing the performance of any two columns, even though they may be operated in completely different manners.

In Table 1, some values of effective peak numbers and, where applicable, the numbers of theoretical plates, are given for a range of column types and modes of operation.

Note added in proof. Since this communication was originally submitted, we have become aware of a publication by Kaiser[3], in which he defines a "separation number" (TZ) in almost exactly identical terms to our definition of EPN. The two concepts are equivalent.

In a recent paper[4], resolution, with respect to consecutive normal alkanes, was specified for a high efficiency column. Besides emphasizing the very limited utility of theoretical plate numbers, the actual value of resolution (68 for nC_6/nC_7) obtained was strikingly high.

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Transmission of Electronic Effects in Transition Metal Complexes

AN interesting aspect of transition metal chemistry relates to the influence which one ligand partner, in a mixed complex, may exert on another. The importance of electronic effects transmitted through a central metal ion is demonstrated, for example, by the directing influence of certain ligands in substitution reactions[1]. We report here some preliminary results on the influence of certain anionic ligands on the nature of the metal–pyridine bond in mixed complexes of cobalt(II). Our evidence for transmitted electronic effects in these complexes rests on certain striking differences in the influence of X on the position of equilibrium in the following configurational transformation:

$$\underset{\text{(tetrahedral)}}{\text{Co py}_2X_2} + 2\text{ py} \rightleftharpoons \underset{\text{(octahedral)}}{\text{Co py}_4X_2} \qquad (1)$$

in chloroform solution. Py = pyridine and X = Cl$^-$, Br$^-$, I$^-$, OCN$^-$, SCN$^-$ or SeCN$^-$. Co(II) complexes are well suited to configurational equilibria investigations of this kind because of the relatively high crystal field stabilization energy expected for the d^7 configuration in a tetrahedral field[2], and experimentally, because of the separate well-defined visible spectral characteristics of the two stereo-chemical arrangements. Equilibrium constants derived from spectrophotometric measurements were coupled with enthalpies determined calorimetrically and the associated entropy changes calculated. Since chloroform is relatively non-co-ordinating, the derived thermodynamic quantities refer, fairly closely, to the actual association reaction as represented in equation (1).

We direct attention to two features of the results: (1) the marked difference in the effect of the halide and OCN$^-$ ions on one hand, and SCN$^-$ and SeCN$^-$ on the other, on the equilibrium constant (K); (2) the predominant importance of the entropy term. The negative entropies are of the order of magnitude expected for an association reaction in which two of the three reacting molecules lose independent translational motion. It is the differences in ΔS, however, which are of greater interest, and to account for them we suggest that the pyridine ligands lose, in addition, a certain amount of rotational freedom (about the cobalt–nitrogen bond) to an extent determined by X. Potential barriers to rotation may arise from steric interactions or from multiple bonding. While some steric interaction must be expected in all the octahedral complexes, steric factors alone cannot be responsible for the dependence of ΔS on X; for example, there is unlikely to be a greater steric effect in Co py$_4$(NCO)$_2$ than in Co py$_4$(NCS)$_2$. Double bonding would arise from back-co-ordination of electrons in non-σ-bonding metal d orbitals to an anti-bonding π-orbital of pyridine; considerable evidence for multiple bonding in metal–pyridine complexes already exists[3]. On this basis, then, it is possible to arrange the ligands X in order of their capacity for inducing dative π-bonding in the Co–pyridine bond. From the ΔS values this order is:

$$\text{I}^- > \text{Br}^- > \text{Cl}^- \gg \text{OCN}^- \gg \text{SCN}^-$$

with SeCN$^-$ probably placed after SCN$^-$.

It seems that both inductive and mesomeric effects are involved. The order of effectiveness of the halide ions indicates that in the halide complexes it is the charge transferred to the metal in the cobalt–X bond that is important in the first instance. This electron drift, since it will decrease the effective electron affinity of the metal, might be expected to have two consequences; (1) decrease the attraction of the meta ion for the electrons in the cobalt–pyridine σ-bon (2) enhance the overlap of the non-σ-bonding metal orbitals with pyridine π-orbitals. By either mean the metal atom becomes partially relieved of excessive electron density close to it. If the inducti effect (1) were the dominant one, the changes in would be reflected mainly in ΔH, which should fall o on passing from chloride to bromide to iodide. In fact not only is a small trend in the opposite sense observed but the entropy of reaction becomes more negative also. This is strong evidence for a transmission of effects by means of both π- and σ-bonding metal orbitals. The much less negative ΔS found for the thiocyanate suggests either very little covalency in the Co–NCS bond (relative to Co–Cl) or a substantial measure of dative π-bonding of the type $d\pi \rightarrow p\pi$ (in Co–NCS) which would render the metal t_{2g} orbital less available for overlap with pyridine π-orbitals The former postulate, however, would conflict with other properties of the SCN$^-$ ion, for example, its reducing character which is comparable with that of Br$^-$ and its position in the nephelauxetic series[4]. Thus it seems that SCN$^-$ (and probably SeCN$^-$ also) when bonded via the nitrogen atom, as is the case here[5], may have a moderately high π-acceptor capacity, OCN$^-$ being less effective in this respect.

These conclusions, which are being tested in further experiments, demonstrate the different ways in which charge may be distributed within a complex molecule so as to maintain the metal atom in a condition close to electrical neutrality.

It is noteworthy that K, which is a sensitive indicator of the tendency of the metal to increase its co-ordination number from 4 to 6, correlates fairly well with the preferred co-ordination number in the solid complex Co py$_2X_2$ (Table 1). Only Co py$_2$(NCO) seems anomalous. However, we believe this anomaly to be apparent rather than real, since the adoption o

Table 1

Complex	K	$-\Delta H$ (kcal mole^{-1})	$-\Delta S$ (cal deg^{-1} mole^{-1})	Preferred configuration in solid state
Co py_2Cl_2	12·6	15·2	47	Bridged octahedral (ref. 7)
Co py_2Br_2	9·04	15·6	49	Tetrahedral (ref. 7)
Co py_2I_2	2·32	16·6	55	Tetrahedral (ref. 7)
Co $py_2(NCO)_2$	23·3	13·7	40	Tetrahedral (ref. 5)
Co $py_2(NCS)_2$	83,000	16·6	84	Bridged octahedral (ref. 7)
Co $py_2(NCSe)_2$	> 100,000*	—	—	Bridged octahedral (ref. 5)

All data refer to measurements at 20° C.

* A reliable value was not obtained because of the low solubility of this complex.

a bridged octahedral configuration depends, among other factors, on the capacity of the anion X for bifunctional linkage. The suggestion[5] that OCN$^-$ might have a low co-ordinating power through the oxygen atom is thus supported by the data presented here. It is probably significant, in this connexion, that while organic thiocyanates are known in both the normal and iso-forms, organic normal-cyanates have not been isolated[6]. It seems that before OCN can be made to bridge in complexes of the kind described here the electron density around the metal atom must be further reduced. One obvious way of attempting this is to replace pyridine by a poorer σ-donor. We have done this using 4-cyanopyridine and find that cobalt(4-cyanopyridine)$_2$(NCO)$_2$ is pink in colour and has a magnetic moment of 5·3 *B.M.* at 18° C, a value in accordance with expectation for 6-co-ordinate Co(II)[7]. The complex is therefore presumed to contain bridged OCN$^-$.

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Electron Affinities of Organic Molecules

ABSOLUTE values of the electron affinity, E, of organic molecules have not been determined experimentally except in a few cases[1,2], and in most of these the uncertainty is several tenths of an electron volt or even more. Some theoretical values have also been calculated[3]. Since the electron affinity is a fundamental molecular property and also is[4] of considerable importance to modern biology it is desirable that more and better values be found.

The deduction of relative values of E from the data of charge-transfer spectra has been possible in principle for some time but absolute values have been unobtainable[2]. However, Jortner and Sokolov[5] have recently used experimental results to relate the affinity of I_2 to the well-known value for atomic iodine. In this way $E(I_2)$ was found to be 1·8 eV. Mulliken had previously estimated[6] 1·8 eV for $E(I_2)$ and indeed such a theoretical value for E could have been made the basis of a table of electron affinity values, which however, to our knowledge, has not been done.

Since I_2 acts as an acceptor in charge-transfer spectra, E of other acceptors can be related to $E(I_2)$. The simplest relationship between the E's of two acceptors depends on a number of assumptions and follows from the expression for the energy, $h\nu$, of the maximum of the charge-transfer band being given by the expression (1).

$$h\nu = I(D) - E(A) + \varphi_{AD} \qquad (1)$$

where $I(D)$ is the ionization potential of the donor molecule D; $E(A)$, the electron affinity of the acceptor, A.

The difference in E for two molecules, i, and j, for a given donor is then:

$$E(A_i) - E(A_j) = h\nu_j - h\nu_i + \varphi_{iD} - \varphi_{jD} \qquad (2)$$

The assumption that the right-hand side of equation (2) is approximately equal to $h\nu_j - h\nu_i$ can be tested by determining $E(A_i) - E(A_j)$ for a series of donors. Data are available for tetrachlorophthalic anhydride with 7 donors; 1,3,5-trinitrobenzene with 12 donors; chloranil (presumably p-chloranil) with 14 donors; and tetracyanoethylene with 14 donors. The affinities of these acceptors relative to $E(I_2)$ are calculated in eV to be respectively $-0·03 \pm 0·10$; $+0·06 \pm 0·13$; $+0·79 \pm 0·17$; $+1·09 \pm 0·10$. The spread of the values so determined is thus only about 0·2 eV in each case and the simplifying assumption accordingly finds some justification.

However, $(\varphi_{iD} - \varphi_{jD})$ need not be zero, but may be a constant with respect to changing the donor, D. We now make the simplest assumption, which was also

Table 1. APPROXIMATE ELECTRON AFFINITIES, E, OF SOME ACCEPTOR MOLECULES AND THE STANDARD DEVIATION, σ

Acceptor	E (eV)	σ (eV)	Donors*	Ref.
Tetracyanoethylene	2·8$_9$	0·10	1–8, 11, 12, 14–17	9, 10, 14
o-Bromanil	2·7$_7$		28	19
o-Chloranil	2·7$_2$		23	19
p-Bromanil	2·6$_4$		14, 21, 28	19–21
p-Chloranil	2·5$_9$	0·17	2–8, 12–28, 21–28	8, 13, 19–21
p-Iodanil	2·5$_8$		14, 21, 23–28	21, 22
2,5-Dichloro-*p*-benzoquinone	2·5$_4$			12
2,6-Dichloro-*p*-benzoquinone	2·3$_4$	0·10	14, 19–22†	16
1,2-Dicyano-1,2-dicarboxyethylene	2·2$_8$		17	9
Chloro-*p*-benzoquinone	2·2$_1$	0·11	14, 19–22	16
p-Benzoquinone	2·0$_2$		17	9
Maleic anhydride‡	2·0$_1$		17	9
Nitric acid	1·9$_3$	0·18	7, 8, 12, 13, 17, 18	18
2,4,6-Trinitrobenzoic acid	1·9$_0$		14	12
1,3,5-Trinitrobenzene	1·8$_6$	0·13	2–10, 13, 14, 18	7, 13
Iodine molecule	(1·8$_0$)§		1–18	6, 11, 13
Tetranitromethane	1·7$_9$	0·16	7, 8, 11, 12, 13, 25–28	17
2,4,6-Trinitroanisole	1·7$_2$		14	12
Tetrachlorophthalic anhydride	1·7$_7$	0·10	7, 8, 12, 14, 17, 18	12, 14
Picryl chloride	1·7$_4$		14	12
2,4,6-Trinitrotoluene	1·6$_9$	0·07	19–22†	12, 16
Maleic anhydride‡	1·5$_8$		7, 8, 16, 18	15
Phthalic anhydride	1·5$_0$	0·07	7, 8, 12, 16, 18	15
Phthalamide	1·5$_0$	0·16	7, 8, 12, 16, 18	15
m-Dinitrobenzene	1·4$_3$	0·10	14, 19–22	16

* 1, Chlorobenzene; 2, benzene; 3, toluene; 4, *m*-xylene, 5, mesitylene; 6, durene; 7, naphthalene; 8, phenanthrene; 9, 2-methylnaphthalene; 10, 1-methylnaphthalene; 11, hexaethylbenzene; 12, biphenyl; 13, stilbene; 14, hexamethylbenzene, 15, triphenylene, 16, chrysene; 17, pyrene, 18, anthracene; 19, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; 20, *N,N,N′,N′*-tetramethyl-*p*-*p′*-diaminodiphenylmethane; 21, methylaniline; 22, dimethylaniline; 23, perylene; 24, aniline; 25, benzylamine; 26, diphenylamine, 27, diethylamine; 28, triethylamine.

† In the case of donors 19–22, values are based on that of trinitrobenzene, taken as 1·86 eV.

‡ There is disagreement in the literature about the experimental result for the perylene–maleic anhydride complex.

§ This value was used to fix the zero of the energy of scale.

implicit in the work of Jortner and Sokolov, that this constant is zero. Consequently, it is possible to compile a table of electron affinities (Table 1) for molecules which act as acceptors in charge-transfer spectra. The above assumption is expected to hold quite well for molecules which form complexes with similar intermolecular separations. For planar molecules such as chloranil, 1,3,5-trinitrobenzene, and for tetracyanoethylene the contribution to $(\varphi_{iD}-\varphi_{jD})$ from a change in intermolecular separation is probably less than 0·2 eV and so may be ignored. For iodine, $(\varphi_{iD}-\varphi_{jD})$ may or may not be significantly larger than 0·2 eV. Since iodine is the basis of the scale it is therefore possible that the electron affinity values given in Table 1 differ from the true values by a constant which, however, is possibly not more than a few tenths of an electron volt. Furthermore, φ_{iD} must vary with the polarizability of the donor and the acceptor. The contribution of such a change to $(\varphi_{iD}-\varphi_{jD})$ for the acceptor pair *p*-chloranil and *p*-bromanil or the pair *p*-chloranil and 1,3,5-trinitrobenzene is no greater than 0·4 eV. In many other cases the contribution must be less than 0·4 eV. The electron affinity values in Table 1 are labelled 'approximate' because it has not been possible to take account of the corrections for lack of sufficient data on polarizabilities and intermolecular distances. In Table 1 $E(\mathrm{I}_2)$ has been taken as 1·80 eV. Those values which are derived from the spectrum with a single donor are subject to a greater uncertainty than those for which many donors have been studied.

Other values of E have been derived from charge-transfer spectra by Briegleb and Czekalla[2] using a different procedure which yields, according to the authors, a lower limit for E. Briegleb and Czekalla's results are: tetracyanoethylene, 1·6 eV; chloranil, 1·35 eV; 1,3,5-trinitrobenzene, 0·6 eV; iodine, I_2, 0·8 eV, and maleic anhydride, 0·5 eV. Compared with values in Table 1, Briegleb and Czekalla's results are indeed lower, as they expected, and by an amount of about 1·2 eV. Our results confirm their relative values.

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Radiation-induced Solid-State Polymerization of Acrylonitrile and Acrylamide in the Presence of an Electric Field

CERTAIN monomers, when irradiated in the solid state, polymerize only to a limited conversion. When acrylonitrile is irradiated with γ-rays at −196° C it appears impossible to polymerize the monomer beyond a conversion of approximately 5 per cent[1–3]. If, however, the irradiated monomer is allowed to melt and then resolidified, a stepwise conversion curve is obtained on further irradiation, with yields of approximately 25 per cent[1]. One explanation of this phenomenon is that initiation occurs at crystal defects and termination occurs at grain boundaries or other surfaces.

It has been reported that during the polymerization the reaction vessels became electronically charged, and subsequent discharging with a grounded electrode caused polymerization to proceed further[4]. A linear conversion of up to 23 per cent was achieved when the solid monomer was irradiated with X-rays in a grounded steel vessel[1]. These results were interpreted as indicating that some electronically charged species are the precursors of the active centres responsible for the initiation of polymerization.

An extension of this work[4] showed that if the positive pole of a cell (1·5–80 V) was placed in the solid, with the negative pole unconnected, yields of up to 40 per cent could be obtained. We have investigated the effect of high electric fields (approximately 1 kV/cm) on the radiation-induced solid-state polymerization of acrylonitrile. The results obtained are summarized in Table 1.

Table 1. POLYMERIZATION OF ACRYLONITRILE AT −196° C BY COBALT-60 γ'S (560 krads/h)

Dose (megarads)	Conversion (percentage polymer)		Voltage (kV)
	No field	Plus electric field	
0·5	—	1·2	1·2
1·0	2·2	1·9	1·4
2·0	7 7	7·8	1·4
3·0	—	5·2	1·4
4·0	3·7	3 9	0·9

Field applied between two parallel platinum plates, about 1 cm apart.

These results show that the electric field has little or no effect on the conversions of acrylonitrile. It has been shown that the polymerization of acrylonitrile at −196° C proceeds by an ionic mechanism[5]. It is felt, therefore, that the results from our experiments show that charge separation cannot be a contributory factor in the electric field enhancement of the polymerization in the case of acrylonitrile.

Similar experiments were performed with acrylamide, this monomer being crystalline at room temperature. 100 per cent conversion of the monomer can be obtained by irradiation with higher doses of γ-radiation at this temperature.

It has been assumed that polymerization of this monomer in the solid state proceeds by a radical mechanism; however, electron spin resonance techniques have shown that observed radicals are not those responsible for polymerization[7]. Further evidence indicates that if a radical mechanism is operative then it is not of a similar nature to that observed in liquids[8].

Table 2. POLYMERIZATION OF ACRYLAMIDE AT ROOM TEMPERATURE BY COBALT-60 γ'S (560 krads/h)

Dose (megarads)	Conversion (percentage polymer)		Voltage (kV)
	No field	Plus electric field	
1·0	10·4	9·6	3·0
1·3	11·1	17·0	1·5
1·6	16·3	16·3	1·3
2·0	40·5	39 5	1·35

The results found in the presence of an electric field are summarized in Table 2.

In the presence of an electric field there is no enhanced conversion; thus it may be assumed that there is no charge separation operative in the polymerization of acrylamide.

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BIOCHEMISTRY

Monoamine Oxidase Inhibition by Ethanol *in vitro*

HYPNOTIC doses of ethanol have been reported by Rosenfeld[1] to prolong the metabolic inactivation of exogenous serotonin (5-hydroxytryptamine) in mice. Olson, Gursey, and Vester[2] found the urinary excretion of 5-hydroxyindolylacetic acid (5–HIAA) to be significantly below normal in alcoholics, even after 6 months of abstinence on rigidly controlled diets. These findings suggest that ethanol may act as a monoamine oxidase (MAO) inhibitor. On the other hand, Gursey and Olson[3] have reported depression of brain-stem serotonin and *nor*epinephrine in rabbits after both acute and chronic ethanol administration; suggesting that ethanol may act to release stores of bound amines. Pscheidt, Issekutz, and Himwich[4] were unable to confirm this finding. In our laboratories, we have found that a single dose of ethanol, given orally to human volunteers, produces a marked, but transitory, increase in the urinary excretion of tryptamine[5].

These reports, although somewhat contradictory and confusing, appear to indicate that ethanol does influence the metabolism of the biogenic amines. The possible importance of such an influence in understanding the pharmacology of ethanol, and in the problem of alcoholism, has been emphasized by Westerfeld and Schulman[6]. As the first part of a systematic study of the effects of ethanol on the biogenic amines, we have investigated the influence of ethanol on mouse liver and brain MAO *in vitro*.

The tissues were homogenized in ice-cold potassium phosphate buffer, *p*H 7·4, 0·1 M. Tryptamine, tyramine, and serotonin (1·0 μmole) were used as substrates. Ethanol was added in the following concentrations: 100, 150, 200, 300, and 400 mg/100 ml. Potassium phosphate buffer, *p*H 7·4, was added to bring the total volume to 3·0 ml. Glass-stoppered flasks, 25 ml. vol., sealed with silicone grease, were used. It was necessary to keep all reagents and glassware chilled until the flasks were sealed, to prevent evaporation of the ethanol. After sealing, the flasks were incubated for 70 min at 37° C in a Dubnoff metabolic incubator. The disappearance of the substrate was measured spectrophotofluorometrically after a butanol extraction procedure modified from that of Udenfriend, Weissbach, and Clark[7]. Duplicate samples were run on the controls and on each of the ethanol concentrations.

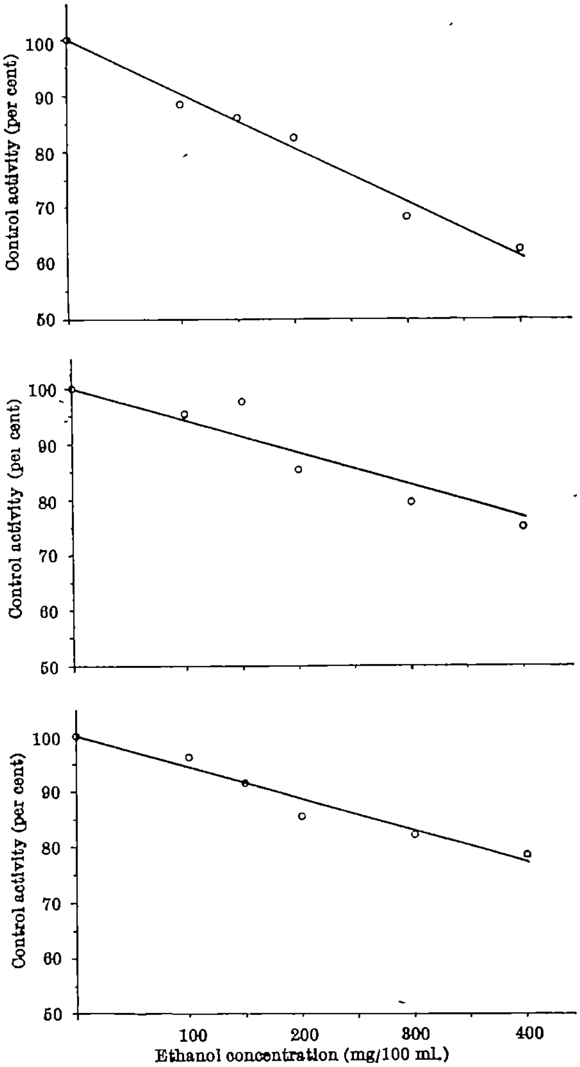

Fig. 1. Inhibition of mouse liver MAO by ethanol *in vitro* using as substrates serotonin (top), tryptamine (middle), tyramine (bottom)

The results obtained, using mouse liver homogenate as the enzyme source, are presented in Fig. 1.

Each of these curves is the average of a minimum of three runs. Straight lines were fitted by the method of least squares. The slopes of these lines are as follows: serotonin, – 0·097 per cent; tyramine, – 0·057 per cent; tryptamine, – 0·058 per cent. The standard deviations of these slopes were calculated. Application of the *t* test indicates that all the slopes differ significantly from a zero slope, *P* being less than 0·01, but do not differ significantly from each other.

Attempts to duplicate these results with brain MAO failed to demonstrate any inhibition of MAO by ethanol, despite the use of a variety of enzyme and substrate concentrations. Purified mitochondria from mouse brain likewise failed to exhibit any inhibition of MAO, although mouse liver mitochondria responded in a manner identical to that of whole liver homogenate (Fig. 1).

These results demonstrate definite and reproducible inhibition by ethanol of mouse liver MAO, but not of mouse brain MAO, when serotonin, tyramine, and tryptamine are used as substrates. The degree of inhibition is of a low order, and its biological and pharmacological significance is questionable. The sharp rise in urinary tryptamine excretion following ethanol ingestion[6] would seem inexplicable on the basis of this degree of MAO inhibition alone. We are therefore led to postulate that ethanol may have an effect on the biogenic amines not unlike that of reserpine, that is, that it can release stores of amines from the bound, inactive state into the free, active state. The depletion of brain-stem serotonin and *nor*epinephrine after the administration of ethanol to rabbits[3] supports this hypothesis. It is intriguing to speculate that ethanol may have a dual action, that of low order MAO inhibition, and that of releasing stores of bound amines. We are investigating this postulated amine releasing action of ethanol.

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Lactate Dehydrogenase Isozymes in the Developing Rat Brain

THE immaturity and the lack of differentiation of the rat brain at birth has been shown some fifty years ago by Sugita[1] in a classic paper on the postnatal morphogenesis of the brain in this animal. The sequence of physiological and biochemical changes which parallel the histological maturation of the rat brain has been investigated, however, only recently.

Biochemical quantitative studies on brain lactate dehydrogenase (LDH) during the post-natal development of the rat have been performed in 1956 by Kuhlman and Lowry[2], who showed that LDH doubles from birth to maturity in the whole rat brain.

A different biochemical approach to the study of the developing brain was made in 1960 by Flexner *et al.*[3] LDH's of cerebral cortex and liver from adult and new-born mice gave different elution patterns when chromatographed on DEAE-cellulose. However, because of variability in loss of activity on the columns, it was impossible to obtain accurate relationships between total anionic and cationic components of the enzyme.

This communication is a preliminary account of quantitative data on LDH isozymes from the whole brain (in the following, the brain without the diencephalon will be referred to as whole brain). Kinetic properties of the enzyme during the ontogenesis will be described elsewhere.

The separation of LDH isozymes was obtained through agar-gel electrophoresis for 3·5 h, at *p*H 8·4, and with a continuous current of 48 m.amp. All other conditions of migration and elution from agar have been previously described by Bonavita and Guarneri[4]. That the technique of elution allowed an almost complete recovery (95 per cent or more) of the enzyme submitted to electrophoretic migration need only be mentioned. In these experimental conditions, LDH from the brain of the adult rat gives the isozymatic pattern illustrated in Fig. 1. This pattern is highly reproducible so far as the migration of the single components and their percentage values are concerned. Table 1 summarizes data on the individual variation of the single components.

The enzyme from the brain of the adult rat gives a pattern quite different from that observed in the new-born animal. Two days after birth, only minor

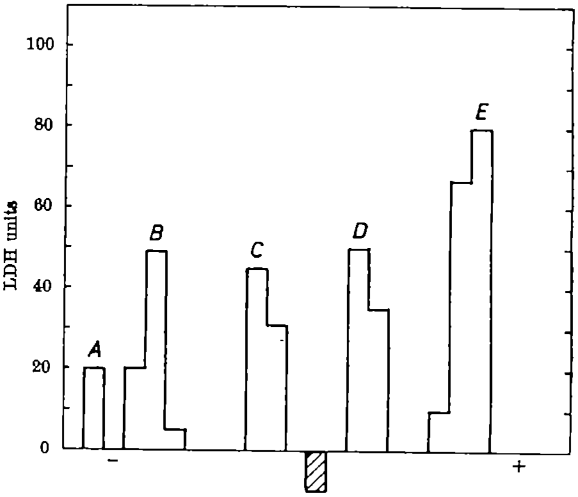

Fig. 1. LDH isozymes from adult rat brain as separated by agar-gel electrophoresis. The starting point is shown below the graph. One LDH unit is the amount of the enzyme which gives an absorbancy decrease of 0·001 in 1 min at 340 mμ

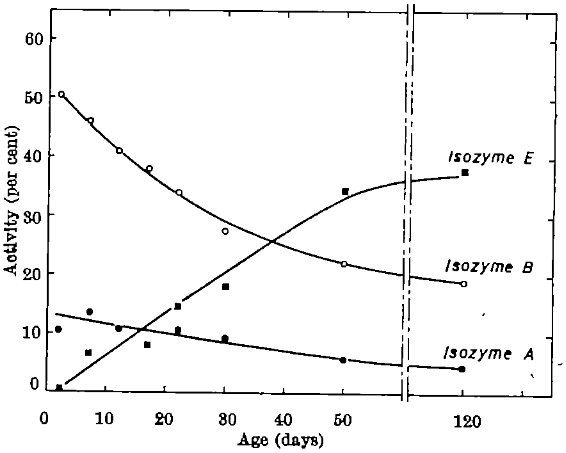

Fig. 2. Changes in the percentage activity of three LDH isozymes from the whole rat brain during the post-natal development. Each experimental point represents the mean of at least three determinations

Table 1. PERCENTAGE MEAN VALUES AND STANDARD DEVIATIONS OF LDH ISOZYMES IN THE WHOLE BRAIN OF THE ADULT RAT*

Isozyme *A*	3·95 ± 0·75
Isozyme *B*	19·45 ± 2·90
Isozyme *C*	19·20 ± 2·80
Isozyme *D*	19·80 ± 2·60
Isozyme *E*	37·60 ± 3·34

* These data have been calculated from the electrophoretic patterns of 12 brain extracts.

amounts of isozyme *E* can be revealed by using the spectrophotometric technique described by Neilands[5] for pig heart enzyme. However, outstanding changes do occur also in the per cent distribution of other enzymatic components. Fig. 2 summarizes data on the quantitative changes of three LDH isozymes in rat brain as a function of the age. Fractions *C* and *D*, shown in Fig. 1, do exhibit only minor modifications with the age of the animals.

The gradual changes in the per cent distribution of LDH isozymes from the developing brain and the developing retina[6] may invite speculation on a possible correlation between morphological and biochemical maturation. It is obvious, however, that such a parallelism may be only approximate. LDH reaches its definitive isozymatic composition only after the twentieth day, that is, when the cortex is already fully organized[1], and when the electrical activity of the brain has been for a long time similar to that of the adult[7,8].

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Transamination of Aromatic Amino-Acids in Nervous Tissue

THE metabolic significance of transaminases in nervous tissues has recently been reviewed by Bonavita[1]. His paper reveals that interest in transaminations of amino-acids in nervous tissues solely comprised the aliphatic compounds. In this communication evidence is presented of transamination of aromatic amino-acids in the nervous tissues, that is, rat brain extracts.

Table 1. PURIFICATION OF RAT BRAIN TRANSAMINASE BY ACETONE PRECIPITATION

3·2 mg protein, 0·5 mM pyridoxalphosphate in each cuvette. Amino acceptor: α-ketoglutaric acid

		Increase in optical density per 30 min		
	Wave-length (mμ)	Brain extract	Purified brain extract	Purification factor
d-*l*-5-OH-tryptophan	328	0 420	0·865	2·0
d-*l*-Tryptophan	328	0·317	0 989	2·7
d-*l*-Dihydroxyphenylalanine	340	0 384	0·174	0 45
d-*l*-Tyrosine	330	0·280	0·837	3·0
d-*l*-Phenylalanine	325	0·174	0·450	2·6
d-*l*-Histidine	293	0 000	0·000	

The enzymatic assay was carried out as follows: rat cerebra were homogenized (20 per cent w/v) in ice-cold 0·57 M borate buffer *p*H 8·2, and afterwards centrifuged at 25,000*g* for 40 min in a refrigerated centrifuge. The clear supernatant was used for enzymatic assay. The assay is based on the ability of the enol tautomer of the aromatic α-keto-acid, which is produced in the transaminase reaction, to combine instantaneously with borate to form a mixed ester-anhydride structure[2,3]. This complex-formation displaces the keto-enol equilibrium in favour of the enol tautomer, which absorbs strongly in the 300 mμ region. The transaminations were read at wavelengths stated in Table 1, using a Beckman *DU* spectrophotometer, fitted with thermospacers at 37° C. Each semimicro cuvette (light path 1 cm) contained 0·4 ml. of the brain extract in the borate buffered reaction mixture of total volume 1·3 ml. Pyridoxalphosphate (Hoffmann–La Roche) was added as stated here. The final concentrations of the amino- and keto-acid were $1·75 \times 10^{-2}$ M and 1×10^{-2} M, respectively. The reaction was started by addition of the keto-acid after a 5 min pre-incubation period.

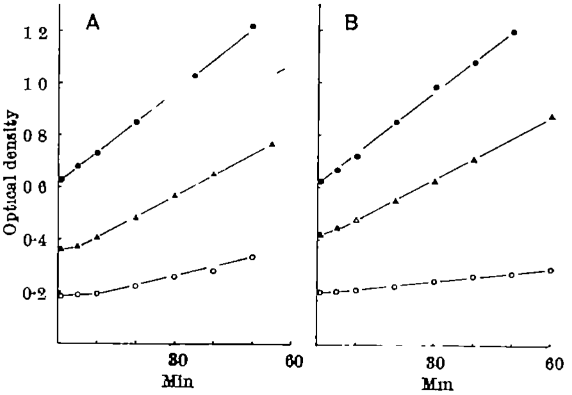

Fig. 1. Transamination of 5-hydroxytryptophan. *A*, α-Ketoglutaric acid as amino acceptor; *B*, pyruvic acid as amino acceptor. ○, 0 00 mM; △, 0 25 mM; ●, 0·5 mM pyridoxal phosphate added

Fig. 1*A* shows typical examples of the data obtained by transamination of 5-hydroxytryptophan with α-ketoglutaric acid. The data demonstrate the pyridoxalphosphate dependency of the transaminase reaction. It can be seen that, providing a sufficient pyridoxalphosphate supply (0·5 mM), a curve is obtained where the increment in optical density is a linear function of time. In Table 1 this increment per 30 min is taken as a measure of enzyme activity. When substituted for α-ketoglutaric acid, pyruvic acid was found to be almost equally effective in the transaminase reaction (Fig. 1*B*). This finding probably reflects low specificity of the enzyme for the keto-acid.

Table 1 reports experiments on the specificity and purification of the enzyme system. It can be seen that both brain extract and a partly purified enzyme preparation transaminated all aromatic amino-acids but histidine. The purified enzyme preparation was obtained as follows: a 20 per cent (w/v) rat cerebra homogenate in 0·15 M phosphate buffer *p*H 7·4 was centrifuged as described here. The clear supernatant was carefully decanted and afterwards

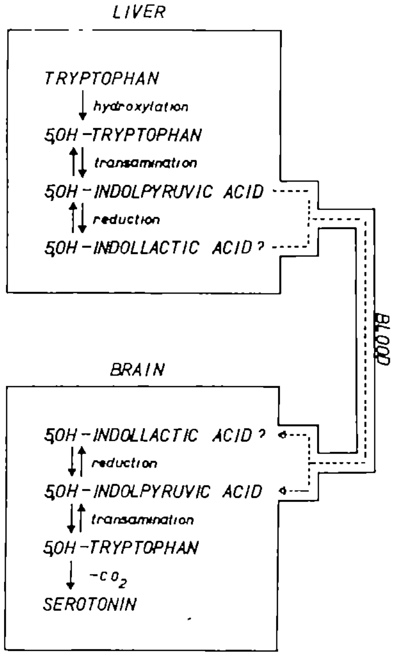

Fig. 2 A hypothetical scheme for the formation of serotonin in brain

precipitated by an equal volume of ice-cold acetone. The precipitate was collected by centrifugation at 25,000*g* for 15 min. The acetone–buffer supernatant was discarded, and the precipitate was extracted with a small volume of the phosphate buffer. Undissolved protein was removed by centrifugation. The protein concentration in the final supernatant was determined by the biuret method, and an aliquot used for enzymatic assay. It appears from Table 1 that the purification procedure gave increased activity for all amino-acids previously transaminated by the brain extract, except dihydroxyphenylalanine, which was transaminated at a significantly slower rate. This reveals that at least two enzymes must be present. However, a higher degree of purification is needed to state the exact number.

Preliminary experiments have shown that the transaminases described here are specific for the *l*-isomer, and further that the catalysis is reversible like other transaminases described[4].

The reactions demonstrated are considered to be of physiological significance for the metabolism of the biological amines. Support for this view is found in the *in vivo* experiments by Pogrund and collaborators[5], who obtained the same pressor response on systemic blood pressure from injection of 3-hydroxy- and 3,4-dihydroxy-phenylpyruvic acid, as from the corresponding amines: *m*-tyramine and dopamine. These findings may well be explained by a transamination and subsequent decarboxylation of the keto-acid directly in the peripheral nervous system. Also in the central nervous system the keto-acids evidently play a prominent part, since patients with phenylketonuria, besides the mental defects, have lowered amine-levels and disturbed amine metabolism[6,7].

The necessary enzymes being present, it seems permissible to postulate that the aromatic keto-acids may act as precursors of the corresponding biological amines. As an example a hypothetical scheme for the formation of serotonin in brain is presented in Fig. 2. This scheme is based on the formation of the appropriate keto (or lactic) derivative in the liver[8,9]. As seen from Fig. 2 a reduced transamination will influence the subsequent steps in the reaction sequence. It would therefore be of interest to investigate the metabolic position of the transaminases described here, in patients with phenylketonuria and other conditions associated with mental defects.

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Effects of Atractyloside and Oligomycin on Magnesium-stimulated Adenosine Triphosphatase and on Adenosine Triphosphate-induced Contraction of Swollen Mitochondria

IN previous reports[1] it was shown that atractyloside inhibits the energy-transfer reactions of oxidative phosphorylation in a manner similar to that of the antibiotic oligomycin[2-4].

From these investigations it appeared that the only difference between the two substances concerned the effect on the magnesium-stimulated adenosine triphosphatase (ATPase). In fact, the magnesium-stimulated ATPase is inhibited by oligomycin and atractyloside using rat-liver mitochondria treated with low concentration of sodium deoxycholate (DOC), but it is not inhibited by atractyloside if the concentration of DOC is increased.

Since the effect of oligomycin on magnesium-stimulated ATPase was not tested on rat-liver mitochondria treated with high concentration of DOC, it was considered of interest to compare the effects of oligomycin and atractyloside under identical experimental conditions. The action of atractyloside on the terminal step of the energy-transfer reactions was also tested by experiments on adenosine triphosphate (ATP)–adenosine diphosphate (ADP) exchange reaction.

Table 1 shows that the effects of atractyloside and oligomycin on magnesium-stimulated ATPase are different: atractyloside strongly inhibits the magnesium-stimulated hydrolysis of ATP when the activity is elicited by a partial damage of the mitochondrial structure (0·1 mM DOC, short-time pre-incubation), while the inhibition is not present, or it is insignificant, when a strong damage is produced by various treatments (1·0 mM DOC, water treatment, mitochondria stored for 72 h); no inhibition was found when the concentration of atractyloside was increased 5- or 10-fold.

In order to explain the difference in the effects of atractyloside and oligomycin, the following possibilities were considered: (*a*) With low concentration

Table 1. Effects of atractyloside and oligomycin on Mg^{++}-stimulated ATPase. Each tube contained 50 mM potassium chloride, 50 mM *tris* buffer, *p*H 7 5, 2·5 mM ATP, 62 mM sucrose, 1 mM $MgSO_4$, atractyloside or oligomycin as indicated, 0·16–0·20 mg of nitrogen rat-liver mitochondria treated as follows: (*a*) 0 1 mM sodium deoxycholate (DOC) added in the medium; (*b*) 10 min of pre-incubation at 25° in 73 mM sucrose, 59 mM potassium chloride, 59 mM *tris* buffer, *p*H 7·5; (*c*) 1·0 mM sodium deoxycholate added in the medium; (*d*) 180 min of pre-treatment at 0° with distilled water; (*e*) ageing 72 h at 4° in 0 25 M sucrose. Final volume, 2 ml.; temperature, 25°; time of incubation, 20 min. Rat-liver mitochondria isolated in 0·25 M sucrose, according to Hogeboom (ref. 5)

	Treatment of mitochondria	μmoles inorganic phosphate liberated		
		Control	Atractyloside 0 05 mM	Oligomycin 0 5 μg/ml.
	—	0·5	0·5	—
(*a*)	0·1 mM DOC	2·6	0·5	0·5
(*b*)	Aged 10 min	2·6	0·6	0·4
(*c*)	1·0 mM DOC	2·4	2·3	0 8
(*d*)	Water at 0°	2 6	2 2	0 8
(*e*)	Aged 72 h	1·8	1·4	0·8

of DOC or with short-time pre-incubation, addition of atractyloside might result in an inhibition of the ageing-process. This hypothesis was in contrast with the observation that the atractyloside-induced inhibition of the magnesium-stimulated ATPase (elicited by pre-incubation in a potassium chloride-*tris*-sucrose medium) was the same when atractyloside was added before or after the preincubation. Furthermore, in Fig. 1 it is seen that atractyloside, as well as oligomycin, does not inhibit the spontaneous or the DOC-induced swelling of mitochondria.

(*b*) The site of hydrolysis or of utilization of the high-energy intermediate during the operation of the magnesium-stimulated ATPase is different in the case of partially and of completely damaged mitochondria. This difference offers a means of distinguishing between the site of action of oligomycin and that of atractyloside.

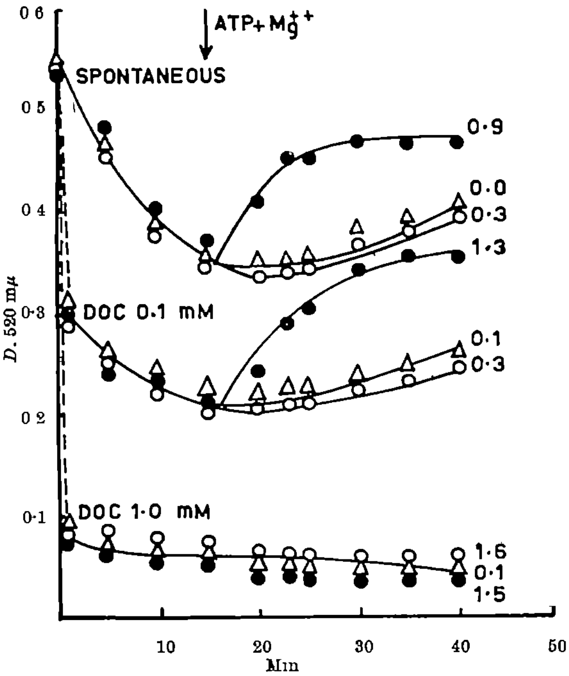

Fig. 1. Effects of atractyloside and oligomycin on ATP-induced contraction of swollen mitochondria and on the accompanying formation of inorganic phosphate. ATP-induced contraction of mitochondria was measured according to Lehninger (ref. 6), using a model *DU* Beckman spectrophotometer and 10 mm × 45 mm cuvettes. Each cuvette contained, in a final volume of 3 ml., 125 mM potassium chloride, 20 mM *tris* buffer, *p*H 7·5, 8·3 mM sucrose, 0 09 mg of nitrogen rat-liver mitochondria and, where indicated, 0·1 or 1·0 mM sodium deoxycholate. Additions: ●, nil; ○, 0 05 mM atractyloside; Δ, 1 0 μg/ml. oligomycin. 2·5 mM ATP and 1 0 mM $MgSO_4$ were added at the time indicated by the arrow. Temperature, 22°. The numbers refer to the μmoles of inorganic phosphate measured at the end of the experimental period

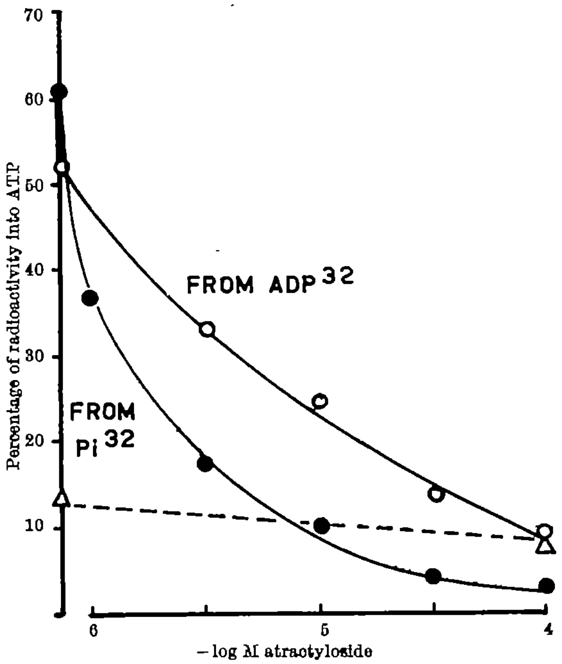

Fig. 2. Effects of atractyloside on ATP–ADP and ATP–inorganic phosphate exchange reactions. Conditions for ATP–ADP exchange: 50 mM *tris* buffer, *p*H 7·5, 4 mM ATP, 1·85 mM ADP (^{32}P) (5 × 10^4 c.p.m.), prepared as described by Cooper and Lehninger (ref. 7), 1 mM EDTA, *p*H 7·2, 62 mM sucrose, 0·08 mg of nitrogen rat-liver mitochondria, atractyloside as indicated. Final volume, 0·4 ml. Δ, per cent of the radioactivity detectable as ATP (^{32}P) in the absence of the ATP addition (adenylate kinase activity). Conditions for ATP–Pi exchange: 50 mM potassium chloride, 40 mM *tris* buffer, *p*H 7·5, 2 5 mM ATP, 1 mM EDTA, *p*H 7·2, 0·7 mM inorganic phosphate (^{32}P) (2 × 10^6 c.p.m.), 0 09 mg of nitrogen rat-liver mitochondria, 50 mM sucrose, atractyloside as indicated. Final volume, 1·0 ml. The samples were chromographed by the methods of Krebs and Hems (ATP–ADP exchange) (ref. 8) and of Cerletti *et al.* (ATP–inorganic phosphate exchange) (ref. 9), following deproteinization with trichloroacetic acid. Temperature, 25°; time of incubation, 20 min

This possibility is strengthened by the results plotted in Fig. 1, showing the effects of atractyloside and oligomycin on ATP-induced contraction of swollen mitochondria in the conditions described by Lehninger[6]. In agreement with the findings of Table 1, it is seen that:

(*a*) When a little damage of mitochondrial structure was produced by 0·1 mM DOC or by pre-incubation in a potassium chloride–*tris* medium, the addition of ATP and magnesium produced a contraction accompanied by formation of inorganic phosphate; both contraction and formation of inorganic phosphate were inhibited by atractyloside or by oligomycin.

(*b*) When a strong damage of mitochondrial structure was produced by 1·0 mM DOC the addition of ATP and magnesium resulted in a release of inorganic phosphate not accompanied by contraction of mitochondria; the release of inorganic phosphate was inhibited by oligomycin, but not by atractyloside.

In Fig. 2 it is seen that atractyloside inhibits both ATP-inorganic phosphate and ATP–ADP exchange reactions, catalysed by fresh liver mitochondria. By omitting ATP from the incubation medium and measuring the ATP formed, it was observed that the adenylate kinase activity of mitochondrial preparation, which was not affected by atractyloside, interfered with the effect of atractyloside on ATP–ADP exchange; ethylenediamine tetraacetate (EDTA) addition strongly reduced the adenylate kinase activity and rendered possible the observation of the atractyloside-induced inhibition of ATP–ADP exchange reaction.

Disruption of mitochondria with 1·0 mM DOC, ageing at 37° or at 4° resulted in an almost complete loss of the part of the ATP–ADP exchange activity that is not to be ascribed to the adenylate kinase. These results are in agreement with those of Cooper and Kulka[10], which also showed that oligomycin inhibits the ATP–ADP exchange activity of digitonin particles of mitochondria.

The atractyloside and oligomycin-induced inhibitions of mitochondrial contraction, together with the high sensitivity of the energy-transfer reactions to these agents, appear to be a convincing proof that the enzyme responsible for contraction is part of, or strictly related to, the coupling system. Furthermore, the findings reported in the present communication indicate that the mechanism or the site of action of atractyloside and oligomycin on the terminal step of energy-transfer reactions is not identical: atractyloside does not inhibit the magnesium-stimulated ATPase when the activity can be ascribed to a splitting of $X \sim P$ and, consequently, it does not interfere with the formation of $X \sim P$ at the expense of ATP; on the other hand, the strong inhibition by oligomycin of the magnesium-stimulated ATPase, in conditions where high concentrations of atractyloside are ineffective, can be explained by assuming that oligomycin inhibits under all conditions the formation of $X \sim P$ at the expense of ATP. In this regard it is interesting to note that atractyloside effect on magnesium-stimulated ATPase is more similar to that of the antibiotic aurovertin[4] than to that of oligomycin.

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Preparation of ^{35}S-Chlorosulphonic Acid from 'Waste', Ba^{35}SO$_4$

SULPHATION procedures involving the use of ^{35}S-chlorosulphonic acid and ^{35}S-sulphuric acid have been widely applied in the chemical synthesis of ^{35}S-labelled sulphate esters of carbohydrates[1], amino-acids[2,3] and phenols[4,5]. A constantly recurring feature of these procedures is that a relatively small proportion only of the ^{35}S-labelled starting material is consumed during the preparation of the required ester, the remainder eventually being removed from the products of reaction as 'waste' Ba^{35}SO$_4$. Thus, in a typical preparation of ^{35}S-tyrosine O-sulphate[2] using 44 mc. of concentrated H$_2{}^{35}$SO$_4$ as sulphating agent, 38·27 mc. of ^{35}S was eventually recovered as Ba^{35}SO$_4$. In general, Ba^{35}SO$_4$ has only limited use in biological tracer experiments, and in consequence, several methods have been devised for the conversion of this material to a form more suitable for such studies[6]. The present report describes a method for the conversion of Ba^{35}SO$_4$ to the useful sulphating agent ^{35}S-chlorosulphonic acid.

Although barium sulphate is almost insoluble in nearly all common solvents, it is soluble in concentrated sulphuric acid (sp. gr. 1·84). For the preparation of ^{35}S-chlorosulphonic acid the Ba^{35}SO$_4$ (2·614 g) was suspended in concentrated sulphuric acid (7·84 ml.) and the whole gently warmed until solution was complete. To the cooled solution was added phosphorus oxychloride (6·72 ml.) and the mixture then distilled in an apparatus designed to exclude moisture. The ^{35}S-chlorosulphonic acid distilling at 157°–158° (6·6 ml.) was collected. Material obtained in this way had a specific gravity 1·760 at 18°. The product is anhydrous and may be used for sulphation procedures without further purification.

The specific activity of the ^{35}S-chlorosulphonic acid obtained is approximately one-fifteenth that of the Ba^{35}SO$_4$ used as starting material. Since it is obtainable in relatively large amounts the ^{35}S-chlorosulphonic acid may be used without dilution with unlabelled acid, a procedure which is usually necessary to meet the quantitative requirements of the sulphation procedure. ^{35}S-chlorosulphonic acid prepared in this way has been used for the preparation of the ^{35}S-labelled sulphate esters of *p*-hydroxyphenylpyruvic acid[7] and a variety of other materials[8]. The products are sufficiently active to enable the general aspects of their *in vivo* metabolism to be studied, and are of general use as marker materials in paper chromatography and paper electrophoresis experiments. 'Waste' Ba^{35}SO$_4$ is now converted into ^{35}S-chlorosulphonic acid as part of the normal laboratory routine. In this way a constant supply of starting material used for tracer studied is provided, and the level of radiation in 'waste' material limited.

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Metabolites of Phenylalanine in the Urine of Children with Kwashiorkor

IN the urine of children with kwashiorkor (protein-calorie malnutrition) there is a significant amount of unidentified nitrogen[1], much of which can be absorbed on to activated charcoal and is therefore associated with molecules containing a ring structure. The isolation of imidazoleacrylic acid[2] indicated an abnormal metabolism of histidine which has been further investigated and confirmed[2]. The findings stimulated the search for abnormalities in the metabolism of other amino-acids containing a ring structure and the present communication describes preliminary results obtained with phenylalanine.

Children suffering from kwashiorkor were given a test dose of 5 g *l*-phenylalanine (L. Light and Co., Ltd.) on admission to the wards of this Unit, and again after treatment for about two weeks. Each child was on a balance bed and urine was collected in consecutive 12-h samples. When a few drops of 10 per cent w/v ferric chloride reagent were added to urine passed in the first days after admission, a marked green colour was produced, indicating the presence of phenylpyruvic acid. The urine was treated with saturated 2 : 4-dinitrophenylhydrazine in 2 N hydrochloric acid and the 2 : 4-dinitrophenylhydrazones of the oxo-acids were isolated by a modification of the method of Isherwood and Niavis[4]. The hydrazones were then separated by paper chromatography in butanol/ethanol/0·5 N ammonia (140 : 20 : 40). Nine compounds were detected and electrolytically reduced to their amino-acid analogues. Two of the compounds, which were present in urine samples taken before and after the feeding of the phenylalanine, were identified by this procedure as α-oxoglutaric and pyruvic acids. Two others which produced very intense spots in samples taken after the test dose gave phenylalanine on reduction and were identified as the two isomers of the 2 : 4-dinitrophenylhydrazone of phenylpyruvic acid. Another, giving a less intense spot, was reduced to material inseparable from an authentic specimen of *p*-tyrosine in two-dimensional co-chromatography. From the chromatographic data and the reaction of the amino-acid analogue with diazotized sulphanilic acid the substance in the spot was tentatively identified as one of the isomers of the 2 : 4-dinitrophenylhydrazone of 4-hydroxyphenylpyruvic acid.

A quantitative estimation of the phenylpyruvic and 4-hydroxyphenylpyruvic acids was made by measuring the ultra-violet extinction at 365 mμ of the eluted 2 : 4-dinitrophenylhydrazones[5]. In a typical case, 490 mg phenylpyruvic acid were excreted during the 36-h period following the ingestion of 5 g phenylalanine; after 10 days treatment the same child excreted only 62 mg in the same period. The corresponding figures for 4-hydroxyphenylpyruvic acid were 45 mg before and 16 mg after treatment. There were also differences in the duration of the excretion of the excess oxo-acids. In the acute stage the excretion was prolonged for up to 48 h, and the maximum occurred 12–36 h after the dose of phenylalanine, but after treatment the greatest excretion was found in the first sample taken.

The results suggest that in kwashiorkor there is a derangement of phenylalanine metabolism which is, in some respects, similar to that previously reported for histidine[3]. Whereas, however, metabolites of histidine are always excreted by sick children, the metabolites of phenylalanine described in this communication were found to be excreted only after a test dose of the amino-acid.

The results indicate that in kwashiorkor there is a decreased ability to hydroxylate phenylalanine to tyrosine. The same defect occurs, possibly to a more severe degree, in the congenital disease, phenylketonuria. In this disease, changes in the colour of the hair have been observed, and there is usually severe and permanent mental retardation. It seems likely that the pale hair and skin, and the profound psychological changes that usually accompany advanced kwashiorkor, but are rapidly reversed with successful treatment[6], may be related to the defects in phenylalanine (and tyrosine) metabolism that we have disclosed.

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Immunological Investigation of Human Growth Hormone

THE immunological impurity of present preparations of human growth hormone has been suggested by Boucher[1], and Hirschfield *et al.*[2], and confirmed by Laron and Assa[3,4].

Further confirmation, with growth hormone extracted by Raben's method[5], is presented and evidence shown that the anti-albumin fraction in the antiserum can be selectively adsorbed out by albumin-coated formolized sheep red cells. The photograph shows a simple method of recording the results of hæmagglutination tests using Perspex plates.

Antiserum was obtained after three weekly subcutaneous injections in rabbits of 1·0 mg of human growth hormone in 1 ml. distilled water and 1 ml. liquid paraffin/lanolin adjuvant, followed by two intravenous booster doses of 1·0 mg and 0·25 mg hormone adsorbed on aluminium phosphate. The total of 4·25 mg hormone injected may have contributed to the high titre of anti-albumin activity. Formolized sheep red cells in 0·9 per cent saline were prepared by Wienbach's method[6], and could be coated with hormone without the need for dilute

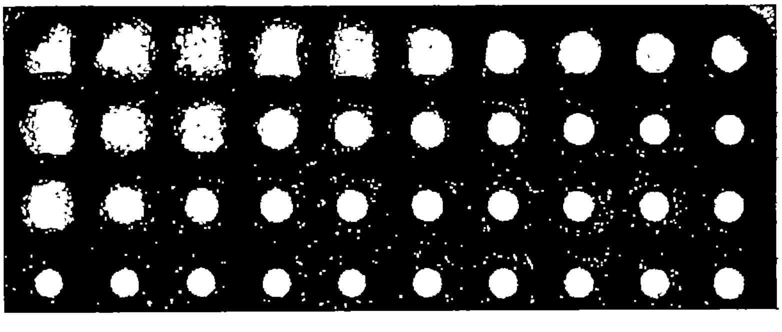

Fig. 1. Contact print of hæmagglutination patterns, using anti-human growth hormone serum and formolized sheep cells coated with human growth hormone (row 1); human serum albumin (row 2); human γ-globulin (row 3). Row 4 is of uncoated formolized sheep cells. Doubling dilutions of antiserum columns 1–9, column 10, 0·9 per cent saline only. (The half-moon shadows in some compartments are due to refraction of light at the spherical interfaces, and are inherent in the photographic technique)

tannic acid or buffered saline solutions. The human growth hormone preparation used showed only one peak on cellulose-acetate electrophoresis.

The record was made by exposing a sheet of bromide paper on which the plate was resting to the enlarger light in the dark-room, for the necessary time for adequate contrast. While the definition of individual settling patterns is poor, the overall trend is clearly seen and the time taken is minimal.

Fig. 1 shows the titre of unadsorbed antiserum (in doubling dilution 1/100 to 1/25,600) against cells coated with human growth hormone (1/6,400), human serum albumin (1/800), and human γ-globulin (1/200). Uncoated formolized cells showed no agglutination.

Adsorption of the antiserum with human serum albumin coated cells reduced the titre to human serum albumin to nil, leaving the human growth hormone titre unchanged at 1/7,000. Adsorption with the growth hormone preparation completely removed both anti-human growth hormone and anti-human serum albumin activity. The antiserum was adsorbed three times in each case, in 1/1,000 dilution, using appropriately coated formolized cells allowed to sediment in a flat-bottomed flask at 4° C for 4 h each time.

This experiment shows that one of the contaminating portions of an anti-serum prepared with an acid-extracted growth hormone fraction can be removed successfully, leaving the anti-human growth hormone activity intact.

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Mucoids of some Micro-organisms

MUCOPROTEINS which are soluble in 0·6 M perchloric acid and precipitated by phosphotungstic acid have been classified as 'mucoids' by Winzler[1]. So far as we know there is no information available in the literature dealing with the mucoid content of micro-organisms; therefore, we were interested to apply the same method previously used by us[2], for animal tissues, to the study of some bacteria and fungi.

The micro-organisms studied were grown for 24 h at 37° C in liquid media without aeration except *Neurospora crassa*, which was cultivated during 4 days at 25° C. *Staphylococcus albus* was cultivated in a beef broth medium with added thiamine hydrochloride (10 μg/100 ml.), nicotinic acid (10 μg/100 ml.) and biotin (5 μg/100 ml.), *Saccharomyces cerevisiae* was grown in yeast–peptone–glucose medium and *Neurospora crassa* in the saccharose medium of Beadle and Tatum[3]. The remaining organisms were grown in beef broth *p*H 7·4.

The organisms grown were collected and washed 3 times with 0·9 per cent sodium chloride solution and treated twice with cold acetone (30 vol.) and twice with cold anhydrous ethyl ether. The cellular material was dried in vacuum and weighed. The mucoids were extracted by shaking 100 mg of the dry acetone powder with 10 ml. of 0·14 M sodium chloride *p*H 6·6 for 30 min at room temperature and left overnight in the ice-box. The clean supernatant extract was then treated with 1·8 M perchloric acid (final concentration of 0·6 M), and to the filtrate phosphotungstic acid was added to precipitate the mucoids which were determined colorimetrically by the orcinol–sulphuric acid reagent[1,2]. The results obtained were compared with a standard solution containing equimolecular amounts of galactose and mannose. Our results are summarized in Table 1.

Table 1. MUCOIDS OF MICRO-ORGANISMS*
(Values expressed in mg of hexose per 100 g of dry acetone powder)

Staphylococcus albus	61
Escherichia coli	114
Bacillus subtilis	182
Bacillus megatherium	454
Salmonella typhi O	554
Salmonella typhi Vi	1,137
Saccharomyces cerevisiae	231
Neurospora crassa	467

* Mean of two observations.

We were unable to obtain any mucoid from acetone powder of *Proteus vulgaris*. Table 1 reveals that the mucoid content varied with the organisms studied. The marked difference between the mucoid content of *Salmonella typhi O* and *Vi* suggests some relation with their antigenic constitution as observed for other biochemical differences previously reported[4].

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PHYSIOLOGY

Hydroxyproline and the Shrinkage Temperature of Collagen

IN a recent publication[1], Drs. L. Klein and P. H. Curtiss criticized a suggestion of Rigby and Spikes[2] concerning a possible explanation for some of the effects observed in the collagen of rheumatic patients.

Their main points were : (1) that our argument was based solely on an extrapolation of the properties of rat-tail tendon ; (2) that, according to their experiments, bovine articular cartilage collagen has a greater thermal stability than bovine tendon collagen, and that this is contrary to our denaturation hypothesis.

Concerning point (1), Drs. Klein and Curtiss stated that such an extrapolation was not possible, since a number of workers have claimed that rat-tail tendon does not behave the same way as other mammalian collagens so far as solubility and other thermal characteristics are concerned. We would like to point out that our suggestions were not based on the properties of rat-tail tendon alone, and in fact Drs. Klein and Curtiss did not mention our main evidence at all. Rat-tail tendon was only one of a number of collagens the shrinkage temperature of which was plotted against hydroxyproline content. Fish and human collagen were included, and a linear relation resulted, in which a low content of hydroxyproline corresponded to a low shrinkage temperature. From this we argued that if for any reason whatsoever[2] the hydroxyproline content in a given collagen is lower than normal for the species, then this collagen will have a lower shrinkage temperature. Again, should the collagen be under mechanical stress, there is an irreversible structural breakdown at a temperature approximately 23° C below shrinkage temperature[3-5], this temperature also being linearly dependent on hydroxyproline content. These results are from human skin collagen, rat-tail tendon and tuna fish skin. With the additional observation[6-9] that temperature and time appear to bear an approximately inverse relation in connexion with the denaturation of collagen (that is, a long time at a low temperature is equivalent to a shorter time at a higher temperature) these results led us to suggest that a human who has less than the normal amount of hydroxyproline in his collagen is likely to develop rheumatic symptoms (and complaints generally referred to as 'collagen diseases'), simply because of a denaturation of his collagen due to temperature and/or mechanical stress.

We turn now to the second point. Drs. Klein and Curtiss list a comparison of the solubilities of bovine tendon and cartilage collagen and show that the tendon collagen is 4–6 times more solubilized than the cartilage collagen at any given temperature. They took the solubilization of the collagen (based on the amount of hydroxyproline that appeared in the filtrate) in the two cases as a measure of the degree of denaturation, but it should be pointed out that the bulk of the collagen could be denatured without going into solution.

Their results further show that the ratio of the solubilities of tendon and cartilage is about the same irrespective of temperature, indicating that the difference in solubilities is due, not so much to a difference in the collagen but to a difference in the relative amounts present (compared with mucopolysaccharide), and to their respective accessibilities to solvent. Cartilage contains a large percentage of chondroitin sulphate intimately associated with the collagen[10,11].

Even though cartilage is not a pure form of collagen, nevertheless, as Partridge[10] has shown, there is a marked correlation between the conditions necessary to obtain good yields of chondromucoid from cartilage and those giving rise to thermal shrinkage of free rat-tail tendon fibres. This may be interpreted as showing that, even in cartilage, collagen will begin to denature at its normal shrinkage temperature (62° C). However, it is likely that large-scale shrinkage would not be observed until a much higher temperature is reached. This would explain the value of 83°–87° C for human costal cartilage quoted by Klein and Curtiss.

Finally, while it is true that our denaturation hypothesis is rather speculative, it must also be remembered that as yet there is no evidence for any causative agent such as bacteria, virus, vitamin deficiency, etc. We have dwelt on denaturation due to temperature and mechanical stress but it is possible that local *p*H changes in tissue could also cause denaturation, and again the process would be accelerated if the collagen were lower in hydroxyproline than normal.

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We did not discuss the suggestions[1] based on the comparative shrinkage temperatures of various collagens since this aspect has been soundly criticized by Bowes and Moss[2]. In our investigations on infectious arthritis[3,4] we purposely used untreated articular cartilage as a source of collagen in order to approach the conditions that exist *in vivo*, being fully aware that a greater thermal stability might exist for the reasons given by Dr. Rigby. Connective tissues (tendon, cartilage) not purified collagens were being compared on a per cent basis[3]. Both tissues were homogenized with *tris* buffer into fine suspensions according to the procedure of Schubert[5]. A comparison of bovine tendon and cartilage collagen in the presence of crystalline trypsin at different temperatures showed that tendon collagen was two to three times more solubilized than the cartilage collagen (unpublished observations).

Rigby and Spikes[1] have assumed that the process of thermal shrinkage ('denaturation') is rate-controlled without referring to the alternative ('phase transition') hypothesis of Flory[6,7], which is independent of rate and thus independent of time. According to this latter view, denaturation would be a function of temperature alone.

Theoretically, denaturation remains a good hypothesis for the explanation of rheumatic diseases; but whether denaturation of collagen actually occurs *in vivo* is different. The amount of peptide-bound hydroxyproline excreted in the urine appears to be an index of the rate of degradation of soluble collagens *in vivo*[8,9]. Ziff *et al.*[10] found no significant difference between the levels of total hydroxyproline excreted by patients with rheumatic diseases and those of normal patients. Persons with accidental thermal burns[11] increased their urinary hydroxyproline ten to twenty-fold and excreted the equivalent of 100–150 g of collagen over a period of four months, thus indicating that urinary peptide hydroxyproline would reflect denaturation of insoluble collagen if it occurred.

Although denaturation of insoluble collagen under physiological conditions does not appear to occur *in vivo*, thermal denaturation and non-specific proteolysis of soluble collagens could be the explanation for the normal presence of peptide hydroxyproline in the urine.

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Long-lasting Changes in the Level of the Electrical Activity of the Cerebral Cortex produced by Polarizing Currents

THE passage of direct current produces changes in the electrical activity of nervous tissue. For example, the polarization of nerve terminals in muscle spindles gives rise to alterations in the membrane potential; a depolarization produces an increased rate of discharge of nerve impulses while hyperpolarization decreases the discharge frequency[1]. A similar effect can be produced by polarization of the spinal cord[2]; the discharge in the ventral roots is altered by the passage of small currents across the thickness of the cord.

We have described elsewhere the effect of polarizing the cerebral cortex of the rat. Potentials evoked in the cortical grey matter by stimulation of the contralateral forepaw are enhanced by radially flowing, surface-positive currents and diminished by surface-negative currents[3]. Surface-positive currents also increase the firing of neurones within the grey matter, in the absence of external stimulation.

In the experiments to be described here, it was found that the change in evoked and spontaneous activity which was produced during the passage of the polarizing current lasted—under certain experimental conditions—for many hours after the current was switched off.

A micro-electrode and a suitable amplification system enabled recordings to be made of the electrical activity of the exposed cerebral cortex of the anæsthetized rat, at any desired depth in the grey matter. Current was passed between a non-polarizable electrode on its surface and an indifferent electrode buried in a distant muscle. The recording micro-electrode could also be used to pass the polarizing current if required.

We found that the negative potential wave in the cortex evoked by sensory stimulation of the contralateral fore-paw was enhanced by surface-positive polarization of 5–50 μamp and that if the duration of passage of current was 5 min or more, this enhancement continued after the polarizing current was switched off. In many cases this effect lasted for 3 h or longer. Similarly, surface-negative currents gave rise to a long-lasting diminution in the size of the evoked potential. In addition to the change in the size of the evoked potential there was an increase in the level of general activity of the cortical neurones during the passage of surface-positive current; this effect also persisted after the polarization was discontinued, in some experiments for longer than 4 h.

The underlying mechanism responsible for this prolonged after-effect could be a local one in the cells actually polarized or it could be due to current

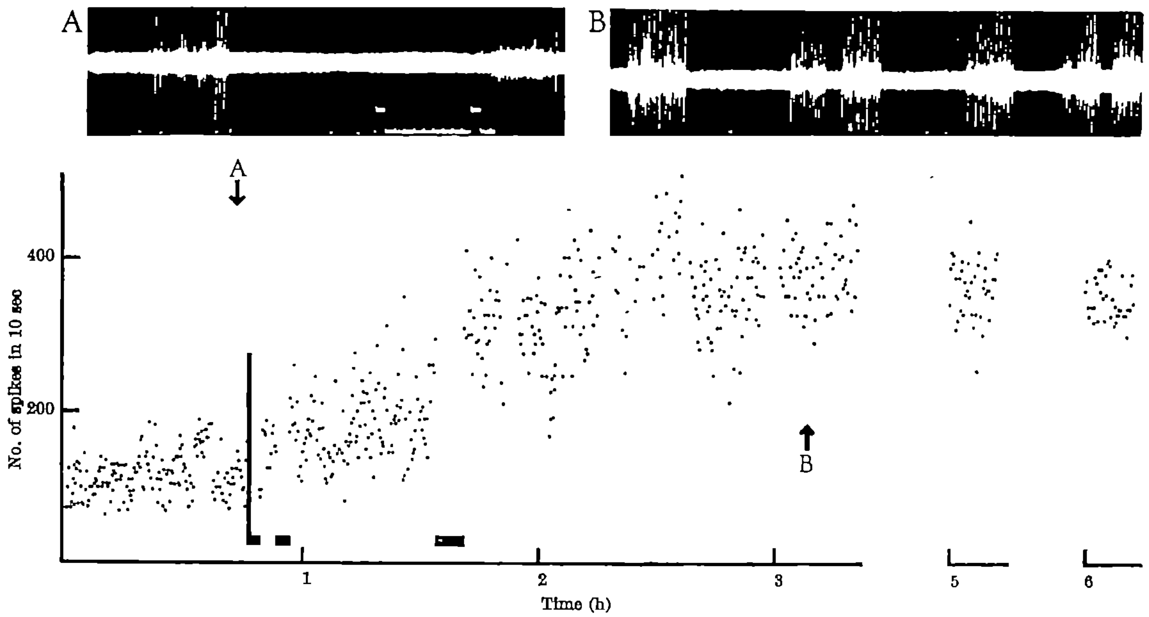

Fig. 1. Graph showing number of action potential spikes before and after the passage of polarizing current. Ordinate number of spikes in 10 sec. Recording made from about seven cells at depth of about 800μ in exposed sensory cortex of a rat anæsthetized with urethane. Micro-electrode of tip diameter 1μ was filled with 10 per cent sodium chloride. Abscissa time. During the three black rectangles a polarizing current of 0 25 μamp was passed from the micro-electrode tip to an indifferent electrode above it on the pia. Electrode tip was positive. Body-temperature, 36·5° C. *A*, Record of spikes taken immediately before (at first arrow), *B*, 1 h 20 min after the end of polarization (at second arrow). Time trace, 1 sec and 1/10 sec. Spikes counted electronically

flowing in other parts of the brain such as the thalamus. However, it appeared that the thalamus was unaffected because no change could be detected in the size of the specific thalamic response while simultaneous recording showed that polarization had produced a considerable alteration in the size of the cortical evoked potential. Moreover, if current flow was confined to a small volume of cortex by passing as little as 0·1 μamp between the recording electrode and an indifferent electrode of relatively large diameter above it on the surface of the pia, a local after-effect was still produced (Fig. 1).

Thus the primary change must be initiated in the cortex in these circumstances although it is possible that there are subsequent effects involving other parts of the brain, such as hyperactivity in neuronal loops.

These local changes in activity of the cerebral cortex were produced by currents of the order of 0·1–0·5 μamp. If the latter value was exceeded, instead of a further increase in the effect, a depression of neuronal activity often resulted. Measurements showed that when currents of about 0·1 μamp were passed through the microelectrode, the maximum potential gradient produced was of the order of 0·25 mV/100μ near to its tip.

Slow potential shifts of up to 2 mV in 0·5 mm, which occur spontaneously in the anæsthetized rat, can be recorded across the thickness of the grey matter. The potential gradients we have used are, therefore, of the order of those which occur naturally.

These observations are of great interest in that they show that it is possible to produce a long-lasting change in the activity of the brain by means of a very small temporary alteration of the physical environment of the nerve cells.

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Mode of Protection with Polyvinylpyrrolidone in Freezing of Bone Marrow

The accepted concept of the action of protective compounds in low-temperature preservation of cells maintains that a small molecular size of the additive is essential to allow its penetration into the cell[1,2]. A previous investigation by us[3], however, demonstrated that polyvinylpyrrolidone (PVP, 30,000 mol. wt.) offers the bone marrow cells good protection (30 per cent) during the freezing and thawing process, being superior to glycerol in this respect. It is obvious that these large molecules cannot enter the cells simply by diffusion. To determine whether the mechanism of this protection involves pinocytosis, hence intracellular penetration, or whether the protective action is extracellular or both extra- and intra-cellular is the object of this investigation.

It is known that pinocytosis can be induced in cells by various salts[4]. We have examined first the effect of the phosphates on the penetration of PVP labelled with iodine-131 ('Raovin') into the bone marrow cells.

Rabbit bone marrow was used to obtain a sufficient quantity of cells. The bone marrow was homogenized and the cells suspended in normal saline. This suspension was divided into four equal parts which were centrifuged at 900 r.p.m. for 5 min and the supernatant decanted. Two parts of this preparation were brought into suspension with 0·1 μc. of labelled PVP per ml. in saline plus serum (3 : 1 ratio) to a total of 2 ml. One of these parts was then kept at 0° C for 45 min. The remaining two parts of the preparation were also treated with labelled PVP except that equal parts of sodium and potassium phosphates were added in 0·03 per cent concentration to one and 0·06 per cent concentration to the other. Both of these were incubated at 37° C for 45 min.

After 45 min, all preparations were centrifuged at 900 r.p.m. for 5 min; the supernatant was drawn off and saved for counting its radioactivity. The cells were washed four times with 10 ml. of 5 per cent PVP, not radioactive, in normal saline. After the final wash the cells were brought back into suspension with 2 ml. of concentrated sulphuric acid. This served to digest the cells so that the solution was uniform for counting. The radioactivity was determined with a Packard liquid scintillation spectrometer.

On the basis of seven experiments we found that the preparations which were kept at 0° C had the lowest amount of radioactivity. The highest radioactivity was observed in the preparations incubated with phosphate ions (Fig. 1).

Since pinocytosis is inhibited at temperatures near 0° C (ref. 5), we have assumed that the higher degree of radioactivity in cell suspensions incubated in the presence of phosphate ion and labelled PVP must be related to an intracellular penetration of the PVP molecule by pinocytosis. The other possible explanation is that phosphate ion enhances the binding of PVP to the cell membrane. We have no evidence for such an assumption, however.

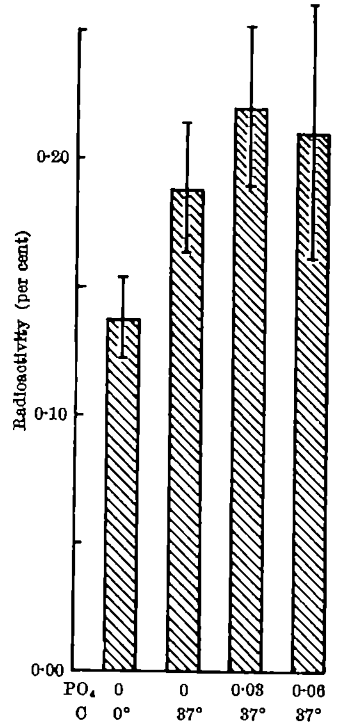

Fig. 1. Percentage of radioactivity in the cell suspensions after incubation with phosphates and after washing. Vertical lines represent standard errors

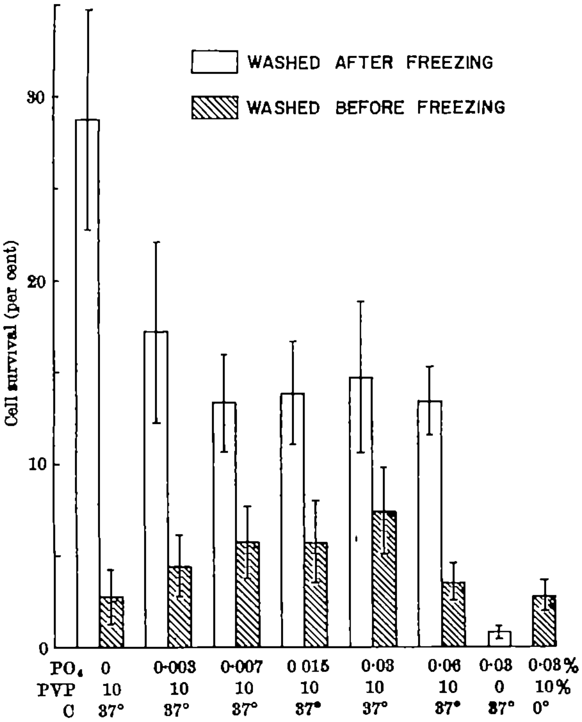

Fig. 2. Percentage of cell survival after incubation with various concentrations of phosphates and 10 per cent PVP, washed and unwashed before freezing. Vertical lines represent standard errors

In the second part of this work, we examined the intra- and extra-cellular protecting effects of PVP during freezing. Since a smaller volume of marrow was required, rat bone marrow was used. After homogenization, the cell suspension was divided into 14 equal parts which were treated as follows: to 13 parts, 10 per cent PVP in normal saline plus serum (in a 3 : 1 ratio) was added. Ten parts were paired, and to each pair the following concentrations of sodium and potassium phosphates were added respectively: 0·003, 0·007, 0·015, 0·030, and 0·060 per cent. To one part without PVP, 0·03 per cent phosphate was added; two parts without phosphate were used as a control. To the remaining one part with 10 per cent PVP, 0·03 per cent phosphate was added. This part was kept at 0° C for the 45 min. All the other preparations were then incubated for 45 min at 37° C. After incubation the pairs were divided into two groups; one was washed before freezing, the other after freezing. Washings were done by diluting the suspensions with an equal volume of normal saline, centrifuging at 900 r.p.m. for 5 min and then decanting the supernatant. The group washed before freezing was frozen in normal saline solution; the group washed after freezing contained 10 per cent PVP during freezing. It should be noted that one preparation washed after freezing never came in contact with PVP. This was the preparation originally suspended and incubated in saline and serum plus 0·03 per cent phosphate. All preparations after thawing were resuspended in 10 per cent PVP plus Hanks solution and serum and then cultured for 24 h at 37° C. Assay of cell viability was carried out by using phase microscopy in accordance with our previous technique[3].

The bar graph in Fig. 2 shows that the cell viability, in the preparations incubated with 10 per cent PVP, washed, and then frozen without preservative, increases as the concentration of the phosphates is increased, reaching 7·2 per cent survival at the 0·03 per cent phosphate concentration. The lowest viability is found in the preparations incubated without phosphates or kept at 0° C. When the preparations are frozen with the preservative, incubation with phosphates lowers the cell survival considerably, the viability being approximately half that in the preparation incubated without phosphates. The incubation and freezing with the phosphates alone has no beneficial effect on the cell survival.

The survival of cells in preparations washed before freezing and frozen without a preservative indicates that these cells were afforded protection during the freezing process. Since the cells were washed and then resuspended in a medium containing no preservative, the protection was presumably intracellular. The first part of this work indicates that labelled PVP gained access to the interior of the cell with the aid of phosphates by the mechanism of pinocytosis. Therefore the survival of cells in preparations washed before freezing is attributed to protection by PVP molecules which apparently were ingested by the cell with the aid of the phosphates. The very low cell survival in the preparation containing both phosphates and PVP but kept at 0° C strengthens this assumption since pinocytosis is inhibited at 0° C. This also speaks against the possibility of protection by the surface binding of PVP. The experimental conditions under which the highest cell survival (29 per cent) was attained are such (absence of phosphates and no incubation prior to freezing) that pinocytosis is unlikely to occur; therefore this protection offered by the PVP molecules during freezing must be extracellular. The addition of intracellular to the extracellular PVP by pinocytosis had no beneficial effect on the cell survival after freezing.

These findings suggest that the present generalization[1,2] of the action of preservatives, which implies only intracellular protection with small molecules, should be modified at least when pertaining to the freezing of bone marrow cells with PVP, which is an effective extracellular preservative.

There are several possible ways PVP may protect the cell from injury during freezing and thawing:

Extracellular effects: (1) Absorption and dilution of electrolytes. (The absorbing property of PVP is well-known.)

(2) Occlusion of cell pores: (*a*) prevents intracellular ice seeding and enhances supercooling; (*b*) reduces the loss of electrolytes; (*c*) reduces excessive intracellular dehydration.

(3) Restoration of the cell permeability after thawing.

Intracellular effects (when PVP enters the cell by pinocytosis): (1) Supercooling to a greater degree. (2) Dilution and absorption of electrolytes.

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Relationship between Air Temperature and Mean Radiant Temperature in Thermal Comfort

Groups of 6–8 apparently healthy persons were exposed to three environmental conditions in a room previously described[1]. The test subjects were lightly dressed (about 343 g/m^2 surface area, excluding shoes), seated and engaged in reading and writing. Though the subjects noted their comfort responses every half-hour, only the last votes of a 3-h session were considered. The conditions were: (*a*) air temperature approximately equal to mean radiant temperature; (*b*) air temperature higher than mean radiant temperature (up to 17·9° F); (*c*) air temperature lower than mean radiant temperature (down to 15·3° F). Humidity was arbitrarily kept at 50 per cent, and air velocity was less than 20 ft./min. Dry bulb and wet bulb temperatures were measured with aspirated and shielded mercury-in-glass thermometers; radiant temperature with the globe thermometer[2], a two-sphere radiometer[3,4] and a modified thermopile[5]; and air velocity with a rhodium-plated kata thermometer[6]. All six room surfaces were kept at approximately the same temperature. The combinations of air temperature and mean radiant temperature were adjusted to yield a mean vote of 'comfortable' ('4') on a seven-point thermal comfort scale[1]. Mean votes, above and below '4' which did not differ by more than 0·5 sensory units, were used as bases for finding, by linear interpolation, the air temperature and mean radiant temperature, which evoke a 'comfortable' vote.

The 'comfortable' votes were entered into a graph, with air temperature and mean radiant temperature as ordinates, and the orthogonal regression line[7] determined:

$$\text{mean radiant temperature} = -1{\cdot}39 \times \text{air temperature} + 182{\cdot}86 \quad (1)$$

This regression line is based on 196 points.

As to estimate limits of confidence, lines perpendicular to the orthogonal regression line were drawn at a distance of 2° F from each other. These lines divided the area into 12 bands of equal width (Table 1).

Table 1. Number of Points in each Band and Ordinates of their Means

Band	No. of points	Air temperature (°F) (mean)	Mean radiant temperature (°F) (mean)
I	3	72·97	87·60
II	4	74·53	86·68
III	7	75·57	84·99
IV	7	76·29	82·59
V	14	77·18	81·00
VI	20	77·66	78·79
VII	28	79·40	77·38
VIII	31	80·90	76·62
IX	27	81·67	74·39
X	33	83·02	72·81
XI	15	84·05	71·26
XII	7	85·83	70·27

Within each band the distance of the points from the orthogonal regression line was determined, and their mean and standard deviation found. One standard deviation was entered in the middle of the respective band on either side of the regression line; thus each band contained two reference points, the distance of which represented plus/minus one standard deviation. Within this distance should fall 2/3 of all observations, if the distribution is normal.

From the reference points representing the standard deviations, two additional regression lines were calculated, one above (2) and one below (3) the original regression line (1):

$$\text{mean radiant temperature} = -1{\cdot}35 \times \text{air temperature} + 187{\cdot}40 \quad (2)$$

$$\text{mean radiant temperature} = -1{\cdot}43 \times \text{air temperature} + 189{\cdot}46 \quad (3)$$

These two lines (2) and (3) encompass 71·94 per cent instead of the expected 68·26 per cent of all points.

From regression line (1) it appears that an increase of 1° F in air temperature can be compensated by a decrease of 1·39° F in mean radiant temperature, and vice versa.

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PHARMACOLOGY

Globulin Permeability Factors without Kininogenase Activity

Among the pharmacologically active proteases in mammalian plasma two, human kallikrein[1,2] and the protease permeability factor of human and guinea pig plasma[3,4], decrease blood pressure and increase the permeability of small blood vessels on intracutaneous injection. Kallikrein is a kininogenase the hypotensive activity of which is due to the release of kinin. It has been assumed that its permeability-increasing activity is due to kinins and that the protease permeability factors are kininogenases of the same kind. Human plasma kallikrein is usually assayed for activity in terms of kinin release, on smooth muscle preparations like the rat uterus[5], using either heparinized plasma[6] or a preparation of ox globulin[7] as substrate; and in terms of its hypotensive effect[8] or its vasodilator effect in the dog's hind limb[4].

Like preparations of human kallikrein, the protease permeability factor in guinea pig plasma, as isolated in the preparation *G*2/1*R*, is hypotensive[4]; and as we have found in a survey of the activity, in the guinea pig, of preparations both of kallikrein and *G*2/1*R* (the former kindly given us by Dr. Marion E. Webster of the National Heart Institute, Bethesda, Md.) the hypotensive potency in each type of preparation is roughly proportional to the permeability-increasing potency; an indication that both effects may be due to the same substance. The indication is strengthened by the fact that the hypotensive and permeability-increasing activities of human and of guinea pig preparations are inhibited to the same degree by prior treatment with appropriate amounts of soya bean trypsin inhibitor and di-isopropyl phosphofluoridate[9].

After exposure to a *p*H of 3·5 for 3 h to destroy peptidase activity, *G*2/1*R* preparations behave like kininogenases when added to 1/600 freshly diluted guinea pig plasma or to the ox globulin substrate. However, when the acid-treated material is heated to 56° C for 30 min, its permeability-increasing potency is retained but it no longer attacks the ox globulin substrate. These doubly treated preparations of $G2/1R$ nevertheless release kinins from fresh plasma. This, however, is not a direct kininogenase effect, for the following reasons.

Guinea pig plasma has its own kininogenase system. When plasma is diluted 1/600 and exposed to glass, the kinins are slowly released, reaching maximum activity in 15–20 min. In siliconed tubes the plasma releases kinins in maximum amounts within 1 min of adding 10 μg/ml. of high molecular weight dextran sulphate[10], of $G2/1R$ or of human kallikrein. But when plasma is preheated for 2 h at 56° C it is still, in the 1/600 dilution in the uterus bath, susceptible to human kallikrein, though no longer so to treated $G2/1R$ or dextran sulphate. It is, however, susceptible to 1/3,000 fresh plasma that has been activated by contact with glass for 20 min or by treatment for 1 min with $G2/1R$ or dextran sulphate. The same results are obtained when ox globulin is substituted for 1/600 heated plasma as a source of kininogen.

It appears, therefore, that kinin production from fresh guinea pig plasma by a preparation of the permeability protease does not necessarily indicate that it has kininogenase activity, since plasma contains an intrinsic kininogenase activable in detectable amounts at 1/3,000, whereas its kininogen content is sufficient for the release of detectable kinin only in concentrations as high as 1/600; if guinea pig plasma is to be used as a substrate for presumed kininogenases it should be preheated to 56° C for 2 h. It also appears that preparations of guinea pig permeability factor behave, like dextran sulphate, as activators of the intrinsic kininogenase. That the permeability factor is the activator in such preparations is evident from our observation that permeability-increasing potency is proportional to kininogenase-activation potency: (*a*) in various fractions of guinea pig globulins; (*b*) in plasma treated with glass ballotini for 2–3 h, a process during which permeability factor is activated and the kininogenase destroyed.

Though the predominant permeability factor in guinea pig plasma is not a kininogenase, it is nevertheless possible that, in the guinea pig, activation of the factor is a necessary precursor of the activation of kininogenase; and that preparations of the factor owe their permeability-increasing potency in the skin to activation of a kininogenase system present in the tissues. It is also possible that hypotensive and vasodilator effects of such preparations are due to the *in vivo* activation of kininogenase.

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Enhancement of Imipramine Mortality in Hyperthyroid Mice

ALTHOUGH the anti-depressant drug imipramine ('Tofranil', Geigy) has received broad clinical acceptance, its mode of action remains uncertain. Nevertheless, potentiation of catecholamine activity is a well-defined pharmacological effect[1-3]. The other major class of anti-depressant drugs, the monoamine oxidase (MAO) inhibitors, have been shown clearly to inhibit the enzyme and to elevate brain levels of monoamines[4]; but it is far from certain that their clinical effect is owing to this biochemical property.

In this connexion Board *et al.* have shown an increase in protein-bound iodine in chronically depressed patients and refer to thyroid changes as a third-order stress response, after adrenal medullary and adrenal cortical activity[5]. Carrier and Buday have demonstrated increased toxicity of both hydrazide and non-hydrazide monoamine oxidase inhibitors in Wistar rats made hyperthyroid[6]. In animals, prolonged imipramine administration has produced loss of weight of thyroid gland and loss of total body-weight[7]. Small doses of imipramine and thyroid extract have been observed apparently to produce paroxysmal auricular tachycardia in a depressed patient while neither substance alone had this effect[8]. In the cat imipramine is known to potentiate the effect of thyroxin on heart-rate and contractility[7].

These observations seemed to indicate the importance of testing systematically the relationship between imipramine and thyroid function. The possibility was held in mind that such a relationship might manifest itself as toxicity in a controlled animal study but might in part contribute to the clinical mode of action of the drug in depressed patients.

Ten Swiss–Webster strain male white mice were assigned at random to each of ten study groups. Half the mice were given a standard laboratory diet; the other half were given the same diet containing 2 per cent desiccated thyroid. All mice were given 1 per cent brewer's yeast supplement. Daily changes in weight are summarized in Table 1. In an analysis of variance, the 'group by day' interaction was statistically significant ($P = < 0{\cdot}001$), supporting the contention that the two groups had shown different patterns of weight gain. At the final weighing the normal mice had a mean weight of 26·6 g and a *S.D.* of 1·49; the hyperthyroid mice had a mean weight of 25·5 g and a *S.D.* of 1·56. One hyperthyroid mouse died from unknown causes. All other mice appeared robust at all times.

Between the 92nd and 94th hours, five physicians were asked to judge which cages contained hyperthyroid and which normal mice. They made a total of 22 correct judgments and 3 incorrect judgments ($P = 0{\cdot}0008$).

At 96 h each mouse was weighed and injected intraperitoneally with lyophilized imipramine in saline (4 mg/ml.). The imipramine was administered as 25, 50, 75, 100, 125, and 150 per cent of the LD_{50} dose (115 mg/kg) previously described[7]. All deaths, except two, occurred within 2 h, and this time span, therefore, was chosen as the operational definition of an 'acute' death. Two hyperthyroid mice died 'late' deaths. The mortality results are shown in Fig. 1.

Table 1. GROUP MEAN WEIGHTS (G)

	Initial weight	24 h	48 h	72 h	96 h	*N*
Standard diet	25·3	25·6	26·4	26·7	26·6	50
2 per cent thyroid diet	25·3	25·4	25·5	25·5	25·5	49

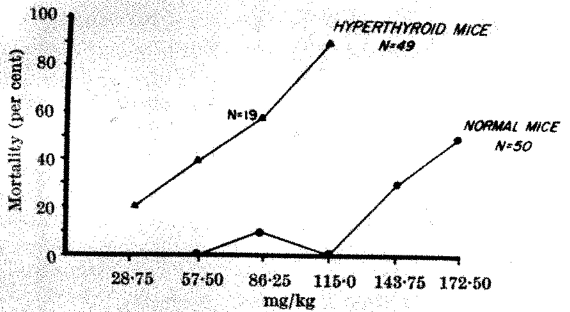

Fig. 1. Intraperitoneal imipramine acute (2 h) mortality in adult male Swiss–Webster white mice

From inspection of the dose-mortality curves it would appear that the LD_{50} for hyperthyroid mice is about 75 mg/kg while the LD_{50} for normal mice is 172·50 mg/kg. The two dose-mortality curves are approximately parallel, with mortality for normal mice starting at a dose slightly greater than 115 mg/kg.

Following imipramine injection hyperthyroid mice seemed grossly overstimulated. They ran about, showed tonic and clonic convulsions, and occasionally opisthotonus. They became quiet only terminally. Thoracotomy was done on several obviously moribund hyperthyroid mice. In all cases cardiac dysrhythmia was noted.

The three groups of normal mice ($N = 30$) that received sub-lethal doses of imipramine appeared drowsy and lethargic. They were easily roused, however, and they recovered within about 1 h. The normal mice receiving larger doses presented the same type behaviour as thyroid mice but to a lesser degree.

Whether hyperthyroidism in man presents a relative contra-indication to the use of imipramine is a moot question. Quite possibly MAO inhibitors should also be used with caution in hyperthyroid patients[6,9].

A number of mechanisms may be involved. Hyperthyroid patients are known to be extremely sensitive to the pressor effect of *nor*epinephrine[10]. In rats thyroxine is known to decrease *O*-methylation of epinephrine[11] and to decrease liver MAO activity while increasing plasma epinephrine and *nor*epinephrine[12]. As stated, at proper dosage imipramine potentiates catecholamines[1-3]. These relationships are being examined in higher animals.

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PATHOLOGY

Glutamic Acid Decarboxylase Activity of *Mycobacterium leprae* and Occurrence of γ-Amino Butyric Acid in Skin Lesions of Leprosy

IN 1872 Hansen characterized *Mycobacterium leprae* as the causative agent in human leprosy. Hitherto, all attempts to cultivate this organism *in vitro* or to transmit it to experimental animals have met with little success, and the metabolism of this micro-organism has remained completely unknown.

The experiments described here form part of a broader investigation designed to investigate the metabolism of *M. leprae*, using concentrates of mycobacteria separated from human lepromatous nodules. The evidence obtained for the presence of glutamic acid decarboxylase in *M. leprae* is discussed in relation to its significance to γ-amino butyric acid (GABA) detected in the skin lesions of patients suffering from lepromatous and tuberculoid forms of leprosy.

Concentrates of *Mycobacteria* were separated from human lepromatous nodules, excised from leprosy patients and kept frozen until required for use. The epidermis was peeled off and the remaining tissue was minced and further crushed in 0·2 M sucrose by passing through a Craigie crusher. The mixture was centrifuged at 35*g* to remove cell debris. The supernatant was then centrifuged at 6,000*g* in 0·3 M sucrose at 0° to sediment the bacteria. The residue was again suspended in 0·2 M sucrose and isotonic potassium chloride and centrifuged at 6,000*g* for 10 min. The sediment containing the micro-organisms was washed with distilled water. On staining by the Ziehl–Neelsen procedure, it could be shown that, whereas the initial fraction contained blue-stained components indicating cell debris, the final fraction consisted entirely of acid-fast material, characteristic of *Mycobacteria*, with only traces of blue-stained fragments.

Samples of normal skin were obtained from the forearm and lower limbs amputated from accident cases and cancer patients. The material was collected within 1 h after the operation. The subcutaneous fat was removed and the tissues were crushed in the Craigie apparatus with distilled water. The proteins were removed by precipitation with 90 per cent alcohol, and the extracts used for paper chromatography. Lepromatous skin nodules and a number of skin biopsies from advanced tuberculoid lesions were subjected to similar treatment as normal skin.

The decarboxylase activity of concentrates of mycobacteria was determined by incubating the suspension of organisms (25 mg protein) with and without L-glutamic acid in presence of pyridoxal phosphate and acetate buffer at *p*H 5·0 for 90 min at 37°. The products of reaction were then extracted with 1·5 ml of ethanol and 200 μl. analysed by ascending paper chromatography. With these amounts of material the R_F values of the ninhydrin-positive spots obtained under various conditions showed the presence of only glutamic acid in the organisms; on incubating the organisms with L-glutamic acid, the products of reaction showed the presence of GABA. The latter compound could not be detected in the non-incubated mixture of glutamic acid and organisms. Glutamic acid decarboxylase has been reported in some strains of *Mycobacteria*[1,2]. Among the strains examined, *M. kedrowsky* and *M. phlei* were found to produce GABA from L-glutamic acid, but this activity

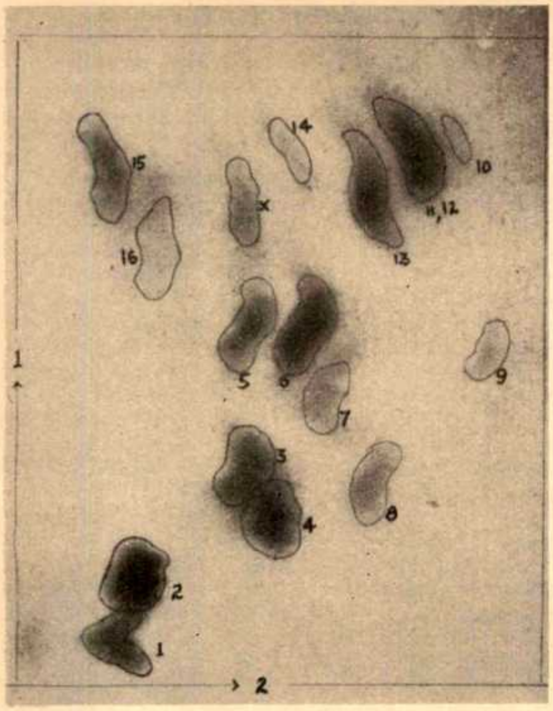

Fig. 1. Free amino-acids in lepromatous nodule extract. Two-dimensional chromatogram. Weight of tissue used: 150 mg. Solvent system: (1) liquid phenol saturated with 10 per cent solution of sodium citrate in water (10 : 1 v/v); (2) a mixture of 2,6-lutidine, 2,4,6-collidine, water and diethylamine (140 : 128 : 132 : 4 v/v). Ninhydrin spots: (1) aspartic acid; (2) glutamic acid; (3) glycine; (4) serine; (5) glutamine; (6) alanine; (7) threonine; (8) taurine; (9) tyrosine; (10) phenyl alanine; (11) leucine; (12) isoleucine; (13) valine; (14) proline; (15) arginine; (16) lysine. *X*, identified as GABA

could not be detected in *M. tuberculosis* (*H* 37 *Ra* or *BCG*) and *M. smegmatis*.

Mammalian brain is reported to be the richest source of GABA in the animal organism. The presence of GABA in human peripheral nerve or skin is not well established[3]. Since *M. leprae* has a predilection for cutaneous nerves[4], a comparative study was carried out on the amino-acids of normal skin tissue and the skin from leprosy lesions. The chromatograms of normal skin and of human ulnar nerve showed the presence of sixteen amino-acids and did not contain GABA. The pattern of free amino-acids in a lepromatous nodule is given in Fig. 1, and shows, in addition to the amino-acids found in normal skin, another spot *X* having the same R_F as GABA. The identity of *X* was confirmed by elution and re-chromatography in phenol using GABA as the control. Three different experiments using a number of skin biopsies from thick lesions of advanced tuberculoid cases of leprosy consistently have shown the spot *X* having the same R_F as GABA.

All preparations of mycobacteria separated from human leprosy nodules were able to decarboxylate L-glutamic acid to form GABA. This activity could not be associated with skin tissue enzymes, since normal human skin was found to be inactive. Nerve fibres in the skin are known to be important sites in which *M. leprae* reside for long periods of time during the course of the disease[4]. These organisms are present in large amounts in the lepromatous nodules and have also been detected in tuberculoid skin lesions[5]. It is therefore conceivable that GABA detected in leprosy skin lesions arises through the decarboxylase activity of *M. leprae* on skin glutamic acid. It is of interest that GABA was also detected in the blood of some cases of lepromatous leprosy. In view of the importance of GABA as an inhibitor of neuronal activity, the foregoing findings raise the question of the possible role of GABA in the involvement of peripheral nerves and anæsthesia closely associated with leprosy[6,7].

We thank Dr. N. Figueredo and his staff at the Acworth Leprosy Home for supplying human leprosy material and the authorities of the Tata Memorial Hospital for the material from human amputated limbs. This work has been aided by a grant from the Indian Council of Medical Research.

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Histamine and its Possible Role in Cytolysis of White Blood Cells in Guinea Pigs sensitized with Tubercle Bacilli

DELAYED or tuberculin-type sensitivity is—apart from some single publications—only transferable by white blood cells or peritoneal cells but not with humoral antibodies of sensitized organisms[1]. *In vitro*, after addition of antigen, a lysis of white blood cells[2] is seen up to 35 per cent[2]. That effect is preceded by morphological changes[3-5]. Cytolysis in the presence of plasma will be discussed elsewhere. Labelled lymphoid cells of sensitized guinea pigs will be found after transfer to normal animals at the side of the dermal tuberculin reaction[6]. The importance of white cells is further revealed by the fact that the tuberculin reaction will be suppressed by a specific anti-lymphocytic serum[7].

An antigen–antibody reaction is followed by liberation of histamine from plasma cells, platelets or by polynuclear leucocytes. On the other hand, it is generally admitted that histamine does not play a part in the tuberculin reaction. An attempt was therefore made to elucidate the possible role of histamine in cytolysis by tuberculin of cells from sensitized guinea pigs.

Male guinea pigs, 300–350 g, were sensitized by 20 mg wet weight of autoclaved (20 min at 120° C) tubercle bacilli *H* 37 *Rv* in paraffin oil. 10 and 20 days later normal and sensitized guinea pigs were injected intraperitoneally after bleeding with about 15 ml heparinized Hanks's solution without phenol red. After 2 min of massaging the solution was taken from the peritoneal cavities. The pooled white cells, cautiously washed and resuspended in Mayer buffer[8], were counted. The final cell concentration was 10^7–5×10^7 cells/ml. Equal volumes of cell suspensions and old-tuberculin (OT) solution or a solution of histamine dihydrochloride with different concentrations were mixed and incubated at 37° C during gentle shaking. After 30–120 min the cell concentration was established by counts, taking the mean value of 5 counts for calculation. The difference of cell concentration in respect to the control (white

Table 1. INFLUENCE OF DIFFERENT OT-CONCENTRATIONS ON CYTOLYSIS OF SENSITIZED CELLS (%)

OT-conc. (T.U./ml.)	60 min	120 min
2,000	—20	—28
1,000	—11	—24
250	—	—20
62	—12	—12
15	— 9	—16

Table 2. INFLUENCE OF DIFFERENT HISTAMINE CONCENTRATIONS ON CYTOLYSIS OF NORMAL CELLS (%)

Hist.-conc. (μg/ml.)	60 min	120 min
40	—39	—37
20	—29	—34
10	—27	—24
5	—13	—16

Table 3. INFLUENCE OF A HISTAMINE-NEUTRALIZING PREPARATION ON OT-CYTOLYSIS (%)

No.	Final concentration	30 min	60 min	120 min
1	OT 1 : 50	—	—15	—17
	OT 1 : 50 ; hip* 1 : 200	—	+ 9	—10
	hip 1 : 200	—	— 8	— 7
2	OT 1 : 50	—31	—	—
	OT 1 : 50 ; hip 1 : 200	— 3	—	—
	hip 1 : 200	— 3	—	—
3	OT 1 : 50	—13	—	—
	OT 1 : 50 ; hip 1 : 200	+ 4	—	—
	hip 1 : 200	± 0	—	—

*hip, histamine-inactivating preparation

cells of non-sensitized animals under otherwise identical conditions) is expressed in per cent. The peritoneal cells consisted of 69 per cent lymphoid cells, 13 per cent polynuclear leucocytes, 10 per cent plasmacells, 2 per cent monocytes, 5 per cent eosinophilic and 1 per cent basophilic cells.

Torantil containing 0·5 U./ml. was taken as a histamine-inactivating preparation in a dilution of 1 : 50, kindly supplied by Hoechst Co. It has been shown in accordance with published data that the limit of normal difference in cell concentration was 10 per cent.

Some typical results are given in Tables 1–3.

There is a marked effect on cytolysis of sensitized cells in comparison to normal cells from non-sensitized animals (Table 1). The effect seems to depend in certain respects on the OT-concentration. The lysis of white cells from sensitized animals by antigen (hapten) as a result of antigen–antibody-reaction with a probable histamine release presumes that histamine plays a part in this reaction. In Table 2 it is shown that normal cells are lysed in the presence of histamine, dependent clearly on the concentration. Therefore it may be that the cytolysis of sensitized cells is initiated by histamine. The cytolysis is (Table 3) suppressed by a histamine-inactivating preparation. This preparation apparently does not affect OT significantly since even a 16-fold higher concentration of histamine-inactivating preparation did not influence OT in the usual concentration for sensitization of sheep erythrocytes (125 T.U./ml.) demonstrated by the Middlebrook–Dubos titre of an antituberculous rabbit serum. These results support the idea of a possible role of histamine in cytolysis.

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BIOLOGY

A Simple Procedure for Freezing and Storage of Tissue Cultures using Liquid Nitrogen

In vitro cultures of many tissue cells have been preserved successfully at low temperatures using techniques of slow freezing in the presence of glycerol or dimethylsulphoxide[1]. Most investigators have recommended cooling at a rate of about 1° C per min[2], and a variety of devices have been constructed to accomplish this condition[2,3].

While studying the effects of cooling-rates on tissue cells (strains HeLa and *L*), we observed equivalent viability after freezing, using rates of cooling from 1° C to about 50° C per min[4]. This finding suggested the possibility of a simple procedure for freezing and storage as a single operation in commercially available liquid nitrogen refrigerators.

One ml. volumes of HeLa or *L* cells (10^6 cells/ml.) in medium containing 5 per cent (by volume) glycerol or dimethylsulphoxide were added to screw-cap glass vials (12 mm. diameter). The vials were placed in perforated cartons and suspended in the vapour above the level of liquid nitrogen in Linde *LNR*–25 or *LNR*–640 refrigerators (supplied by Linde Co., Division of Union Carbide Corp., New York; and Union Carbide, Ltd., 8 Grafton Street, London, W.1). Thermocouples were fixed at the wall, the centre, and midway between the wall and centre of a representative vial. Recordings established that cooling-rates, except at the centre of the specimen, did not exceed 50° C per min. Rates of cooling for various regions of temperature and position are shown in Table 1. More than 80 per cent of the total specimen volume occurs within the annular volume extending 3 mm inward from the walls, where the cooling-rate does not exceed 50° C per min.

Table 1. RATES OF COOLING MEASURED IN VARIOUS REGIONS OF FROZEN CELL SUSPENSIONS

Temperature range (° C)	Cooling-rate ° C/min in vapour		
	Wall*	Midway*	Axis*
0 to −10	10	6	5
−10 to −25	36	42	90
−25 to −50	37	50	50

* Position of thermocouples along radius of a glass vial 12 mm diam.

After thawing in a 37° C water bath, viability was assayed by trypan blue exclusion, growth and metabolism in serial dilutions of cells, containing from 120 to $7 \cdot 5 \times 10^3$ cells per ml. This procedure enables one to view microscopically well separated cells and to evaluate growth in terms of cell attachment and extension of cell processes. Results are shown in Table 2. For both HeLa and *L* cells, viability by all three criteria was essentially equivalent to unfrozen controls. Very little growth occurred when cells were frozen directly in liquid nitrogen.

In addition to strains HeLa and *L*, eight established cell lines and five types of primary cell suspensions (not previously cultured *in vitro*) have been successfully preserved by this technique. These included established cells of human embryonic intestine (Henle), skin and muscle (*MAF*, Gray), human adult liver (Chang), human amnion (*AV*–3, Robbins and Lepow), human epidermoid carcinoma (*HEp*–2, Toolan), human adult sternal marrow (Detroit 98, Berman and Stulberg), mouse sarcoma (sarcoma 180, Foley), and melanoma (*S*–91, Foley). Primary cell suspensions included monkey kidney, rabbit spleen, lymphoid and peritoneal exudate cells, and human thyroid cells. Frozen cultures have been thawed and used after almost two years of storage. Quantitative

Table 2. VIABILITY OF TISSUE CELLS AFTER FREEZING IN LIQUID NITROGEN REFRIGERATOR

		Frozen in	Percentage excluding trypan blue	Growth and pH in serial dilution cultures (cells × 10^3/ml.) 120	60	30	15	7·5
HeLa	Frozen	Vapour	96	+ + + +* 6·5†	+ + + + 6·5	+ + + + 6·8	+ + + + 7·0	+ + + + 7·4
	Unfrozen		98	+ + + + 6·5	+ + + + 6·5	+ + + + 6·8	+ + + + 7·2	+ + + + 7·4
L	Frozen	Vapour	92	+ + + + 6·8	+ + + + 7·0	+ + + + 7·2	+ + + + 7·3	+ + + + 7·4
	Frozen	Liquid nitrogen	81	+ + 7·0	+ 7·8	+ 7·8	– 7·8	– 7·8
	Unfrozen		97	+ + + + 6·8	+ + + + 6·8	+ + + + 6·9	+ + + + 7·4	+ + + + 7·4

* Growth, read microscopically (– to + + + +) after 2 days incubation.
† pH, read after 5 days incubation.

studies of viability after prolonged storage have not been done. Since storage was between –170 and –196° C, it is very unlikely that viability would differ with time of storage[3,5].

It is possible to freeze and store a variety of tissue cell lines easily, safely and relatively economically in nitrogen vapour above liquid nitrogen in liquid nitrogen refrigerators as available at present.

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Frequency of Ciliary Beat measured with a Photo-sensitive Cell

A REVIEW of the present knowledge of cilia has been made by Rivera[1]. Various methods have been used for estimating the frequency and testing the effects of different substances on ciliary beat. A common method has been to drop particles such as coal and seed on ciliated epithelium and to count the time required for the particle to travel a certain distance[2]. This method gives a rough value for the speed of the cilia, but other factors such as viscosity and thickness of the mucous layer interfere severely with the results. The 'Flimmeruhr'[3] and the 'Cilioscribe'[4] were other methods described by earlier authors. With the aid of the Leitz Ultra-Pak vertical light microscope better measuring methods have been developed. Cinematographic recording and stroboscopy combine greater accuracy with less traumatic effect on the cilia when measuring. Dalhamn examines the two methods systematically[5] and describes a cinematographic equipment which makes it possible to determine quite small changes in frequency of ciliary beat in living rabbits[6]. The stroboscopic method is rather severely criticized and not recommended by this author. Microscopic counting of the vibratory activity of small ciliated epithelium fragments in tissue cultures is another method which has been used for testing the influence of various agents[7]. A disadvantage of the cinematographic method is that the results are not available immediately, and, as continuous measurement is not possible, minor effects of substances tested might be overlooked.

With the aid of a photosensitive cell and a microscope with vertical illumination, it has been possible, when observing cilia, to obtain a variation in voltage caused by variations in light intensity. The frequency of the signal corresponds to ciliary-beat frequencies obtained with the cinematographic method.

An Ultra-Pak microscope is fitted with the standard camera house equipment. The plate holder is replaced by a black 'Plexiglass' plate with a central hole. A photo-sensitive cadmium sulphide cell (Philips *ORP* 61) is fastened to the 'Plexiglass' plate with the photo-sensitive surface protruding over the hole. A Philips tone lamp (6 amp 8 W, type 6019*M*) is used for illumination. The impulses from the photo-sensitive cell are recorded on an oscilloscope via an amplifier. Block diagram (Fig. 2). The magnification of the microscopic picture on the 'Plexiglass' plate is 110. As one group or wave of cilia has a surface of about $3\mu^2$ (ref. 5) this surface will measure 0·3 mm² after enlarging. The sensitive area of the photo-conductive cell is 0·25 mm² and it is reasonable to assume that it does not cover more than a few waves at the same time. The cilia waves are seen when the vertical light is reflected from their surfaces into the microscope. As the aperture of the objective is rather small, one group of cilia in motion soon leaves the angle where reflexion is possible and the site turns dark. This change in light intensity corresponds to a decrease in the registered voltage. Adjacent groups of cilia do not interfere, as their angle, when they are seen by the photo-sensitive cell, does not make reflexion possible.

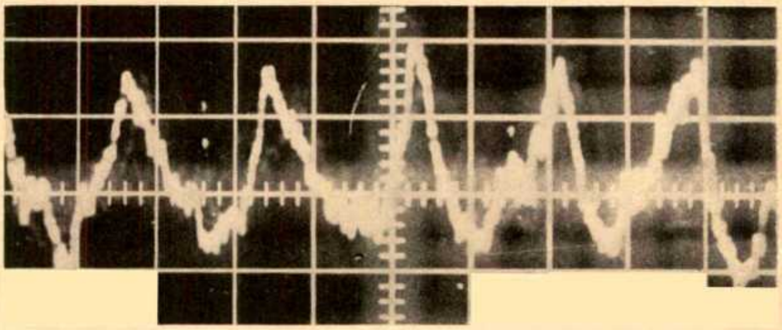

Fig. 1. Voltage variation obtained with photo-sensitive cell on a level with enlarged microscopic image of cilia

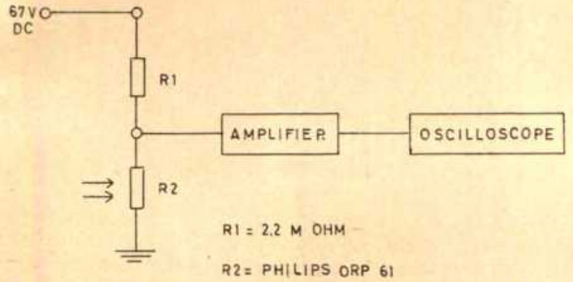

Fig. 2. Diagram of apparatus for measuring ciliary activity by means of a photo-sensitive cell

The impulses from the photo-sensitive cell can also be registered on a ratemeter and the frequency is thus obtained immediately. The oscilloscope, however, should always be used as a control as variations in amplitude occur. These variations might be caused by cell fragments or other particles which have been shed and which cause a temporary decrease in light reflex intensity when they are moved forward by the cilia.

With the method described here it seems to be possible to record changes in frequency of ciliary beat, and the effects of various agents can be studied. The method is suitable when observing ciliary motion in mammals both *in vivo* and *in vitro*.

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Isolation of and Direct Analysis of Uncontaminated Muscle Cell Contents

IT has not been possible hitherto to separate muscle cell contents from the cell walls and the extracellular fluid[1,2], so that important factors, such as the concentration of intracellular electrolytes, have had to be arrived at indirectly. The number of workers who have resorted to studying the diffusion-rates of electrolytes into or out of whole muscles in lieu of direct determination is an indication of the wide interest in this field.

Although cod (*Gadus callarias* L.) muscle cells are very short, they can be as much as 350μ in diameter in a large fish[3]. Further, they can with practice be separated undamaged from a piece of muscle. They appear suitable, therefore, as material for the mechanical separation of cell contents.

Very large live cod (around 125 cm long) were obtained by trawling off south-east Iceland in June 1961. They were killed by a blow on the head and filleted. A piece of muscle was dissected from a wide myotome, and single cells were stripped off and laid straight and parallel on glass microscope slides (25 × 76 mm), two rows of 5 cells being placed on each slide. The slides were kept chilled to prevent the cells from drying. They were frozen rapidly, wrapped in aluminium foil and stored at −80° to −100°. Cells were only collected within the period 10–30 min after death.

Separation of cell constituents was carried out in a laboratory at −20°, the operator wearing a heated suit. The slides, after unwrapping, were held on an adapted microscope stage, above which knives of hardened steel could be raised and lowered with precision, the operation being observed under a stereoscopic microscope (× 35).

In the first operation, a wide flat cutter, parallel to the surface of the slide, was lowered until it cut into one end of the cell. The stage was then

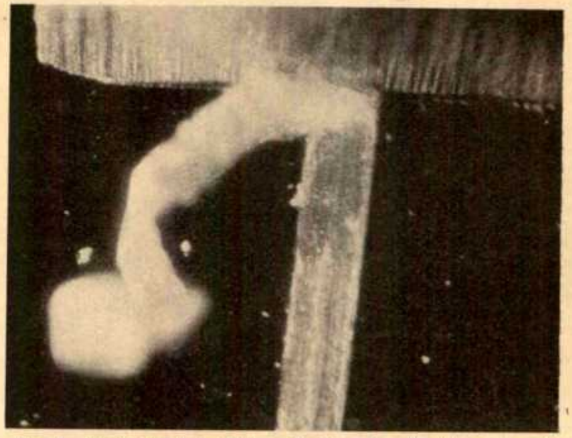

Fig. 1. Slicing the top off the cell with a broad cutter; the cell is moved upwards towards the knife, and the cell wall, etc., is shown curling away to the left

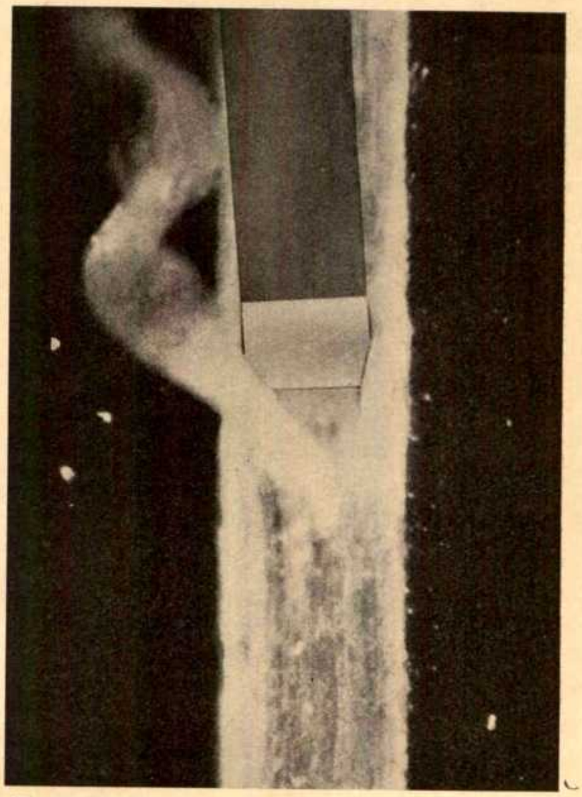

Fig. 2. Ploughing out the cell contents, shown curling towards the left. The hollow-ground chisel-edged plough (width, 130μ) has been withdrawn slightly to show part of the cutting edge. The cell moves upwards towards the plough

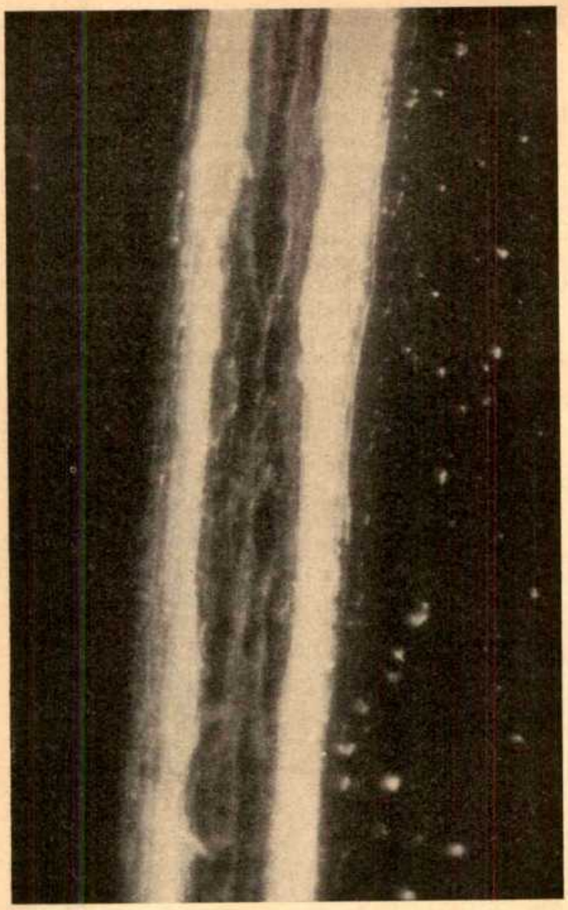

Fig. 3. Cell remainder after removal of contents. Note clean separation

operated to move the entire cell past the cutter, which sliced off the wall and some intracellular material all along the uppermost surface of the cell (Fig. 1). The contents of the cell were then ploughed out with a cutter of suitable width (Figs. 2 and 3), the cell being guided along 2 or 3 times under the plough, which was progressively lowered but was not allowed to break through the part of the cell adhering to the glass.

Provided that the cell had been frozen in a second or two, the contents came out cleanly and easily, and they were then stored in a silica tube at −80° to −100° until sufficient material had been accumulated. The sample was then weighed, dried at 100° and dry ashed at 500°. The wet weights of the three samples obtained in this work ranged from 2 to 5 mg, a yield of 50–70 μg of material from each cell.

Composition. The amount of sodium and potassium in the cell contents, with standard deviations, is compared with that of cells from which some of the contents have been removed, as in Fig. 3. The composition of the latter is of slight value, but it serves as a control, showing less potassium and more sodium than the cell contents. The rather low water value shown may have been caused by drying out—whole cod muscle contains 80·5 per cent of water or more[4]—although the small scatter is noteworthy. This appears to be the first direct analysis of intracellular material, apart from squid axoplasm[5]. The sodium value (Table 1) is close to that obtained indirectly[6] from dog muscle, 35·9 mg per cent.

Table 1

	Water (per cent)	Na (mg per cent)	K (mg per cent)
Cell contents (three samples)	70·9 ± 1·1	43·3 ± 14·1	719 ± 58
Cell residues plus extra-cellular fluid	—	80·2 ± 8·1	594 ± 34

I thank Dr. R. A. Chalmers and Mr. I. Marr of the Department of Chemistry, University of Aberdeen, who did the flame photometric analysis, and Mr. Harry Bruce, who made the dissection apparatus. The work was carried out as part of the programme of the Department of Scientific and Industrial Research.

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Influence of Incandescent and Coloured Light on Chicken Embryos during Incubation

Jensen and Matson[1] reported increased weight of the eye and flattening of the cornea (probably due to increased intra-ocular pressure) in chicks exposed to continuous incandescent light. Histological studies of affected eyes, reported by Lauber *et al.*[2], showed thinning of the retinal layers when chicks were subjected to continuous incandescent light. Shutze *et al.*[3] reported that hens reared under continuous incandescent light until 20 weeks of age showed a subsequent effect from continuous incandescent light which was manifest as depressed egg production and blindness. When silkworms were exposed to light at various stages of development, they either spun larger than normal quantities of silk or laid larger numbers of eggs (M. Kato, personal communication, 1961).

Experiments have been conducted here to examine the influence of continuous incandescent and/or coloured light on Leghorn chicken embryos during incubation. In a preliminary trial, a forced-draught incubator was equipped with light fixtures and provided with red, yellow, and blue 'Cellophane' filters between the lights and the eggs. Eggs for a control group were maintained in a dark incubator. Four chicks were selected at random from each of the five different treatments; dark control, unfiltered incandescent, filtered red, yellow, and blue lights. Body-weight, eye-weight, and eye dimensions were measured at hatching.

No differences were observed among treatments with respect to eye measurements or body-weights. However, the eggs exposed to continuous incandescent light hatched sooner than eggs in a dark incubator.

Two experiments were conducted to test the influence of continuous incandescent light and coloured light on incubation time required for hatching White Leghorn chicken eggs. These experiments were replicated in three incubators. Both the eggs and incubators were at room temperature, and all machines were started at the same time. Eggs for the second experiment had been stored at 55° F from 2–4 weeks and in the third experiment from 1–3 days before setting. The chicks were removed from the incubator at 8-h intervals, beginning after 456 h of

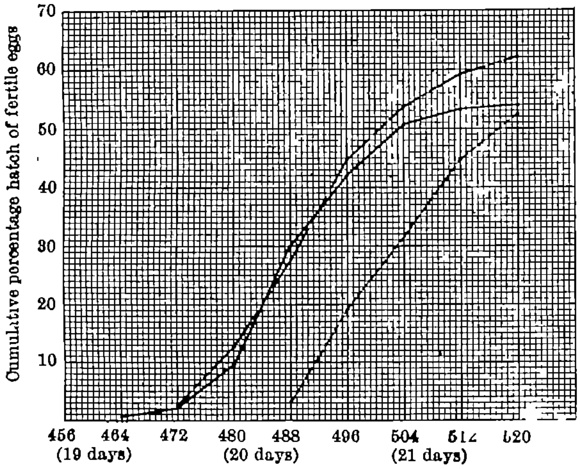

Fig. 1. Accumulative percentage hatch at 8-h intervals of chicken eggs exposed to different lighting treatments during incubation (Exp. 2). ——, continuous incandescent; – – – –, continuous coloured, - - - - -, dark

incubation and continued through 520 h. Chicks which were completely free from the shell were removed at the various periods. Apart from lighting variations, standard incubation procedures were followed.

The cumulative percentages of chicks hatched from the different treatments based on percentage hatch of fertile eggs at each period in experiment 2 are presented in Fig. 1. The lines on the graph represent 540, 180, and 180 eggs for the incandescent, coloured, and dark control treatments, respectively. Some of the eggs exposed to continuous incandescent light were hatched after 464 h, which was 24 h before any of the control eggs and 8 h before the eggs exposed to coloured lights. There were no differences attributable to colour of light; therefore, these three treatments were grouped together. Eggs exposed to the continuous incandescent light hatched approximately 16 h earlier, on the average, than those incubated in darkness throughout the experiment.

The differences observed in the third experiment were more pronounced than in the second experiment (Fig. 2). The lines on the graph represent 540, 180, and 540 eggs for the incandescent, coloured, and dark control treatments, respectively. The difference in hatchability was attributed to the difference in time the eggs were stored prior to incubation; in the second trial they were held 2–4 weeks and in the third trial 1–3 days before being placed in the incubator. The lower hatchability observed in the second experiment was therefore attributed to the age of the eggs[5].

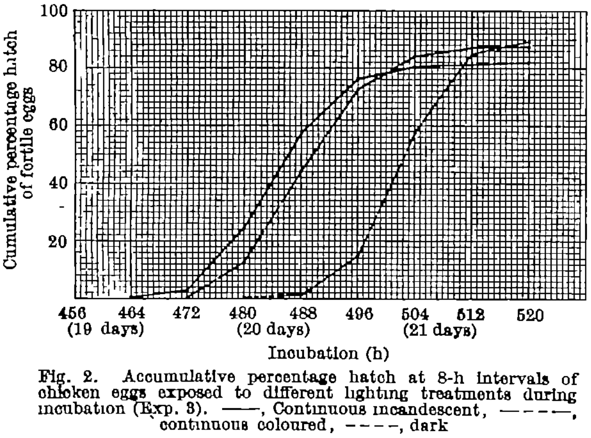

Fig. 2. Accumulative percentage hatch at 8-h intervals of chicken eggs exposed to different lighting treatments during incubation (Exp. 3). ——, Continuous incandescent, – – – –, continuous coloured, - - - -, dark

More variation in hatchability was observed among the incandescent light treatments than the controls, but this difference was not statistically significant.

The radiant energy in the incubators where light was used was measured. Low readings were obtained on 20–25 per cent of the eggs which were closest to the light. The eggs not directly under the lights did not receive enough radiant energy to register on the instrument. The readings obtained (g.cal/cm^2/min) were 0–0·1, 0–0·2, 0–0·1 and 0–0·2 for the yellow, red, blue and incandescent light, respectively. No differences were observed in hatching time that could be correlated with position of the eggs in the hatching trays.

The chicks from the second experiment were maintained in battery brooders for two weeks and no differences in viability were observed. The detailed procedure and spectral analysis of the coloured filters are available from me (J. V. S.).

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Movement of Salmon

In June and July, 1962, 156 'clean' salmon *Salmo salar* L. caught at sea by drift nets off the North Mayo coast were marked with a Lea's hydrostatic tag and released at once. One of these (length 65 cm) released on June 29 was recaptured on July 28 near Ängelholm in southern Sweden, having travelled a distance of more than 1,750 km. While a number of Irish tagged salmon have been recovered in Great Britain, mostly in Scotland, this is the first occasion on which a recapture has been made in Continental Europe.

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Drosopterins in the Throat-Fans of Some Puerto Rican Lizards

Two major groups of pigments were discovered recently in the throat-fans of Puerto Rican lizards of the genus *Anolis*[1]. Yellow carotenoid pigments were extracted with organic solvents. In addition, a family of highly fluorescent pteridines were readily extractable from the dewlaps with aqueous solvents. Only colourless or faintly buff-coloured pteridines were detected in those species of *Anolis* the throat-fans of which appear yellow or white. But in those anoles with orange- or crimson-coloured dewlaps (such as *A. pulchellus* and *A. cristatellus*) three principal red-coloured pteridines which fluoresced orange when exposed to ultra-violet light were also present. Paper chromatography of the latter pigments in five different solvent systems showed that they migrated to the same positions as three red-coloured and orange-fluorescent pigments present in the eyes of *Drosophila melanogaster*, and which were designated by Viscontini *et al.*[2-4] as neodrosopterin, drosopterin and isodrosopterin respectively.

We wish to report that this close similarity between the red pigments in the dewlap of *A. pulchellus* and the drosopterins described by Viscontini is substantiated by their electrophoretic behaviour and spectral properties.

Dewlaps of *A. pulchellus*, and flies of the species *D. melanogaster* Oregon *R*, were homogenized, and extracted side by side at 25° with a solution consisting of equal parts of 1 per cent (v/v) formic acid and of acetone. The extracts were centrifuged, and the supernatant fluids were dried *in vacuo* in the frozen state. The dried material was dissolved in 1 per cent formic acid (*p*H 2·3), applied to sheets of paper, and then subjected to paper electrophoresis in this medium (72 V/cm; 35 m.amp) for 2 h at − 5° in a Wieland-Pfleiderer apparatus. This procedure separated three major orange-fluorescent bands from extracts of both the lizard dewlaps and the flies. These three red substances travelled 20, 40 and 55 mm toward the cathode, and resemble closely the neodrosopterin, drosopterin and isodrosopterin described by Viscontini[2,3], and the drosopterins *A*, *B* and *C* described by one of us[5]. The red pteridines were eluted from the dried paper sheets with 1 per cent formic acid, dried *in vacuo*, and their spectra recorded with the aid of a Cary model 11 spectrophotometer. All the pigments appear yellow in dilute acid and are coloured pink in dilute base. In 0·1 N hydrochloric acid, the two faster-moving substances from both *A. pulchellus* and *D. melanogaster* exhibited two major absorption bands with maxima at 260 mμ and 479 mμ respectively. But in 0·1 N potassium hydroxide, both the latter substances derived from the anole dewlap showed bands with maxima at 245 mμ and 505 mμ, whereas the corresponding red compounds from *D. melanogaster* possessed absorption bands with maxima at 260 mμ and 505 mμ in the dilute base. A fourth red substance which fluoresced orange and migrated 65 mm toward the cathode was found only in extracts from *D. melanogaster*. The latter compound was previously described[5-7] as drosopterin *D*. It exhibits absorption bands with maxima at 258 mμ and 475 mμ in 0·1 N hydrochloric acid, and at 243 mμ and 497 mμ in 0·1 N potassium hydroxide. This drosopterin *D* is not identical with a fourth red-coloured and orange-fluorescent pigment present in traces in the dewlap of *A. pulchellus*, and which can be separated from the pteridines resembling neodrosopterin, drosopterin and isodrosopterin by paper chromatography with 3 per cent aqueous ammonium chloride as solvent[1].

Evidence was obtained that the dewlap of the North American anole *Anolis carolinensis*, which is pink in colour, contains the same drosopterins found in the throat-fan of *A. pulchellus*.

Previous experiments[1] suggested that extracts of the dewlaps of a variety of Puerto Rican anoles (including *A. pulchellus*) contained xanthopterin, isoxanthopterin, biopterin and 2-amino-4-hydroxypteridine, in addition to the red pteridines described here and a number of other unidentified colourless substances which fluoresce strongly in ultra-violet light. The identity of the latter four pteridines has now been corroborated by electrophoresis on paper, by repeated paper chromatography in a variety of solvent systems[1], and by examination of the ultraviolet absorption spectra in dilute acid and base of the separated substances. Fresh extracts of the dewlap of *A. pulchellus* appear to be devoid of intensely yellow-coloured and fluorescent substances resembling sepiapteridines[8,9] and also of 2-amino-4-hydroxy-pteridine-6-carboxylic acid.

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MICROBIOLOGY

Utilization of Biuret by *Pseudomonas aeruginosa*

IN the course of investigating the dissimilation of biuret by micro-organisms, we found that all of seven strains of *Pseudomonas aeruginosa* examined were inducible for an enzyme, or enzyme system, capable of hydrolysing biuret. Den Dooren de Jong[1] isolated some 40 pseudomonads, only two of which showed good growth when biuret was provided as sole carbon, nitrogen and energy source. Although biuret does not seem to be a good nitrogen source for any of the strains which we examined, conditions eventually were achieved in which cell growth, using glucose as an energy source, was sufficiently fast and predictable for study. Biuret does not serve as the sole source of carbon and energy. The mean generation time in biuret media (Fig. 1) is characteristically about twice that observed in urea media under similar conditions; this relation is maintained when absolute growth-rates higher than those shown are obtained by increasing the aeration. The curves shown describe only about three generations; but growth curves followed by plate counts, and extending over some 20 generations, are in agreement with those presented; however, for smaller, unadapted inocula, the adaptation is more erratic, and the curves cannot at present be analysed with confidence.

Evidence is available that biuret readily forms a molecular compound with *p*-aminobenzoic acid[2]; it was suggested[2] that this mechanism might explain both the mild bacteriostasis which biuret exerts against *Escherichia coli*, and the synergistic action of

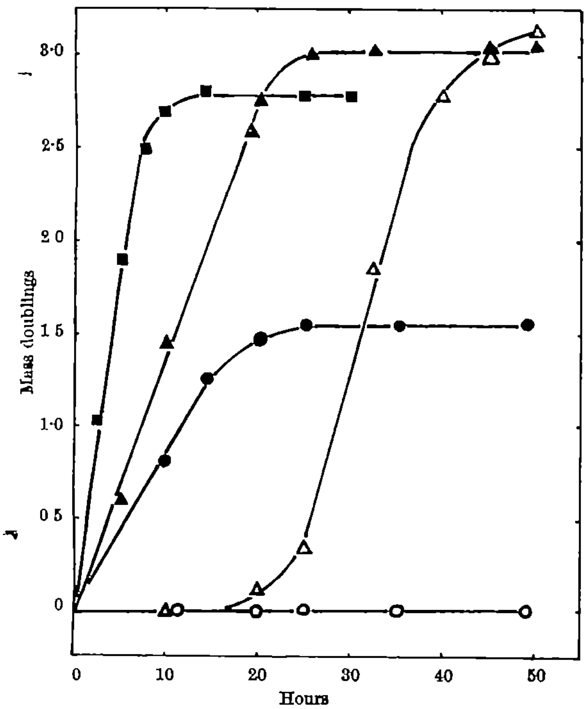

Fig. 1. Growth curves obtained with initial cell concentrations, *c.* 10^6 cells/ml. The nitrogen sources below were added to a glucose–salts basal medium, *p*H 6·0. Other conditions (temperature, aeration) were the same for all cultures. ▲, 0 1 per cent biuret, adapted inoculum; ●, 0·01 per cent biuret, adapted inoculum; △, 0·1 per cent biuret, unadapted inoculum; ○, control, no nitrogen source; ■, 0·1 per cent urea. Ordinates were calculated as 2·19 + $\log_e$ (cell concentration), where cell concentration was expressed as mg/dry weight/ml.

biuret with sulphanilamide against the same organism. We are conducting experiments with *Ps. aeruginosa* which should decide whether or not the foregoing phenomenon is responsible for the slow growth-rate, and for the long lag-phase consistently observed when unadapted cells are inoculated into media containing biuret.

Since den Dooren de Jong reported that a number of common organisms (including *E. coli*) grow well on a simple glucose–biuret–salts medium, we surmise that the bacteriostatic effects of biuret are relatively slight. In our work, they have apparently materialized occasionally as lengthy lag periods and erratic growth curves, while complete suppression of growth is effected by higher levels (1·0 per cent) of biuret.

Ammonium ion (as ammonium sulphate) represses the synthesis of the biuretolytic enzyme, and the inclusion of additional organic nitrogen (peptones or urea) in the medium with biuret results only in the preferential utilization of the added material; growth on biuret, with concomitant induction, does not consistently follow this first growth period, the deciding factors remaining unknown. Ammonium ion represents the synthesis of urease in *Ps. aeruginosa* in a similar manner[4].

Induction is most reproducible in growth media containing biuret as sole nitrogen source. Under these conditions, enzyme activity-levels in cells gathered at 12, 15, 17 and 20 h do not differ significantly. The earliest growth of unadapted inocula in biuret-media occurs when the initial *p*H is near 6·0, although dissimilation of biuret by resting cells has a maximum rate at about *p*H 7·5. The former optimum is not explicable in terms of substrate properties, since Kumler and Lee[5] have obtained strong evidence that biuret exists in solution as a hydrogen-bonded ring-structure without ionic character. (It is pertinent to note that enol tautomers are also disallowed by their data.)

The enzyme activity in washed cells and in cell-free extracts was measured by Conway's microdiffusion method, essentially as described by Kelly and Clarke[6]. The levels of enzyme activity found were much lower (about 1 μmole substrate/100 min/mg dry weight of cells) than those of the inducible amidase studied by Kelly and Clarke (in the order of 500 times the above figure), and remain of the same order when glucose in the growth medium is replaced by other energy sources. Nevertheless, the low levels observed account for the rate of turnover of nitrogen calculated from the growth-rate, so that a great loss in activity, during collecting cells and preparing extracts, is not considered likely. When broken-cell preparations are centrifuged at 15,000*g* for 10 min, all activity remains in the supernatant, and is not increased upon addition of the precipitated material. Biuret is hydrolysed by broken cell preparations with the stoichiometric release of 3 moles ammonia per mole of biuret. Occam's razor justifies the provisional conclusion that a single enzyme, biuretase, is probably responsible. It must be emphasized, however, that a two-step mechanism for the hydrolysis of biuret, with urease catalysing the second reaction, has not yet been excluded.

Malonamide and *N*-acetyl urea, which are structural analogues of biuret and probably exist as similar hydrogen-bonded ring systems, induce enzymes active on both the inducing compound and on biuret. Propionamide and urea are not inducers.

Somewhat surprisingly, biuret-grown cells and extracts thereof were found inactive on malonamide and *N*-acetyl urea. Fractionation of cell extracts, which is at present in progress, should further elucidate the relationship involved here.

Ps. aeruginosa recently has been reported to elaborate an inducible amidase in response to some ten different amides[6]. All the inducers and substrates (propionamide being the best of the latter) contained only one nitrogen atom; also, since high activities were found in cells grown on media containing ammonium ion as nitrogen source, it appears that ammonium ion is not an active repressor. We conclude that this amidase and biuretase are not closely related; also, that the dissimilation of biuret has little in common with that of urea, with the exception that ammonium ion represses the synthesis of each enzyme. Biuret dissimilation can perhaps be more fruitfully studied within the context of amides capable of forming similar hydrogen-bonded ring-structures

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Viable Micro-organisms in a Fifty-Year-Old Yeast Preparation in Antarctica

During the course of our recent investigations of the microbiota of air and soil in Antarctica, a food cache which was partially covered by a snow embankment was found at Cape Evans, Ross Island. The cache was left at this base camp by Captain Robert Falcon Scott of the British Royal Navy prior to his ill-fated expedition to the South Pole in 1911, and consisted of various types of tinned foods together with a glass container of granular, dehydrated bakers' yeast (1 lb. capacity).

The bottle of yeast retained the original label, which read in part: 'Rising-up Yeast', 'Dauerhefe', and 'Levure Inalterable'. The remaining portion of the label was illegible. A wax seal covering the cork stopper was intact. The bottle of yeast was shipped under refrigeration along with other biological collections to the Department of Microbiology, University of Texas, where it was held for two months at 4° C until the laboratory investigation was initiated.

The wax seal was removed, and the outer surface of the cork stopper and mouth of the bottle were sterilized by several successive treatments with ethanol followed by merthiolate solution. The cork stopper together with the piece of 'glassine-like' paper, immediately beneath the cork, were placed in a sterile covered dish for replacement later. This exposed a 1-in.-thick layer of rancid tallow covering the surface of the yeast completely, which was used evidently for water-proofing and for preserving the yeast.

The centre portion of the tallow was removed with a sterile scalpel, so that a plug of yeast material could be withdrawn with a sterile cork borer. The bulk of the yeast material consisted of yeast cells in a plant starch medium. The tallow had permeated to within 1 in. of the bottom of the bottle, causing the granules of the yeast material to adhere together. A 5-in. plug was removed to a sterile covered dish and divided into five equal parts. Pieces of material from the centre of each of the five parts were inoculated into malt extract (Difco), plain nutrient (Difco), and prune broth tubes; and inoculated directly to malt (Difco), plain nutrient (Difco), peptone glucose-acid, prune (Difco), and Sabouraud's dextrose agar plates. All broth and agar cultures were incubated at 25° C. The broth tubes showing growth were afterwards streaked to plates of the 5 different agar media, and also incubated at 25° C. Standard bacteriological methods were used in the handling of the yeast material to ensure that the isolated organisms originated from the yeast preparation.

The uppermost part of the plug material, immediately beneath the tallow cover, produced no growth in any of the media used. The second part produced several colonies of a single species of *Rhodotorula*; no other organisms were recovered from this particular portion. From the third, fourth, and fifth parts, the following were isolated:

Yeasts[1]:
- *Saccharomyces cerevisiae* Hansen. Many cells containing four ascospores can be seen in culture. The isolates agree well with the standard description.
- *Rhodotorula pallida* Lodder.

Moulds:
- *Absidia corymbifera* Lichtheim.
- *Rhizopus arrhizus* Fischer.

Bacteria[2]:
- *Bacillus* sp. Rods, Gram-positive, very long chains, motile, spores.
- *Pseudomonas* sp. Rods, Gram-negative, motile, slime produced.
- Micrococcaceae family. Cocci, Gram-positive, tetrads.

The fact that *Saccharomyces cerevisiae* remained viable in a yeast preparation which had been abandoned in the Antarctic for more than 50 years is unique. It is no less unique that another species of yeast, two moulds, and three species of bacteria also survived. Not any of these organisms were single isolates. The growth of *Saccharomyces cerevisiae* was noted in most of the cultures from all the lower three pieces of yeast material. The three bacterial species were also seen in most of the cultures from all the lower pieces. *Rhizopus* and *Absidia* were encountered only infrequently.

Records of longevity of micro-organisms are scanty, and opportunities for examining materials as reported here are rare.

It might have been predicted that a few viable yeast cells would be recovered from the preparation, and perhaps that contaminating bacterial endospores would survive fifty years of cold storage. It is surprising, however, that the contaminants which must have been present as a minor component in this yeast preparation have retained sufficient viability to be readily detected.

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Lung Retention in Mice exposed to Airborne Micro-organisms

The respiratory minute volume of mice can be obtained from Guyton's[1] paper; but this does not tell us how much particulate material is retained in the lungs, a measurement of importance in calculating doses in respiratory infectivity experiments with micro-organisms. Harper and Morton[2] found a mean lung retention of 2·7 ml./min when mice weighing 20–22 g were exposed to clouds of single-cell particles of *Bacillus globigii* spores labelled with phosphorus-32. The exposure method used at that time was unsatisfactory in that it involved considerable restriction of the mice by neck yokes. Later (1956, unpublished work) a few tests using less restrictive methods indicated a lung retention value of approximately 7·5 ml./min.

Lung retention (the amount found in the lungs separated from the trachea at the bifurcation of the bronchi) has since been measured using improved exposure methods in which restriction is minimal.

Mice (20–22 g) contained either in small boxes from which the heads protruded, or in Perspex tubes from which the noses protruded, were exposed by inserting the heads or noses into the exposure tube of a modified Henderson[3] apparatus. Animals were exposed for 5 or 10 min to clouds of mainly single cell particles of either phosphorus-32 labelled *Bacillus globigii* spores or *Brucella suis*. Cloud and lung samples were assessed using the methods described by Harper and Morton[2]. Three tests, in each of which 10 mice were exposed, were carried out with *Bacillus globigii* spores, and two tests, each with 16 mice, with *Brucella suis*.

The mean lung retention under these conditions was 6·9 ml./min (95 per cent confidence limits 6·4–7·4) with the spore clouds and 6·8 ml./min (95 per cent confidence limits 5·3–8·4) with the *Brucella* clouds. There was no significant difference in mean lung retention when mice held in boxes were exposed with their noses through thin rubber diaphragms in the wall of the exposure tube to prevent leakage around the animals' heads during exposure. Other exposure methods involving pressure on the neck or face of mice resulted in reduced and irregular lung retention.

A mean lung retention value of 7·0 ml./min for mice weighing 20–22 g, which represents about 27 per cent of the respiratory minute volume, is now used in this Establishment for calculating the lung retention of groups of mice in this weight-range when exposed to clouds of micro-organisms in the size-range encompassing *Brucella suis* cells and *Bacillus globigii* spores.

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GENETICS

Frequency of Atypical Pseudocholinesterase in British and Mediterranean Populations

Considerable interest has been taken over the past twenty years in the level of human serum pseudocholinesterase, because it was known that it was altered in a number of pathological conditions. It has been recognized, however, in the past ten years that considerable differences may also exist in healthy people, and that this difference is genetically determined[1]. Several variants of the enzyme are now known[2–5], but the variants which give rise to the most frequently found phenotypes are the 'typical' and 'atypical' enzyme of Kalow and Genest[2]. These can be distinguished by their sensitivity to the pseudocholinesterase inhibitor dibucaine. Under given conditions the 'typical' enzyme is inhibited by about 80 per cent ('Dibucaine Number' or $DN = 80$), and the 'atypical' enzyme by about 20 per cent ($DN = 20$). The three phenotypes, 'typical' homozygotes, 'atypical' homozygotes and heterozygotes show a DN of about 80, 20 and 60 respectively. Kalow and Gunn[6] found the gene frequency of the 'atypical' gene to be 0·0188 in a healthy Canadian population. 'Atypical' homozygotes would therefore be expected at a frequency of 1 in 3,000. This is of practical importance because they respond to the usually short-acting, and in modern anæsthetics widely used muscle relaxant suxamethonium with a prolonged and dangerous apnœa, the normally short action of the drug being due to its rapid destruction by the 'typical' serum pseudocholinesterase. We had the impression that this anæsthetic hazard was more often encountered in persons of Mediterranean origin than in British patients. We have, therefore, determined the gene frequency of the 'atypical' gene in British and various Mediterranean populations by measuring the DN. The results are shown in Table 1. It may be noted that only heterozygotes and no 'atypical' homozygotes were discovered.

Table 1. Incidence of 'Atypical' Pseudocholinesterase

Population	No examined	No. of heterozygotes	Frequency of heterozygotes (per cent)	Gene frequency
British	703	27	3·8	0·019
Greek	360	13	3·6	0·018
Portuguese	179	6	3·4	0·017
Berber	55	2	4	—
Moroccan-Jewish	51	1	2	—

The details of the various populations were as follows, with the number of heterozygotes given in brackets. The 703 British consisted of 483 presumably healthy blood donors (17), and 220 hospital patients (10); the 179 Portuguese were all blood donors; the 360 Greeks were children from Athens and from the region of Lake Kopais known for its high incidence of the sickle-cell gene. There were 69 presumably healthy school children from Athens (3), 118 from the Lake Kopais region (6), and 173 hospital patients from Athens (4). The sera from 55 Berbers and 51 South Moroccan Jews were supplied by Dr. A. E. Mourant, Dr. R. Johnson and Mr. R. A. P. Scott, respectively.

A polymorphism implies the existence of selective pressure for or against the various phenotypes. Geographical differences in gene frequency might well supply a clue to the nature of these selective forces. The fact that we have found no significant differences in these various populations suggests that either the selective forces operating are only slight at the present time or that they are uniform in Britain and in the Mediterranean regions studied.

If our impression is correct that suxamethonium apnœa is seen more often in Mediterranean than in British patients, it cannot be explained on the basis of a higher frequency of the gene responsible for 'atypical' pseudocholinesterase. However, the rare 'silent' pseudocholinesterase gene which causes in the homozygote complete absence of pseudocholinesterase activity has been seen in three families in Britain[5,7] of which one was British, one Belgian and one Greek. The Greek family included the only known homozygote with a complete absence of serum pseudocholinesterase activity. The heterozygotes for this 'silent' gene and for the 'typical' gene are not easily discovered. They have a 'typical' DN but the serum enzyme activity is lower than normal. We did find a number of sera among the Greek samples in which the enzyme activity was low, and where there seemed to be no obvious explanation such as malnutrition or liver disease. Notably we saw one family, all with 'typical' DN, where the propositus, a healthy girl, had a pseudocholinesterase activity of 42 units (normal 65–120). Her seemingly healthy parents had enzyme activities of 36 and 43 units respectively, and her only brother one of 50 units. The presence of the 'silent' gene can only be satisfactorily proved when families are found where it either interacts with the 'atypical' gene, or where a homozygote can be discovered. Nevertheless we think it likely that at least the present family, and possibly several others in Greece, may be harbouring the 'silent' pseudocholinesterase gene.

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Distribution of Alkaline Phosphatase Variants in a Heteroploid Strain of Human Cells in Tissue Culture

Attempts to demonstrate possible genetic interactions among mammalian cells grown in tissue culture have been hampered by the lack of suitable markers which may be conveniently detected and scored among large cellular populations. The difficulty in initiating genetic studies with this material is due, in large part, to the considerable biochemical and morphological uniformity which characterizes mammalian cell strains, of whatever origin, established *in vitro*. Recently, however, it has been shown that there exists striking differences in the alkaline phosphatase activity of a sub-line of HeLa cells as compared with the parent culture[1] and between several human cell strains of different origins[2,3]. We describe here a simple technique which permits the detection and isolation of variant clones of human tissue culture cells which lack alkaline phosphatase or which show diminished amounts of the enzyme activity. In addition, we give some preliminary results pertaining to the clonal distribution and properties of some alkaline phosphatase variants which were obtained by use of this technique.

Several cell strains of human and animal origin have been examined; the results of this investigation will be reported in detail elsewhere. The experiments reported here were conducted using strain *EUE*, an epithelioid cell strain derived from the subcutaneous tissue of a human embryo. This strain has been maintained in this laboratory for 4 years.

The cells were grown in Melnick's medium or '*TC* Medium 199' (Difco); both media were supplemented with 10 per cent calf serum and 3 per cent human serum. Monodisperse suspensions were prepared by trypsinizing monolayer cultures and the cells plated in 10-cm Petri plates. The inocula varied from 500 to 1,000 cells per plate. The plates were incubated at 37° C in an atmosphere of 5 per cent carbon dioxide–95 per cent air until most of the colonies had acquired a diameter of about 2 mm (about 15–20 days).

Hanks's double strength salt solution containing 0·1 M *tris* was adjusted to *p*H 9·4 with concentrated hydrochloric acid. Sigma 104 phosphatase substrate (*p*-nitrophenyl phosphate (*p*-NPP) disodium salt) was then added to a concentration of 2 mg/ml., and the solution sterilized by filtration. An equal volume of sterile, melted, and slightly cooled 3 per cent 'Bacto-Agar' (Difco) was then added and the agar-*p*-NPP solution maintained at 45° C in a water bath.

The colonies were washed once in normal strength Hanks's salt solution (*p*H 7·4) and 10 ml. of the agar-*p*-NPP solution were then added to each plate. The results could be read after about 2–5 min incubation at 25° C. Positive colonies are characterized by the appearance of a brilliant yellow colour which is due to the release of *p*-nitrophenol in the immediate vicinity of the colony. Negative colonies remain colourless. Surprisingly enough, neither the high *p*H nor the presence of *p*-nitrophenol (or its phosphate) seems to exert a markedly toxic effect on the cells: viable cells may be obtained from selected clones and transferred to fresh medium for further culture for as long as 15–30 min after the addition of the agar.

Table 1. INDUCTION OF ALKALINE PHOSPHATASE ACTIVITY IN NEGATIVE STRAIN *EUE*6

Cell strain	Inducer	Specific activity
EUE	No inducer	18·50
*EUE*6	No inducer	<0·07
*EUE*6	Prednisolone, 0·5 μg/l.	0·59
*EUE*6	Phenyl phosphate, 5 × 10^{-3} M	0·88
*EUE*6	Phenyl phosphate, 2 × 10^{-3} M	1·38
Human skin fibroblasts	No inducer	0·43

Cells were cultured for 5 days in '*TC* Medium 199' (10 per cent calf serum, 3 per cent human serum) in the presence of inducers (refs 2 and 3) as shown. Specific activity determinations were performed according to the method of Torriani (ref. 4) using extracts prepared by repeated cycles of rapid freeze-thawing of heavy cellular suspensions. Results are expressed in terms of micromoles of *p*-NPP hydrolysed per minute per mg protein at 35° C. For purposes of comparison, the specific alkaline phosphatase activity of fibroblastic cells derived from a human skin explant and maintained in culture for 2 months is also presented.

The specific alkaline phosphatase activity of strain *EUE* cells is about 18·50 (Table 1). However, an examination of individual clones obtained by plating monodisperse suspensions of *EUE* revealed that there exists considerable heterogeneity of the alkaline phosphatase activity within the cellular population. Thus the pooled data from several experiments showed that of 7,000 clones examined, 69 were negative, that is, no hydrolysis of *p*-NPP could be detected in the vicinity of the colony even after prolonged incubation at 37° C. Many intermediate levels of enzyme activity could also be observed among various clones. Confirmation of the absence of alkaline phosphatase activity among the negative clones was provided by histochemical staining using α-naphthol phosphate as substrate with fast blue *RR* as coupler.

Thirty-six sub-clonings of individual positive and negative colonies have indicated that at least two types of clonal derivatives may be distinguished: (1) those which continue to produce mixtures of positive and negative colonies on replating; (2) those which produce only negative colonies. Derivative lines of type 1 may be obtained from either positive or negative clones. Thus, three sub-lines initially derived from negative clones and one sub-line derived from a positive clone have produced both positive and negative colonies in about equal numbers on replating.

However, nearly all positive colonies derived from the negative clones showed only faint activity. We are unable at present to explain the appearance of these faintly positive colonies. Clonal derivatives of type 2 have only been obtained from negative colonies. One of these derivatives, designated strain *EUE*6, has been chosen for a more detailed examination since it has produced no positive colonies among more than 600 examined.

The specific alkaline phosphatase activity of strain *EUE*6 is too low to be measured accurately. However, measurable amounts of alkaline phosphatase activity may be induced in this strain by cultivation in the presence of either prednisolone or phenyl phosphate[2,3] (Table 1). Under the experimental conditions, phenyl phosphate was much more effective as an inducer than prednisolone. It may also be seen from Table 1 that induction of *EUE*6 resulted in the appearance of less than one-tenth of the enzymatic activity of the parent strain, *EUE*. Induced colonies of *EUE*6 may be detected using the agar–*p*-NPP method, a fact which suggests that it may be possible to select variants which are completely uninducible or, conversely, which may be induced to very high levels of enzyme activity.

It is interesting to note that strain *EUE*, which has high levels of constitutive alkaline phosphatase activity, could not be cultured in the presence of 2×10^{-2} M phenyl phosphate, whereas growth of *EUE*6 was little affected by this concentration of the compound. This may be explained by the fact that strain *EUE* cultures rapidly hydrolysed the inducer, phenyl phosphate, as evidenced by the strong odour of phenol in such cultures.

We do not know at present whether all negative *EUE* strains selected by this technique are more effectively induced by phenyl phosphate than by prednisolone. Human fibroblast cultures are induced by phenyl phosphate whereas the HeLa strains (epithelioid) respond more effectively to prednisolone[2,3]. Our strain *EUE* has a decidedly epithelioid morphology.

It is apparent that the ability to synthesize alkaline phosphatase is not lacking in strain *EUE*6, and that the variation in this case is correlated with the loss of 'constitutivity'. Some preliminary experiments indicate that there are at least three electrophoretically distinct alkaline phosphatases in strain *EUE*. It will be of considerable interest to study the role of these enzymes in the induction process and their distribution among clonal lines showing low or intermediate enzyme activity.

A further problem posed by these findings concerns the relatively high frequency of clonal variants revealed by this technique. Whether this variation may be correlated with a high frequency of aberrant chromosomal segregations in heteroploid tissue culture strains, alterations at specific intrachromosomal loci, or more strictly physiological factors remains to be explored.

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[1] Portelius, P., Saksela, E., and Saxon, E., *Exp. Cell Res.*, 21, 616 (1960).

[2] Cox, R. P., and MacLeod, C. M., *Nature*, 190, 85 (1961).

[3] Cox, R. P., and Pontecorvo, G., *Proc. U.S. Nat. Acad. Sci.*, 47, 839 (1961).

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PSYCHOLOGY

Intelligence, Divergence and Potential Originality

RECENTLY, many attempts have been made to isolate the intellectual and personal qualities which enable people to do original work, particularly in science. One of the most interesting results so far is Getzels and Jackson's[1] discovery of the "High Creative" type among American school-children. (These are children who are successful academically, whose intelligence quotients are relatively low, but who excel in open-ended tests—for example, "How many uses can you think of for each of the following objects?") The contrast between "High Creative" and "High Intelligence Quotient" types is seen to support a more general distinction between 'divergent' and 'convergent' styles of thinking, the former—it is argued—being the style which leads to original work among adults.

Getzels and Jackson's work has been repeated, and in certain respects amplified, on a sample of 95 English sixth-form pupils, aged 15–17. The results, in one respect at least, were unexpected. As in the American work, the contrast between intelligence quotient and open-ended scores correlates with a variety of personal differences; but these differences are overshadowed by a broad distinction between arts and science subjects. Boys specializing in history and English literature are strong on the open-ended tests and relatively weak on intelligence quotient, while those specializing in physical science are the reverse (Table 1, $P = 0 \cdot 001$; χ^2 test with Yates's correction). Specialists in classics, modern languages and biology seem—although the samples are small—to be distributed between these two extremes.

This arts/science discrimination adds to the available means of measuring schoolboys' academic bias[2]. It also throws light on the nature of ability in the arts; one would not expect to find, for example, that unpractical young historians are significantly better ($P = 0 \cdot 05$) than practically minded young physical scientists at thinking of uses for everyday objects. On the other hand, its implications are puzzling. Does it imply, as American theorizing would suggest, that most potentially creative sixth-form pupils dislike science—or, at least, the way it is taught in English schools—and turn to the arts instead ? (If this is even partly true, the consequences for English science are disturbing.) The personal differences between the two groups are certainly consistent. 'Divergent' scorers (that is, those who are relatively strong on the open-ended tests and tend towards arts subjects) are significantly more likely than 'convergent' scorers to express unusual attitudes ($P = 0 \cdot 025$); to have liberal, non-authoritarian views about politics and school life ($P = 0 \cdot 005$); to show emotional involvement in their opinions ($P = 0 \cdot 005$); to say that they are unhappy at school ($P = 0 \cdot 005$); to have a broad rather than narrow range of interests and hobbies ($P = 0 \cdot 025$); to choose unusual themes for drawings ($P = 0 \cdot 025$) and to include people ($P = 0 \cdot 025$) and violence ($P = 0 \cdot 05$) in them; to make errors in doing the intelligence

Table 1. SIXTH-FORM SPECIALISTS IN HISTORY AND PHYSICAL SCIENCE CONTRASTED IN TERMS OF THEIR PSYCHOLOGICAL TEST SCORES

('Divergent': open-ended scores greater than intelligence quotient; 'Convergent': intelligence quotient greater than open-ended scores)

	'Divergent'	Neutral	'Convergent'
History	$n = 13$	5	0
Physical science	4	12	25

quotient test ($P=0\cdot01$); and so on. For the most part, these differences are in keeping with those found in America. Although there are many individual exceptions, they do suggest that young physical scientists tend to be less intellectually flexible than young arts specialists, and more restricted emotionally. On the other hand, there is at this stage no means of telling which boys will eventually do original work—and therefore no empirical proof that original work is more likely to follow one emotional configuration than the other.

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Theoretical and Empirical Confusion Functions

The following table should replace Table 1 of my communication that appeared under the above title on p. 836 of the August 25 issue of *Nature*.

Table 1

	x_1/x_2							
Author	0	0·5	0·67	0·75	0·80	0·83	0·86	0·88
Crossman	0·00	1·00	1·71	2·41	3·18	3·82	4·51	5·18
Welford	0·33	1·00	1·67	2·33	3·00	3·67	4·33	5·00
Hammerton								
$k=1$	0·34	1·00	1·64	2·28	2·92	3·57	4·35	4·86
$k=1/14$	1·00	1·00	1·00	1·05	1·11	1·25	1·39	1·54

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STATISTICS

Partition Correlation Matrices for Heterogeneous Quantitative Data

A form of data which occurs occasionally in sociology, and very commonly in ecology, is that in which the attributes, though measurable when they occur, do not run through the whole population, so that the data matrix contains many zeros. Such a population consists in reality of a number of more or less discrete sub-populations, each defined by some only of the attributes, and the application of traditional multivariate methods to such data encounters two main difficulties. First, even though the non-zero values of an attribute are normally distributed, the addition of zeros causes the mean/variance relationship of the whole attribute to approximate to that of a qualitative (0,1) distribution; if component analysis is used in an attempt to separate the sub-populations the normal $(x-\bar{x})/\sigma$ transformation then results in excessive weight being given to the absence of a common attribute or the presence of a rare one. Secondly, if factor analysis with communalities is attempted, factors after the first—since all factors unrealistically involve all attributes—cannot be interpreted. Data which are heterogeneous in this sense are better subdivided on a presence-or-absence basis; and a series of papers from this laboratory[1-6] has explored one statistical technique for this purpose. However, if the data are not intrinsically entirely qualitative this involves discarding information, and there appears to exist no method of assessing the relative importance of the qualitative and quantitative elements of a given set of data. This communication outlines such a method.

Let the set of values for an attribute x, some of which are zeros, be denoted by a vector $[0, x]$, and let m_x be the mean of the non-zero values. Let this vector be regarded as the sum of a qualitative (L) vector $[0, m_x]$, in which all non-zero values are replaced by m_x, and a quantitative (N) vector $[0,(x-m_x)]$. The sum of the squares of the deviations of the N-vector elements from their (zero) mean is the same as that of the non-zero values of x from their own mean m_x; and the sum of the variances of the L and N vectors is identically equal to that of the original vector. The division may thus be regarded as a true partition between the qualitative and quantitative elements of the attribute. If two such vectors $[0, x]$ and $[0, y]$ are so partitioned, it is easily shown that the sum of the four covariances L/L, L/N, N/L and N/N is also identically equal to that between the original vectors. These covariances may therefore be used, with the corresponding L and N variances, to generate a set of four correlation coefficients (indeterminate values being treated as zeros), which in turn define three correlation matrices.

The first of these, L/L, is a normal correlation matrix; it contains all the purely qualitative information, and is in fact the matrix which would have been obtained if the original data had been replaced by [0,1]. The second, N/N, is also a normal correlation matrix, summarizing the numerical relationships between pairs of attributes when, and only when, both are present in the same individual (one common individual will suffice since what is under examination is the extent to which either deviates from its non-zero mean). The third ($L/N + N/L$) is asymmetric, with zeros in the principal diagonal, and we suggest the convention of L for rows and N for columns. This matrix summarizes the extent to which changes in the quantity of one variable are accompanied by the appearance or disappearance of others.

Inspection of the three matrices shows very clearly where most of the information resides; as an objective measure of the total information content of each matrix we tentatively propose to use the sum of the squares of all the correlation coefficients, excluding the principal diagonal; but this is not easy to justify, and we may wish to modify the proposal with more experience. If most of the information is in the L/L or L/N matrices, then subdivision of the population is clearly indicated; only when, after successive subdivisions, the bulk of the information appears in the N/N matrix is factor analysis indicated, and certain of success. The method has proved extremely effective when applied to specially constructed artificial populations, and we hope to use it to analyse data which have proved intractable by traditional methods.

One of us (M. B. D.) acknowledges the support of a Department of Scientific and Industrial Research maintenance award.

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Department of Botany,
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[1] Williams, W. T., and Lance, G. N., *Nature*, 182, 1755 (1958).

[2] Williams, W. T., and Lambert, J. M., *J. Ecol.*, 47, 83 (1959).

[3] Williams, W. T., and Lambert, J. M., *J. Ecol*., 48, 689 (1960).

[4] Williams, W. T., and Lambert, J. M., *Nature*, 191, 202 (1961).

[5] Williams, W. T., and Lambert, J. M., *J. Ecol.*, 49, 717 (1961).

[6] Lambert, J. M., and Williams, W. T., *J. Ecol.* (in the press).

FORTHCOMING EVENTS

(*Meetings marked with an asterisk are open to the public*)

Monday, November 12

University of London (at Friends' House, Euston Road, London, N.W.1), at 5.30 p.m.—Sir Vivian Fuchs: "The Human Element in Exploration" (Rickman Godlee Lecture).*

Plastics Institute (at the Royal Institution, Albemarle Street, London, W.1), at 6.30 p.m.—Dr. J. C. Swallow: Second Swinburne Award Address.

Society for Visiting Scientists (at the English-Speaking Union, Dartmouth House, 37 Charles Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Matching Men and Machines". Chairman: Dr. W. F. Floyd. Speakers: Dr. D. J. K. Stuart, Dr. H. P. Ruffell and Mr. D. E. Broadbent.

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Dr. I. G. Gass: "Return to Tristan da Cunha".

Tuesday, November 13

Institute of Metal Finishing, London Branch (at the Rembrandt Hotel, Thurloe Place, London, S.W.7), at 9.30 a.m.—Symposium on "Barrel Processes in Metal Finishing".

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. J. C. West and Dr. B. V. Jayawant: "A New Linear Oscillating Motor".

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. F. Roth: "Some Aspects of the Use of Computers in Process-Control Applications".

Institution of the Rubber Industry (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—"A Look at the Soviet Rubber Industry" by a member of the RAPRA Staff; 7 p.m.—Dr. W. C. Wake and Mr. D. I. James: "Tyre Cord Adhesion; a Study of Filament Damage".

Parliamentary and Scientific Committee, General Committee (in Committee Room 11, House of Commons, London, S.W.1), at 5.30 p.m.—Dr. D. A. Senior: "Soviet Science".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr F. Sanger, F.R.S.: "Proteins".* (Ninth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. M. G. Bekker: "Mechanics of Off-the-Road Locomotion" (James Clayton Lecture).

Society of Chemical Industry, Agriculture Group (joint meeting with the Oils and Fats Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. D. Lewis: "Fats in Animal Feeding".

Royal Institute of Chemistry, London Section (at Norwood Technical College, Knights' Hill, London, S.E.27), at 7 p.m.—Film Show.

Tuesday, November 13—Wednesday, November 14

Society of Cosmetic Chemists of Great Britain (at the Queen's Hotel, Cheltenham)—Symposium on "Perfumery".

Wednesday, November 14

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. G. J. Phillips: "Stereophonic Broadcasting Systems".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. L. Dudley Stamp: "Some Aspects of Medical Geography. 3: The Mapping of Mortality and Morbidity".* (Third of four Heath Clark Lectures.)

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Papers on "Radio Transmission of Digital Data".

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Mr. S. D. Hall: "Analysing the Results of Vibration Tests with FM Tape Recordings".

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. N. A. T. Fisher: "Studies on the Lipids of Flours from Single Wheat Varieties of Widely Differing Baking Qualities".

Institute of Metal Finishing, Organic Finishing Group (at the British Institute of Management, 80 Fetter Lane, London, E.C.4), at 6.30 p.m.—Mr. R. F. Drysdale: "Modern Methods of Pretreatment for Metals".

Society of Instrument Technology, Automation Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. W. F. Floyd: "The Ergonomics of Keyboards with special reference to Letter Sorting Machines"; Mr. G. N. Davison: "The Coding and Automatic Sorting of Letters".

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7.30 p.m.—Mr. H. J. Nation: "Modern Developments in the Use of Engineering in Agriculture".

Thursday, November 15

East Malling Research Station (at Withersdane, Wye College, Ashford, Kent), at 3 p.m.—Mr. J. M. S. Potter: "The National Fruit Trials" (Amos Memorial Lecture).

Royal Institution of Naval Architects (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Dr. G. Hughes. "Correlation of Model Resistance and Application to Ships" and "Results of Resistance Tests with a Standard Model".

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. E. Douglas: "Derivation of a Basic Efficiency Formula for Concentrating Operations"; Mr. S. C. Newman: "Revision of the Mine Accounting and Costing Procedures in the Rand Mines Group".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Annual General Meeting. Prof. W. K. Hayman: "On Successive Coefficients of Univalent Functions".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. D. W. Moss: "Isoenzymes".* (Tenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. L. Dudley Stamp: "Some Aspects of Medical Geography. 4: The Way Ahead". (Last of four Heath Clark Lectures).*

British Institute of Radiology (in the Reid-Knox Hall, 32 Welbeck Street, London, W.1), at 6 p.m.—Discussion Group meeting on "Fractionation of Treatment". 8 p.m.—Ordinary Meeting, followed by Mr. Frank Ellis: "The Philosophy of Radiotherapy" (Presidential Address).

Chemical Society (in the Anatomy Lecture Theatre, King's College, Strand, London, W.C.2), at 6 p.m.—Dr. R. E. Richards, F.R.S.: "Nuclear Magnetic Resonance Spectroscopy" (Tilden Lecture).

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. A. Please and Dr. T. Sugawara: "The Use of Mechanical Tests in the Design of Bituminous Road Surfacing Mixtures. III: Relation of the Results of Mechanical Tests to Road Behaviour".

Royal Society of Tropical Medicine and Hygiene (at the Liverpool School of Tropical Medicine, Pembroke Place, Liverpool 3), at 7.30 p.m.—Laboratory Meeting. Chairman: Sir George McRobert, C.I.E., F.R.C.P.

Thursday, November 15—Friday, November 16

Royal Society (at Burlington House, Piccadilly, London, W.1) at 10.30 a.m. on Thursday and 10 a.m. on Friday—Discussion Meeting on "Demography" (the application of vital and social statistics to some problems of human biology). Organized by Prof. P. B. Medawar, F.R.S., and Mr. D. V. Glass.

Friday, November 16

Institute of Navigation (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Mr. Curtis T. Clayton: "Automation and Marine Navigation".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. A. Silverleaf: "Experiments with Ship Models".

Friday, November 16—Saturday, November 17

British Society for Immunology (at the Wellcome Building, Euston Road, London, N.W.1)—Autumn Meeting.

Friday, November 16

At 2.30 p.m.—Symposium on "Pharmacology of Allergic Reactions and Related Inflammatory States". Chairman: Dr. J. H. Humphrey.

Saturday, November 17

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Peter Crossley-Holland: "Music Among the Tibetans".*

Monday, November 19

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. D. W. McKenzie. "The Twisting Land".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Future of Global Communications" opened by Mr. R. J. Halsey.

Society of Chemical Industry, Pesticides Group (joint meeting with the Agriculture and Fine Chemical Groups, at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. J. F. Scott: "The Design and Analysis of Experiments".

University of London (in the Anatomy Theatre, University College, Gower Street, London, W. C.1), at 5.30 p.m.—Prof. W. R. Mead: "The Geographical Tradition in Finland" (Inaugural Lecture).*

Royal Meteorological Society (in the Conference Hall, County Hall, Westminster Bridge, London, S.E.1), at 6 p.m.—Dr. R. Frith: "The Upper Atmosphere" (Popular Lecture; to be repeated November 20).

Royal Institute of Chemistry (joint meeting with the Dartford and District Branch of the Pharmaceutical Society, at the North-West Kent College of Technology, Miskin Road, Dartford), at 7.45 p.m.—Dr. R. E. Bowman: "Some Aspects of Research in the Pharmaceutical Industry".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Experimental Research Physicist, for the development and use of an electron spin resonance machine in the Physics Department—The Secretary, Board of Governors, Hammersmith Hospital, Du Cane Road, London, W.12 (November 15).

Assistant Lecturer in Physiology—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (November 17).

Assistant Lecturer in Agriculture concerned with the biology of farm management—The Registrar, Room 22 (O.R.B.), The University, Reading (November 19).

Lecturer (with a degree in agricultural economics or in economics or agriculture with postgraduate experience in agricultural economics) in Agricultural Economics—The Registrar, University College of North Wales, Bangor, North Wales (November 19).

Lecturer or Assistant Lecturer in Applied Mathematics—The Registrar, The University, Sheffield (November 24).

Lecturer in Biochemistry in the Department of Physiology—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (November 30).

Lecturer in Veterinary Pathology—The Registrar, The University, Liverpool, quoting Ref. CV/198 (November 30).

Senior Research Officer, to take charge of a new programme of research on cryogenic engineering, with special reference to the cryogenic pumping of gases or problems of mechanical engineering at low temperatures—The Secretary, Engineering Laboratory, The University, 19 Parks Road, Oxford (December 1).

Lecturer in Statistics in the Department of Mathematics, to give particular help in connexion with biological statistics—The Registrar and Secretary, King's College (University of Durham) Newcastle upon Tyne (December 3).

Senior Lecturer or Lecturer (preferably with a knowledge of tropical livestock and feeding-stuffs, especially of grasslands) in Agricultural Biochemistry and Nutrition in the Department of Agricultural Chemistry, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 7).

Chair of Physics in the University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W.C.2; and The Appointments Section, University of New South Wales, P.O. Box 1, Kensington, N.S.W., Australia (December 14).

Lecturer/Senior Lecturer in Zoology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 14).

Lecturers or Assistant Lecturers in Chemistry (4); Geography (1); Mathematics (4); Physics (3); and Zoology (3) at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 15).

Senior Lecturer in Mathematics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 7).

Assistant Experimental Officer, for work on the physiology of the fungal diseases of stored fruit—The Secretary, Agricultural Research Council, Ditton Laboratory, Larkfield, Maidstone, Kent.

Assistant (preferably graduate) in Plant Physiology, to prepare material for practical classes and act as part-time research assistant—Prof. H. K. Porter, Imperial College of Science and Technology, London, S.W.7.

Biochemist (probationary or basic grade, with a first- or second-class honours degree in biochemistry or chemistry or equivalent qualifications) in the Department of Pathology—The Group Secretary, The Royal Hospital, Wolverhampton.

Lecturer (with an honours degree or equivalent in physics and research or industrial experience in a field involving a practical knowledge of electronics) in Physics—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7, quoting Ref. CST.62/65.

Research Assistant (with a good honours degree and an interest in microbial metabolism) in the Department of Biological Sciences, for work on the photometabolism of organic acids in algae—The Registrar, Bradford Institute of Technology, Bradford 7.

Research Biochemist (with a higher degree), for work on human serum and tumour proteins—Professor of Chemical Pathology, Westminster Medical School, 17 Horseferry Road, London, S.W.1.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

The Empire Cotton Growing Corporation. Progress Reports from Experiment Stations, Season 1960–61. Tanganyika Territory—Coastal Provinces. By R. Smith and T. Bradley. Pp. 14. 2*s*. 6*d*. The West Indies. By J. R. Spence, C. T. Cave, M. A. Archibald and C. F. Nicholls. Pp. 14. 2*s*. 6*d*. Survey of Reports. By H. E. King. Pp. 6. (London: The Empire Cotton Growing Corporation, 1962.) [129

Commonwealth Institute of Entomology. The African Army Worm *Spodoptera exempta* (Walker) (Lepidoptera, Noctuidae): a Review of the Literature. By E. S. Brown. Pp. iv+69. (London: Commonealth Institute of Entomology, 1962.) 7*s*. 6*d*. [129

Annual Report on the Meteorological Office for the year 1 January to 31 December 1961. Pp. viii+65+4 plates. (M.O. 726). (London: H.M. Stationery Office, 1962.) 5*s*. 6*d*. net. [219

Agricultural Research Station. Annual Report of the Low Temperature Research Station, Cambridge, for 1961. Pp. 40. (London: H.M. Stationery Office, 1962.) 2*s*. 6*d*. net [219

University of Cambridge and Agricultural Research Council. The Low Temperature Research Station, Cambridge. Pp. 48+8 plates. (London: H.M. Stationery Office, 1962.) 2*s*. 6*d*. net. [219

Department of Scientific and Industrial Research: Building Research Station. National Building Studies—Special Report No. 32: Simplified Tables of External Loads on Buried Pipelines. By N. W. B. Clarke. Pp. iv+12. (London: H.M. Stationery Office, 1962.) 1*s*. 6*d*. net. [219

The Royal Institute of Chemistry. Remuneration Survey, 1962. Pp. 9. (London: The Royal Institute of Chemistry, 1962.) [219

Imperial College of Science and Technology, (University of London). Shorter Postgraduate Courses, Autumn Term, Session 1962–63. Pp. 19. (London: Imperial College of Science and Technology, 1962.) [219

The Institute of Navigation. Annual Report, 1961–62. Pp. 18. (London: Institute of Navigation, 1962.) [219

Ministry of Health. Memorandum on Measures for the Control of Mosquito Nuisances in Great Britain. Revised 1962. Pp. 38 (2 plates). (Memo. 238/Med.) (London: H.M. Stationery Office, 1962.) 2*s*. 6*d*. net. [219

Report by the Hydrographer of the Navy for the year 1961. Pp. 31+1 chart. (H.D. 523). (London: Admiralty, 1962.) [219

Agricultural Research Council: Radiobiological Laboratory. Annual Report, 1961–62. Pp. xv+96. (London: H.M. Stationery Office, 1962.) 6*s*. 6*d*. net. [219

Other Countries

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SUPPORT FOR SCIENTIFIC RESEARCH AND TRAINING IN BRITAIN

THE long-awaited report on the support given by the Department of Scientific and Industrial Research for research and training in universities and colleges* scarcely makes the contribution to the discussion of the balance of the nation's research effort, and more particularly to the scale of fundamental research, that might have been hoped. However, in fairness, it must be said that it could only be adequately covered by a report from the Advisory Council on Scientific Policy or the Minister for Science himself. Although the report only covers the years 1956–60, it indicates clearly enough the magnitude of the contribution which the Department is already making to university research, and indeed to the nation's research effort as a whole through the support given to the training of research workers. It is no less clear that this support is likely to continue to grow, and that the magnitude of the contribution which should be made through the Council for Scientific and Industrial Research and the other Research Councils by way of postgraduate training awards, studentships and fellowships and by research grants is a matter which calls for considered decision at highest level. It bears vitally on the allocation which should be made for the expansion of the universities and colleges of technology and for their normal operation.

Whereas in 1929–30 the Department's expenditure on 39 research grants and 155 training awards was £37,000, and in 1939–40 only £24,000 on 39 research grants and 119 training awards, by 1949–50 it was spending £344,000 on 95 research grants and £212,000 on 844 training awards; for 1959–60 these figures had risen to £916,000 on 525 research grants and £904,000 on 2,464 training awards, and in the Council's last report, for 1960–61, research grants totalled nearly £1·5 million and training awards more than £1·1 million. Already, however, the Department is reviewing its plans for training awards in the light of the proposed expansion of the universities to some 170,000 places by the late 1960's or early 1970's, and four considerations point to the need of increasing further the number of studentships offered annually over the next four years.

First, it is anticipated that the universities alone, apart from the colleges of technology and other technical institutions, will need some 6,000 more teachers of science and technology in the next decade or so. Secondly, the output of undergraduates in science and technology has been increasing at roughly twice the rate expected when plans were being laid in 1959, and the intake of undergraduates into university faculties of science and technology shows that this trend is likely to increase. Thirdly, there is some evidence that the proportion of graduates achieving good honours degrees in science and technology is also increasing. Fourthly, the progress of knowledge in science and technology and the resulting crowded state of undergraduate syllabuses is putting a heavier burden on postgraduate training and may necessitate more postgraduate courses and, in consequence, advanced course studentships.

There is room for some examination of both the last two contentions, though it can be accepted that they will increase the demand for studentships. What is no less important is the extent to which the Department is now supporting postgraduate students in science and technology. In 1958 the Department was supporting 39 per cent of all British research students, working in departments the subject of which lay wholly or partly within its province, and 45 per cent of those in their first year. These figures compare with 6·0 per cent and 4·5 per cent, respectively, supported by the other research councils, 14·5 per cent and 12·9 per cent by industry and 5·2 per cent and 4·5 per cent by charitable trusts, learned societies, etc. For first-year advanced course students in 1958–59 the figure was 32·9 per cent compared with 18·0 per cent for industry, 0·4 per cent for the other Research Councils and 3·3 per cent for charitable trusts and learned societies, etc.

In view of this dominant position of the Department, the policy of the Department in administering the awards is of great importance. It does not appear that the awards have had any considerable effect on migration between universities, and there are very few individual university departments or research schools consistently attracting students with the Department's awards from other universities. The tendency to migrate appears to depend somewhat on the subject of research, but it would appear possible that the awards could be administered to foster migration where desirable, as they are already used in general terms to encourage fields of research which call for special attention. This is perhaps more important in checking the marked movement away from the colleges of technology. The Department is well aware of the opportunities for research offered by the colleges of advanced technology, but the prestige and status of the universities, the high reputation of research in British universities and the doctorate offered as a reward for successful training in research have hitherto combined to make the demand for awards tenable in these colleges and other technical colleges small. Although the Department's technological awards now total nearly £450,000, the demand reflects to some extent the research effort in the colleges of technology and even in 1959–60 research grants for technology numbered only 57 and totalled more than £300,000.

The encouragement of postgraduate training and research in these institutions is so desirable that more positive action by the Council for Scientific and Industrial Research might well be justified, at least as part of a considered policy of expanding the

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* Department of Scientific and Industrial Research. *D.S.I.R., Universities and Colleges 1956–60: a Report on D.S.I.R. Support for Research and Training in Universities and Colleges, 1956–60.* Pp. viii + 232 + 15 plates. (London: H.M.S.O., 1962.) 15s. net. (See also p. 623 of this issue.)

British research effort. The value of the awards could, of course, have some effect, and on this the most important section of the report relating to training awards is that which outlines the recommendations of the sub-committee set up by the Postgraduate Training Grants Committee with Sir Eric Ashby as chairman to review the value of these awards and which indicates the action being taken to implement them. The sub-committee presented its final report in November 1960 and in its last report the Research Council indicated that it was hoped to implement the recommendations in 1962. There is one exception: the Council did not consider that the recommendations intended to give such students national insurance privileges received by persons in employment should be adopted at present.

The sub-committee reaffirmed that the purpose of the Department's awards was to give opportunities to the most promising graduates in science and technology each year to pursue advanced study, or to learn the techniques of research, in both their own interest and that of the country as a whole. The philosophy underlying the early awards, then called 'maintenance allowances', was that they should be just enough to support a student (taking into account other sources of income available to him). This philosophy the sub-committee regarded as being unsound in view of the quality of the men who receive awards and of the widespread opinion that young scientists should be encouraged to cultivate interests outside science, and it recommended that the value of these studentships should be sufficiently high to allow the students a standard of living rather higher than that enjoyed by most undergraduates of comparable ability.

It is clearly in the public interest that there should be a sufficient inducement to accept these awards, and it is as well that the recommendations should come from a sub-committee on which Sir Eric Ashby had colleagues of the standing of Prof. P. M. S. Blackett, Dr. J. W. Cook, J. S. Fulton, Prof. E. C. Stoner and Prof. C. E. Tilley. The rates of award, it considered, should be kept in steady relation to starting salaries in employment, and for students living in lodgings in London the sub-committee estimated that the rate of award should not differ markedly for 70–75 per cent of the average net salary that they would be likely to obtain in employment. It recommended as a matter of principle that a Department student should be able to afford roughly the same standard of living regardless of the university he chooses to attend, and for this purpose the rate in London of £400 per annum was accepted as standard. Accordingly, it was recommended that the Department should make periodic assessments of what it costs to maintain a given standard of living in each town. For students living at home, the sub-committee recommended that the value of the award should be increased to £300 a year wherever the homes might be.

On deductions from awards the sub-committee took a reasonable view. It regarded as essentially sound the general principle that the awards are intended primarily to supplement and not to replace the students' other income, in order to bring the total to a sum which provides an adequate standard of living, but recognized also that, strictly applied, the principle would discourage students from undertaking work that is often beneficial to their studies. Accordingly, the sub-committee recommended an overall limit, initially of £75 on the amount a student may freely retain from private income and all earnings combined, but in addition a student should be allowed to retain the first £50 from prizes and half the next £50 in any year. On the research fellowships the sub-committee took the view that the Department should be able to offer the able Fellow enough to encourage him to stay in Britain, and recommended that the scale of payments should be fixed having regard to the value of university assistant lectureships and to the range of values of most of the post-doctoral fellowships in science and technology awarded by other bodies in the United Kingdom. With these principles in mind, the sub-committee arrived at a scale of £800–£950 per annum, with family allowances of £50 a child, up to £150, an extra £60 for Fellows working in Central London, and a maximum of £1,200, including allowances for Fellows of outstanding ability. In these scales it is assumed that superannuation can be provided.

The Department, however, also fosters research in universities through its research grants and some 95 per cent of the Department's research grants are made in response to applications from university scientists. The Research Grants Committee believes, however, that there is a large reserve of research potential in the colleges of technology and other institutions of higher education, and that the expenditure of comparatively modest sums could help scientists in these laboratories to make fuller use of their own abilities and the facilities provided—with benefit also to their teaching. The Committee has, in fact, appointed a sub-committee with Sir Willis Jackson as chairman to give special attention to research in the field of technology, and it recognizes that the tendency to place technological research in compartments not only in universities and colleges but also in industry presents an outstanding problem of organization. In this report, however, it offers no fresh specific proposals for encouraging research in the colleges of technology and only discusses the encouragement of technological research as a particular aspect of the balance of the nation's research effort. Some stress is indeed laid on the importance of good collaboration, both between the universities (and, by implication, the colleges of technology) in science and technology, and the national and industrial laboratories in application and development. The Committee fully recognizes that more voluntary co-operation is required within and between universities for broader and deeper study of promising fields, in ways that may cut across existing boundaries of departments and faculties.

This applies equally to co-operation between the universities and the colleges of technology, and specifically the Committee suggests that such co-ordinated studies would be particularly valuable in the basic properties of materials, the mechanics of metal forming, and automatic control. Moreover, it is a matter of effective communication, as well as of organization, and here the Committee recognizes that the Department of Scientific and Industrial Research is well placed to strengthen the links between academic and industrial research. The Development Committee set up in 1959 to advise the Department on its policy of providing further support of research and development in industry, and the joint committee with the National Research Development Corporation are two steps to that end, and the series of detailed surveys of important industries being made under the Department's Economics Committee are also illuminating the areas in which the nation's research and development effort requires strengthening. Greater interchange of staff between university, college of technology and industrial and Government laboratories could also assist the establishment of stronger links between industrial and other research establishments. The Committee notes that such interchange could be assisted by recognition of Government laboratories for advanced study and their use by universities for projects requiring large-scale equipment, and the use of their specialist staff for teaching in universities and technical colleges.

The Department would welcome the extension of such collaboration and this is a matter in which a major responsibility obviously rests on the universities. It is, however, related to the use of large-scale research facilities and it may well be hoped that practices that are already becoming common in nuclear physics and radio astronomy, for example, may encourage a general willingness to co-operate in this way. These problems are discussed in a separate section of the report, but little is added to what has already been said in reports from the Advisory Council on Scientific Policy and the University Grants Committee. However, it is clear that the Research Grants Committee is fully aware of the particular difficulties which arise in sharing special research equipment, and that though it may be made a condition of a grant for providing equipment for a university or similar institution that the equipment must be made available for use by other workers in the field, or that if changes in staff lead to inadequate use of a large piece of equipment, the equipment must be made available to workers from other institutions, such conditions are not likely to be enforced unreasonably or without imagination.

Broadly speaking, the Research Grants Committee reflects in this report the considerations in regard to location and staff which were expressed so admirably in the Zuckerman Report on the control and management of research and development and justify the confidence that due regard will be had to the possibilities of co-operation in the financing and the organization of Britain's research effort in the next few years. Some reference is also made to the major extension of the Department's arrangements for supporting research in universities introduced in 1959 under the names 'longer-term' or 'renewable' grants. These grants are intended to increase the flexibility of the general scheme and a few such grants have now been made. These are mostly for an initial period ending in 1962 so that their first review for possible research can be synchronized with the universities' estimates of the University Grants Committee funds for the quinquennium 1962–67. It is the expectation of this scheme for longer-term grants that towards the end of each such quinquennium, and in consultation with the university, the Department will consider whether, in the light of the scientific progress and its cost, the work so supported should be continued and, if so, whether the full cost should fall on the Department, or be shared between the Department and the university, or be assimilated by the university into its established work.

Such a decision will turn on whether the work is sufficiently well established to form an integral part of the university's work; whether other universities have decided that scientifically similar and otherwise comparable work is suitable for support from general university funds; whether the magnitude or unpredictability of the cost will unduly disturb university finances; and whether the cost, in relation to the importance and promise of the new line of research, is such that continuing support is not justified. These longer-term grants clearly offer a valuable means of arranging support for expensive new lines of research without distorting the financial framework of a university, and they add to the importance of the influence which the Department is already exerting on the magnitude and balance of Britain's research effort. Indeed, the Research Grants Committee comments in this report on certain fields where some increased effort is required if the research effort in pure science is to be reasonably balanced. For example, it considered that there is insufficient effort in the fringe areas of biophysics, geophysics, meteorology and oceanography, and in some aspects of the main areas of astronomy, biology, chemistry, geology and physics; but the report gives no countenance to excessive expenditure on space research or research with satellites.

What seems lacking is the recognition that the Advisory Council on Scientific Policy and not any particular Research Council, even the Council for Scientific and Industrial Research, is the best placed to advise on the balance of the nation's research effort. It is true none the less that the Department's allocation of research grants has risen from £824,000 in 1956–57 to £2,142,000 in 1959–60 and its actual expenditure on such grants from £360,000 in 1956–57 to £1,433,309, excluding that on the human sciences and space research. The Department has been scrupulously careful in administering its grants for research and training to avoid interfering with academic freedom or endangering the conditions most favourable to creative work; there is no reason to doubt that the Department's valuable contribution to the research effort of Britain has also been made with due regard to its responsibility for the considerable public funds involved. Nevertheless, it seems desirable that consideration for university autonomy should no longer be allowed to hinder the provision of a fuller picture of the actual expenditure on academic research than is as yet obtainable either from the reports of the University Grants Committee or those of the Research Councils. The Minister for Science seems the appropriate person to look to for a picture that is required as a basis for ultimate decisions on the balance of Britain's effort in pure and applied research. It should at least be possible for the information to be available in his own Office and that his Office should be staffed appropriately.

The present report, however, is intended to answer such questions as whether the Department's research schemes are serving their purpose and whether the expenditure represents a good investment of public money; whether these are the best ways of helping research and training, and what are the likely future developments. To the first two questions the report gives an unhesitating affirmative answer, subject to an assessment of the value of science and education and to a belief in the long-term value of basic scientific and technological research. To the second pair of questions the answer would come better from the Advisory Council for Scientific Policy—the Research Grants Committee does not attempt an answer. It points out, indeed, that the success so far has been based on a good flow of research problems, good advice on the selection of projects to support, a good supply of men and women of the highest ability to tackle them, adequate funds from the Exchequer and efficient and flexible administration. Failure of any one of these factors could obviously prejudice the success of the schemes. Furthermore, the Research Grants Committee stresses the desirability that the substantial help coming from other sources will also grow and provide the university scientist with a healthy independence of Government support as well as valuable links between the academic and the industrial scientists.

As to the value of such alternative sources of support there can be no doubt, but whether it can provide in future substantial independence of Government support seems unlikely. The Research Grants Committee itself recognizes that the Government must still be prepared to receive an increasing flow of demands for support and to carry an increasing financial responsibility. The limits at which that financial responsibility must be fixed and the distribution of resources between different fields of effort are to some extent matters of policy to be determined at Cabinet level. However, those decisions should be taken in accordance with the principles laid down so clearly in the Zuckerman report if the financial resources are to be efficiently used and waste and frustration avoided. If this report does not represent as useful a contribution to informed discussion on the balance of the research effort as might have been hoped, it strongly supports much that was recommended in the Zuckerman report and provides the Minister for Science with further evidence of the futility and wastefulness of some recent Government action regarding the universities and colleges of technology.

JOHNSON FOR MATHEMATICIANS

Mathematisches Wörterbuch mit Einbeziehung der Theoretischen Physik

Bearbeitet und Herausgegeben unter Mitwirkung zahlreicher Fachgelehrter von Josef Naas und Hermann Ludwig Schmid. Band 1: A–K. Pp. xi + 1043. Band 2: L–Z. Pp. vi + 952. (London and New York: Pergamon Press, 1961.) £40 the set.

GERMAN scholarship, with extensive view, surveys mathematics and mathematicians, from 'Abacist' to 'Zylinderfunktionen', from Abel to Zygmund, in these two volumes containing nearly 2,000 closely printed, double-columned pages. Some of the larger entries are small monographs, with useful short bibliographies of texts and papers. Cross-referencing is extremely thorough, so that the multiplicity and the over-lapping of technical terms do not prevent rapid identification of any particular topic: a list of symbols used in the text is a sensible auxiliary, though it might well have been given in duplicate, since the physical labour of turning from one volume to the other is not inconsiderable.

Theoretical physics has a fair proportion of space, dealing with general dynamics, celestial mechanics, mechanics of deformable media, electrodynamics, thermodynamics, relativity, quantum theory and nuclear physics. The appearance of the item 'turbine' does not imply a lapse into engineering, but refers to the plane figure formed by tangents from points on a circle drawn to a concentric circle.

Biographical notices are brief and to the point, giving dates, place of work and principal interests. The boundary here must have been difficult to draw; there is a case for including William Thomson (Kelvin) and excluding J. J. Thomson, but scarcely for including Moritz Cantor and omitting T. L. Heath.

The thoroughness of the major articles may be judged from the 18 pages devoted to Legendre functions. The Laplace equation in spherical coordinates is shown as the origin of the Legendre ordinary differential equation containing two parameters μ, ν, related to the hypergeometric equation. The solutions are defined; results are then stated concerning the recurrence formulæ, Hobson's expressions in terms of Pochhammer loop integrals, the simpler asymptotic formulæ. The parameters μ, ν, which in the general case are complex, are now specialized: (*a*) μ an integer; (*b*) ν an integer, μ zero (the Legendre polynomials and the functions of the second kind); (*c*) μ, ν both integers. In each of these cases, the specialized forms of the general results are given, with further details in important instances, such as the Christoffel–Darboux summation formula for the Legendre polynomials. All this makes a comprehensive and usable précis of results and formulæ, arranged conveniently for reference. There is a bibliography, listing standard treatises such as Hobson, Whittaker and Watson, and the Bateman Project volumes, as well as several memoirs of basic importance; the recent 3-volume comprehensive treatise by Robin (1957–59) may have appeared too late to be mentioned.

The editors have seen that recently developed topics, such as, for example, fibre bundles, and programming, receive full attention, but elementary topics are not dismissed too lightly. Thus π receives two pages. It is defined geometrically, with a note on Archimedes' method of exhaustion and the related recurrence formula; it is also defined in terms of the zeros of the circular functions. There are a short paragraph on the quadrature problem, and an account of the calculation of π, mentioning the infinite products of Vieta and Wallis, the Gregory series, the Machin formula and the Shanks determination of 707 decimal places. Ferguson's discovery of an error in Shanks's figures is not mentioned, but this does not affect the 40 decimal places given; machine calculations to 100,000 decimal places have come too recently to be referred to.

The printing is very good, even in small-type symbolic work, and there are few misprints, though some proper names are mis-spelt. It is difficult to name any topic which is not dealt with, but there is a noticeable variation in scale, both in the main text and in the bibliographies, a defect which could perhaps scarcely have been avoided by the most careful editorial supervision in such an enterprise as this, wherein more than 120 mathematicians have collaborated. It is indeed a solid German monument to mathematical scholarship. T. A. A. BROADBENT

PROPAGATION THEORY

Electromagnetic Waves

Proceedings of a Symposium conducted by the Mathematics Research Center, United States Army, at the University of Wisconsin, Madison, April 10–12, 1961. Edited by Rudolph E. Langer. Pp. xii + 396. (Madison: University of Wisconsin Press, 1962.) 6 dollars.

THIS volume is the record of a symposium conducted by the Mathematics Research Center, United States Army, in the University of Wisconsin, during April 10–12, 1961.

The title of the work suggests probably a greater generality in the contents than is intended, for the matter contained relates almost exclusively to the propagation of waves in free space and to the attendant problems of diffraction, scattering and aerial theory. Some sixteen papers by a selection of eminent authorities are included although none of the discussions which took place during the conference is reported.

Although the appeal of the book will be mainly to the mathematical specialist in the field defined, a number of the constituent papers are in the nature of reviews and may well serve as introduction to present-day thinking for a rather wider audience. For example, the paper by M. Kline on "Electromagnetic Theory and Geometrical Optics" includes a useful review of the historical aspects of the two approaches, in addition to delineating the fields of application of either technique, and discussing some of the remaining "open problems". In a similar manner the longest of the papers included, "The Propagation of Electromagnetic Waves along the Earth's Surface", by J. R. Wait, while mainly presenting an up-to-date account of the author's own investigations of the subject, implicitly summarizes much of the earlier work in this field.

The greatest number of papers on any one specific topic relate to diffraction theory. Of these the essentially 'pure' mathematician's approach is represented by "The Mathematical Foundations of Diffraction Theory" by C. H. Willcox, in which the general diffraction problem is formulated in precise mathematical terms together with appropriate existence and uniqueness theorems, and further theorems governing the functional properties of the solution. The use of "Abstract Operator Methods in Electromagnetic Diffraction" is discussed in a paper by Marcuvitz, who points out that despite the extensive use of this approach in quantum mechanical investigations the technique has been but little utilized in diffraction theory. Further papers in this field include an extensive discussion of "A Mathematical Model for Diffraction by Convex Surfaces", and a general treatment of diffraction by polygonal cylinders.

Several of the papers refer to aerial calculations, of particular interest being a discussion of "The Pulse Solution Connected with the Sommerfeld Problem for a Dipole in the Interface between Two Dielectrics" by H. Bremmer, and a treatment of "Dipoles in Dissipative Media" by R. W. P. King. This latter treatment could relate, for example, to such cases as radiation from a submarine aerial or from a rocket antenna probe in an ionized region or plasma sheath.

A useful addition to the analytical techniques of scattering theory is provided by "The Quasi Static Radar Cross Sections of Complex Bodies of Radiation" by K. M. Siegel, which extends the author's earlier methods to obtain higher order solutions than previously.

Mention should also be made of a short, but interesting, paper by G. Goubau entitled "Some Characteristics of Electromagnetic Wave Beams", which makes some pertinent comments on the currently interesting subject of 'coherent-' and 'incoherent-' optics.

In summary, this collection of papers is full of stimulating, if esoteric, interest, and is certainly deserving of a place in any library serving the needs of those concerned with the theory of aerials and wave propagation. G. D. SIMS

MEDICINAL AND POISONOUS PLANTS

Piante Medicinali

Chimica Farmacologia e Terapia, Vol. 1. Da Dr. Benigni, Dr. C. Capra e Dr. P. E. Cattorini. Pp. vii + 730. (Milano: Inverno and Della Beffa, 1962.) 8,000 lire.

The Medicinal and Poisonous Plants of Southern and Eastern Africa

Being an Account of Their Medicinal and Other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. By Prof. John Mitchell Watt and Dr. Maria Gerdina Breyer-Brandwijk. Second edition. Pp. xii + 1457. (Edinburgh and London: E. and S. Livingstone, Ltd., 1962.) 252*s*.

TWO interesting new books on medicinal or poisonous plants have recently appeared, one in Italy and the other in South Africa.

The Italian book, *Piante Medicinali*, is the first volume of a new two-volume work and deals with some 144 species arranged according to their common Italian names and covering the letters *A* to *H*. The second volume (due to appear this year) will deal with another 120 species under the letters *I* to *Z*. Each species is dealt with in similar fashion, the space devoted to each depending on its importance medicinally. There are notes or information on etymology and nomenclature, habitat, part used, active principles, therapeutical properties, pharmacology and chemical composition. With the less-important medicinal plants one or two pages only are devoted to them, but with the more important species a great deal more space is given, especially to their chemistry, all the latest information having been assembled or brought together. As an example, one may take digitalis (*Digitalis purpurea*), to which 30 pages are devoted, including an up-to-date bibliography of 215 references. *Digitalis lanata* occupies 23 pages with 95 references. Among the non-European or tropical drug plants dealt with in this volume are star anise, boldo, buchu, cannabis, cardamom, cascara, cinchona, coca, cola, Calabar bean and ginseng. To anyone engaged in pharmacological work and able to read Italian, this should prove a useful reference work.

The South African book is a revised and greatly enlarged edition of the now well-known work by Watt and Breyer-Brandwijk (*The Medicinal and Poisonous Plants of Southern Africa*) that first appeared in 1932. Since that time a great deal of knowledge has accumulated on the poisonous and medicinal plants of South and East Africa, and it is in every way desirable that a new edition of this book should now appear. The book shows little resemblance to its illustrious predecessor, being more than four times as large and consisting of 1,455 pages, as against 314 pages for the original edition. It now weighs 7¼ lb.! Unfortunately, this greatly increased size has to be reflected in the price of the book.

As indicated in the title and subtitle, the work deals with the medicinal and other uses, chemical composition, pharmacological effects and toxicology in man and animal of the medicinal and poisonous plants of southern and eastern Africa. The appearance of this new exhaustive and scholarly work is especially appropriate at the present time in view of the fact that much of the folk-medicine of the indigenous peoples of Africa is undoubtedly disappearing before the advancing tide of civilization. Some feel that much of it may have disappeared altogether within measurable time. This is also happening in other parts of the world. The detailed index (occupying 258 pages) is a valuable feature of the book, including as it does native, Afrikaans and English names of plants. This new edition is much more freely illustrated than the first edition and includes pleasing coloured plates. F. N. Howes

BIRDS OF NORTH AMERICA

Handbook of North American Birds

Edited by Ralph S. Palmer. Vol. 1: Loons through Flamingos. Pp. vii + 567 + 6 plates. (New Haven, Conn., and London: Yale University Press, 1962.) 105*s*.

For some thirty years British, and indeed European, ornithologists have had a standard work of reference in the five volumes of the *Handbook of British Birds* by H. F. Witherby and others. Now an equivalent work for North America has been launched under the auspices of the American Ornithologists' Union, in association with the New York State Museum and Science Service. This is expected to run to at least six volumes, over a number of years; the first has now been published. Important advances in ornithological knowledge have been made since the older work was published, and in the new one it is interesting to note the shifts of emphasis on different facets. In particular, the systematic treatment in this work is based on species, whereas the earlier one was the victim of a passing fashion for regarding the subspecies (or geographical race) as the biological entity.

The area covered comprises Greenland, Canada, the United States (including Alaska, but, of course, not Hawaii), and a few islands such as the Bermudas. This is something less than the classical Nearctic region, which takes in the northern part of Mexico; even the isolated Mexican peninsula of Baja California, in the area of the *American Ornithologists' Union Check-list*, is here excluded. Following a practice that is likewise common in British bird-books, species that are only rare vagrants to the area are given full treatment. The relevant subspecies are dealt with quite briefly: extralimital forms are just listed.

Commendable care is taken at the outset to define terms. An illustrated section on colour specification is especially noteworthy. The terminology of successive plumages and moults is explained, equating new and old names. Methods of measurement and the topography of the body-surface are the subjects of clear diagrams. The definitions of vowel and consonant sounds in vocalization follow the late Bernard Tucker of Oxford. Other definitions relate to types of pair-bond (including multiple sexual nexus) and shapes of 'egg profile'.

The systematic treatment includes, for each species, detailed description of plumage, points for field identification, and the characteristics of voice; habitat, range and migration (with a statement of 'banding status'); reproductive behaviour, eggs, young and 'survival data'; general habits and food. The last mentioned is based mainly on the ascertained stomach contents or castings and with as quantitative an approach as the evidence permits.

Although no policy as regards illustrations is explicit, a new tendency for a work of this kind is evident. No attempt is made to portray every species, but some are shown in drawings that give a good impression of the character of the bird. Colour is used only to bring out special points; a plate illustrating seasonal changes in the bill, iris and foot in certain heron species is particularly valuable. On the other hand, black-and-white maps of distribution are used lavishly; and there are some whole pages of useful diagrammatic drawings of various display postures of single species. These reflect a modern geographical and ethological approach to bird study.

Dr. Ralph Palmer (of the State Museum at Albany) is to be congratulated on his first volume, and on the manner in which he has integrated the numerous contributions by specialists. At this end of the taxonomic spectrum there is an absence of peculiarly New World groups. Thus the loons (that is, divers) are circumpolar, the grebes are at most generically distinct, the petrels inhabit the oceans of the world, and the totipalmate families are cosmopolitan. In North America the last mentioned include quite a number of cormorant species, an anhinga not unlike the darter of Africa, and a marine pelican that plunges on its prey from flight like a gannet. The herons are strongly represented, the storks poorly, and there are a few ibises. Finally, there is one flamingo—usually regarded as conspecific with that found in parts of Europe and Africa, but showing a deeper pink. Landsborough Thomson

INTERNATIONAL HORIZONS IN THE LIFE SCIENCES*

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AT this moment we are living in one of the most critical and exciting periods of the history of biology, of mankind, and of life as a whole. In biology itself, the breaking of the DNA code is giving us a key to the nature of life. This research is establishing a standard of biological activity which is just as fundamental to biology as is the Periodic Table to chemistry. At the same time, biologists in many fields are reaching beyond the barriers which constrained their predecessors, and are making spectacular advances by combining data from different disciplines. Taxonomists are enriching and modernizing their field with chemistry and statistics. Morphologists are supplementing their images of dead, fixed and stained tissues with those obtained from living tissues raised under carefully controlled conditions, and by means of the electron microscope are establishing connexions between overall structure and the molecular elements of which cells are composed. Physiologists are becoming increasingly aware of the importance to them not only of a more complete understanding of life-processes in terms of chemistry and physics but also of the value of comparing similar processes in different organisms. As a result of such efforts, disciplines of biology which are basically synthetic, such as cell biology, morphogenesis, and evolution, are flourishing as never before.

In the history of mankind our Earth has entered not only the atomic age but also the age of negligible distances. With jet transportation, a person can go from one part of the Earth to any other so rapidly that neither mileage nor time is a limiting factor. As one scientist remarked to me, modern distances should be reckoned not in miles or even hours but in dollars. (Another scientist has remarked on hearing this comment that "unless the distance in dollars is a minus quantity, I don't go".)

We have also entered the age of potential freedom from drudgery for all mankind. If known inventions and technological accomplishments are exploited to their fullest extent, if the world's resources can be fully utilized and conserved, and if the growth of the human population can be controlled so as to maintain a stable, permanent equilibrium with our resources, a society can be created in which no man will need to work for long hours in order to receive enough return to keep him barely above starvation. Men can no longer say, as did philosophers from Plato and Aristotle to Tolstoi, that many of their fellow men must work for long hours with little hope of reward in order that a small group of fortunate intellectuals may be free to enrich the lives of mankind in the present by literature, music, and the arts, and to make plans for improving the lot of future generations by studying history, political science and philosophy.

At the same time, this potential freedom from drudgery is accompanied by actual threats to our society more serious than any which humanity has faced in its past history. The ever-present threat of wholesale atomic destruction is only one of these. Equally dangerous, but more subtle and insidious, is the lack of balance caused by the rapid increase of social and economic standards of living in many parts of the world, particularly in temperate regions, while other regions are unable to break loose from an age-old pattern of poverty and social inequality, just as their populations are increasing in an explosive manner. This lack of balance threatens to bring to the world a new inequity of social structure, in which nations of economic aristocrats, mostly in temperate Europe and America, will be opposed by geographical slums covering hundreds of thousands of square miles, and including millions of human beings who will be living in conditions of degradation, and semi-starvation, and hopelessness as severe as those which were endured by the slave classes of past empires. The natural concern over this possibility on the part of leaders and informed citizens of these less-fortunate nations, and their desperate attempts to avoid disaster by any means available to them, are causing much of the world's present instability.

From the point of view of organic evolution as a whole, the present instant in the history of life on Earth, as Sir Julian Huxley has so aptly stated in many publications, is one in which for the first time a single species, man, has gained, at least potentially, complete dominance over all other species of living things. Our experiences with species such as the buffalo and passenger pigeon, and with the control of pests such as the malarial mosquito and the organisms causing cholera and smallpox, tell us that if men should desire this urgently enough, and should devote as much time, thought, energy and money to destroying any other species of living organism as they are devoting to means for destroying each other, they could render such species extinct or negligible in importance in a relatively short time.

Furthermore, man is using his reign of dominance to produce the most far-reaching, sudden and drastic upset of natural communities which the world has ever seen. During the past ten thousand years, which represents a split-second of evolutionary time, man has changed at least 80 per cent of the world's biotic communities in one way or another, and the great bulk of the land surface in temperate regions and in the drier parts of the tropics has been altered beyond recognition. The knowledge of evolution which biologists have acquired during the past quarter of a century tells us that evolutionary change is closely correlated with habitat disturbance. Evolution means the extinction of some species, accompanied by modification and spread of others. Many of the recently extinct species, such as the dodo, passenger pigeon, and heath hen, are familiar to all. In other instances nearly extinct species, such as the plains antelope and the sea otter, have shown an astonishing and encouraging ability to regain lost ground. The species which have become modified and more widely spread are the cultivated plants and domestic animals which are evolving under the direct guidance of man and the weeds and pests which have filled the new niches created by the human revolution. I use this latter term advisedly, to express an upset in the Earth's stability which is of far greater consequence to living organisms than were the geological 'revolutions' of the past, such as the Laramide and the Appalachian revolution. The complex course which the evolution of weeds and cultivated plants has taken is well expressed in the analyses by Heiser and Anderson of the post-human evolution of the common sunflower, those by Kihara, Sears and others on the evolution of wheat and its weedy relatives, and by Mangelsdorf and his students on the evolution of maize and teosinte.

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* Substance of an address delivered at Corvallis, Oregon, on August 30, under the auspices of the American Institute of Biological Sciences.

Even if we wanted to, we could not resist or reverse these changes. Men have, however, shown that they can guide the evolution of communities of organisms in such a way that the result is both satisfying to our æsthetic sense and provides a stable source of natural resources. The restful mosaic of carefully tilled fields, enriched pasture lands, and conserved woodlands which characterizes the landscape of England, many parts of northern Europe, and parts of the middle Atlantic States in America, form the best-known example of this stabilized transformation. On the other side of the Earth, the brilliant green carefully ordered rice paddies of Japan, which alternate with regularly planted and carefully harvested *Cryptomeria* forests on the steeper hills, provide an equally impressive example. We know, therefore, that given a favourable climate, an industrious people, and a deeply ingrained sense of beauty, mankind can transform Nature into a source of æsthetic satisfaction and undiminished natural resources. What we must find out, however, is how to produce similar transformations in regions where natural conditions are less favourable to them, and where traditions of pioneer advancement, with its accompanying exploitation, greed and wastefulness, have weighed against the establishment of stable communities of organisms, or where a tired land, exhausted from centuries of thoughtless exploitation, will respond only to the most ingenious and thorough plans for restoration and conservation. Such constructive transformations have been begun in many regions, particularly in those like the American south-west, where large-scale irrigation projects have greatly raised production in previously unproductive lands. Nevertheless, reconstruction of biological resources, and the development on a world-wide scale of biotic communities which will have a maximum stability and usefulness to mankind—this is a task which has been barely begun, and which will require for its successful completion all the resourcefulness, organizing ability, and knowledge of living organisms which we can acquire.

The role of biologists in such a constructive enterprise is clear. We must supply the basic information needed by the architects and planners of these new and more productive, man-directed communities of organisms. Just as the urban architect, who designs a new civic centre or surburban housing project, cannot proceed without a thorough knowledge of the terrain on which the buildings will be constructed, the tensile strength and other properties of the building materials at his disposal, the major directions of traffic flow through the area, and many other facts, so the conservationists, foresters, agriculturists and other applied scientists who must take the actual responsibility for building these new biotic communities will require a great quantity of facts, now unknown, both about the individual species which must form these communities, and their actual or potential relationships to each other.

At this moment in the Earth's history, therefore, biologists have two equally essential and complementary tasks. One is to probe deeply into the nature of life itself, and to understand as well as possible the properties which living things have in common, and which distinguish them from inanimate matter. The structure and synthesis of the nucleic acids and their role in protein synthesis are basic to this understanding, but many other facts, such as the relationship between structure and activity of proteins, and the properties of living membranes are almost equally important. Our other task is to explore the diversity of life. We must learn about the population structure of individual species, the ways in which species interact with each other in communities, and how both particular species groups and communities have evolved. This latter type of knowledge is equally or more important than the former if we are to re-orient organic evolution in such a way as to make the world a better home for mankind.

For both of these tasks, international co-operation among biologists is necessary. The most suitable form of this co-operation is, however, very different for the two types of research. The nature of life is best studied by teams of research workers in laboratories which are equipped with the most modern apparatus, much of which is highly complex and expensive both to acquire and maintain. Exchange of information between laboratories, both within countries and on an international scale, is best carried out through conferences between workers who have available the carefully checked results of repeated experiments. Consequently, the best form of international co-operation in these fields is the promotion of symposia, conferences between small groups of specialists, and exchange of personnel between laboratories.

On the other hand, for exploring the diversity of life, field studies are as essential as are those in the laboratory. Since similar biotic communities exist in widely separated areas, since many individual species have spread throughout the Earth under the influence of man, and since biotic communities in many parts of the world are simultaneously influenced by changes in world climate, co-ordinated research in those fields is essential. International organizations for promoting biology must become increasingly aware of the diversity of biological research, and of the different kinds of needs which it creates.

International Organization in the Life Sciences

Before saying what various international organizations are doing and plan to do to help biology, I must give a brief account of the different organizations involved, and of their relationships to each other. The best known of the international organizations, the United Nations, has done much indirectly to promote basic biology on an international scale; but its direct functions include only the applications of biology and biological education. The agencies of the U.N. for applied biology are the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the International Atomic Energy Agency (IAEA). In addition, its Educational, Scientific and Cultural Organization (UNESCO) is doing much for biological education, particularly in the less-developed nations. In the basic sciences, the general organization for promoting research on an international scale is the International Council of Scientific Unions (ICSU). It is a federation of fourteen different unions, which between them cover all branches of the natural sciences. The financial support of ICSU and its member unions is derived partly from UNESCO; but both ICSU itself and the member unions are becoming increasingly self-supporting through direct contributions from their member nations. Of the fourteen unions belonging to ICSU, three are of particular interest to biologists: the International Union of Biochemistry, the International Union of Physiology, and the International Union of Biological Sciences (IUBS). In addition, two independent organizations deal with the applications of biology on an international scale: the Council for International Organizations in the Medical Sciences (CIOMS) and the International Union for the Conservation of Nature (IUCN).

International Union of Biological Sciences

The principal organization for promoting the interests of biologists on an international scale is the International Union of Biological Sciences, one of the fourteen unions federated under ICSU. This Union is one of the oldest of the fourteen, having been founded in 1919 with the original League of Nations. Its affiliation with ICSU dates from 1947. Thirty-two nations belong to IUBS, by virtue of making to its treasury an annual contribution, which until this year varied from 46 dollars (200 Swiss francs) for the smaller nations to 2,300 dollars for the United States. At the General Assembly of the Union in Amsterdam in July 1961, these amounts were doubled.

The business of the Union is carried on by an executive committee, which meets annually; by the officers of twenty-two different sections, by commissions, and other permanent bodies, representing various fields of biology, and by a general assembly, which meets every three years. The officers elected at the last General Assembly (Amsterdam, 1961) are as follow: *president*, C. H. Waddington (Great Britain); *vice-president*, P. Chouard (France); *secretary-general*, G. L. Stebbins (United States); *treasurer*, J. Lanjouw (Netherlands); *publications secretary*, R. Ulrich (France); *past-president*, G. Montalenti (Italy). The remaining six members of the executive committee are M. Chèvremont (Belgium); C. G. Hedén (Sweden); A. Kursanov (U.S.S.R.); Sir George Taylor (England); J. Baer (Switzerland); and K. Grell (Germany).

The numerous sections of the Union reflect the diversity of the field of biology. In many instances, as in botany, genetics, cell biology and microbiology, officers of sections of IUBS are closely affiliated with or actually the same people as the officers of permanent committees for international congresses. Sections have been established whenever a group of biologists, usually organized into an international society or federation for the purpose of holding congresses, have desired to become affiliated with IUBS, and their application has been approved by the delegates at a general assembly. The Union may, therefore, be regarded as a federation of international societies which have united to promote their common interests.

The general objective of the International Union of Biological Sciences is to provide an international unifying force in biology such as is provided for the United States by the AIBS. This objective is being carried out in three ways: aid in standardization of the methods and language of biology, helping biologists to organize symposia and congresses, and organizing research projects in basic biology which will contribute to human welfare. The first of these functions is the oldest and most firmly established. The Union has, for many years, given financial support to the Committees of Botanical, Zoological, and Bacteriological Nomenclature. It has supported the maintenance of permanent cultures of *Drosophila* and of various micro-organisms. It has represented biology on the International Abstracting Board, which aims at establishing international standards for abstracts of scientific publications. Other tasks of standardization have been supported from time to time, as needs have arisen.

In recent years the sponsorship of congresses and symposia has been the major function of the Union. The relatively limited funds obtained from membership dues have been generously increased by an annual subvention from the International Council of Scientific Unions, which has been supported in turn by UNESCO. Nevertheless, the Union is able to give only partial support of the meetings which it sponsors; most of its subventions have been 1,500–2,000 dollars. Although no rigid rules exist regarding the use of these funds, the Union prefers to have them spent on travel and subsistence of scientists attending the congresses and symposia.

The interest of IUBS in gatherings of scientists extends beyond sponsorship and financial support of individual meetings. The Union maintains a calendar of biological congresses and symposia for three years in advance, and is in a position to suggest dates for holding meetings which will not conflict with those in related fields. Furthermore, at each of its general assemblies, it has been much concerned with how large international congresses should be conducted, and what useful purpose they may serve. At the last assembly in Amsterdam, for example, secretaries and other officers of international congresses which have recently been held in the fields of botany, genetics and microbiology exchanged experiences with officers who were organizing future congresses in these fields, as well as in zoology, limnology, entomology and biometry. The liveliness of their discussion as well as of those which have preceded in its past general assemblies is ample testimony of the value of such exchanges of experiences and opinions between biologists who have similar tasks to perform for aiding different segments of the life sciences.

International Biological Programme

Direct participation in organizing biological research on an international scale is the newest activity of IUBS, and is still in an embryonic stage. Its principal feature is participation by IUBS in an effort organized by ICSU for which formal plans were begun last May, and is entitled the International Biological Programme. The need for some programme of biological research similar to the International Geophysical Year has been felt by biologists on both sides of the Atlantic for some time, and various informal discussions have been held on the subject. In July 1960, Drs. Hiden Cox and John Olive of AIBS met with interested biologists in the United States and, as a result of their discussions, a television programme on the subject was held in Washington, D.C., in May 1961. A warm response to this programme indicated that a well-conceived and practical plan for international research in biology would arouse considerable interest in the United States. In the meanwhile, the problem was discussed by the Executive Committee of IUBS at its annual meetings in 1959 and 1960, and by the Executive Board of ICSU in the spring and fall of 1960. The consensus of these discussions was that a research programme which would embrace all biology would be far too vast, amorphous, and unwieldy to be of practical execution. Before definite plans could be made, the leaders of the programme would have to decide how it could be limited so that it would be practical and at the same time broad enough so that it would fulfil a real need commensurate with the expense involved, and would capture the imagination so as to enlist the support of the non-biological public, particularly heads of Governments and directors of fund-granting agencies. After much deliberation, the following basic rules were established, which any project would have to fulfil before it could be considered acceptable as part of an international programme of biological research: (1) Is the research contemplated of a basic biological nature, or is it of an applied type which is best carried out under the auspices of FAO, WHO, IUCN or some similar agency ? (2) Is it truly relevant to human welfare ? (3) Is it something which needs to be done now ? (4) Does it require co-operation between biologists on an international scale for the actual research itself ?

These thoughts were uppermost in the minds of the officers of IUBS when making plans for the general assembly which was held in Amsterdam in July 1961. One day of this assembly was devoted to addresses on this subject. Prof. G. Montalenti, who was then president of the Union, discussed projects in human genetics and gene ecology. The vice-president, Prof. P. Chouard, discussed the possibilities for international co-operation opened up by the building of large-scale phytotrons for growing plants under carefully controlled conditions, like those at Pasadena and Gif-sur-Yvett, France. Dr. Paul Weiss discussed possibilities for international co-operation in research in experimental biology. Dr. C. G. Heden presented a broad discussion of the potential role of microbiological research in contributing toward an increase in production of biological communities. Prof. A. J. Hasler of Wisconsin spoke on opportunities in fresh-water biology. The symposium ended with a round-table discussion in which the participants were C. H. Waddington (Great Britain), A. Kursanov (U.S.S.R.), M. Siniscalco (Italy), and H. Roderick (United States), who spoke for the Division of Natural Sciences of UNESCO. On the following day, the facts and opinions presented in the symposium were discussed by the delegates to the assembly. As a result of its discussion, the assembly passed a resolution recommending that the whole problem of an International Biological Programme be carefully considered by a special committee appointed for this purpose, and suggesting that research could be centred around the following topics:

Investigation of biological communities menaced by transformation or destruction.

Investigation of the consequences of the rapidly changing pressure exerted by the environment on the genetic constitution of mankind, and on the dynamics of populations.

Encouragement given to research directed toward a better understanding of various means of modifying the global equilibrium and balance sheet of organic materials and resources, so as to improve the balance sheet by increasing production and reducing losses.

In September 1961 the General Assembly of its parent organization, ICSU, reviewed the IUBS resolution. In agreement with it, it authorized Dr. Montalenti to convene a planning committee, which would include representatives of the International Unions of Biochemistry, Biological Sciences, Geography and Physiology, as well as all other international organizations interested. This committee met for the first time at Morges, Switzerland, in May. It decided first that although biologists might gain inspiration and a knowledge of procedural techniques from the International Geophysical Year, the needs of biology are so different from those of geophysical sciences that a single International Biological Year would be neither practical nor desirable. On the other hand, the need for continuing International Biological Programme of co-ordinated research became increasingly evident as the discussion of the committee progressed.

Consequently, the committee decided on an overall title: "The Biological Basis of Productivity and Human Welfare". Under this title it established six sub-divisions for each of which a special planning sub-committee was set up.

The first of these sub-committees is making plans for a world-wide survey of natural terrestrial communities. Its chairman, Prof. H. Ellenberg (Zurich), is in contact with ecologists throughout the world, many of whom have expressed a keen desire to take part in the programme. The foundations for this survey have already been laid by the Commission on Ecology of the International Union for the Conservation of Nature, at a symposium held in Zurich in May 1961. Plans were made there for a comparison of the natural productivity and the energy systems of forests in climatically and floristically diverse regions of the north temperate zone. Natural, essentially undisturbed communities will be examined first, to serve as a standard of comparison. Uniform methods of research have already been outlined, and members of the sub-committee are busy assembling literature on studies which have already been made of these communities. Some of the specific objectives of this research are the creation of an international classification of habitat types; correlation of programmes for mapping vegetation throughout the world; a world-wide census of large wild mammals, with notes on their past numbers and future prospects; and a particular study of the ecological relationships and present condition of those species of plants and animals which are threatened with extinction or are becoming rare.

Plans are under way to extend the survey of communities from the more familiar ones of temperate regions to the vastly more complex and poorly known communities of the tropics. Surveys of communities which show various degrees of alteration by man, both uncontrolled degradation through man's thoughtless capacity for destruction and controlled modification dictated by the needs of farming and grazing, will also be undertaken. Plans for such surveys will require close co-operation between field biologists and practical agriculturists for both their formulation and execution. Consequently, they are proceeding more slowly, and I cannot predict at this time how soon co-ordinated research of this sort can be profitably undertaken.

A second sub-committee is organizing research on the metabolism of natural communities. As stated by its chairman, Prof. M. Florkin, this sub-committee aims at extending our knowledge of such basic processes as photosynthesis and nitrogen metabolism as they occur in cell-free systems and in controlled cultures of organisms. It hopes to develop an understanding of the dynamics and results of these same processes as they occur in those communities of organisms on which we depend for our subsistence. Some of the problems to be attacked by the research which this sub-committee will organize are the following:

In what types of communities—natural, semi-natural, pastoral, or agricultural—is the conversion by photosynthesis of light-energy to useful organic products the most efficient ? What controllable and uncontrollable factors modify this efficiency ? What organisms, both plant and animal, are most important in maintaining a proper balance of available nitrogen compounds in various types of communities under diverse conditions ? What agricultural practices are beneficial to and what practices are particularly harmful to these organisms ? How does the photosynthetic efficiency of natural and agricultural communities compare with that which might be expected from strictly artificial cultures of algæ growing in tanks ?

In this field, as in the general survey of communities, one of the most important tasks of the research units will obviously be to bring together the large store of existing information, and to build on this by concentrating on the major gaps in our knowledge.

A third sub-committee, under the direction of E. M. Nicholson (Nature Conservancy, London), is concerned with conservation and productivity of communities. It is directing its attention to the following problems:

What natural communities most urgently need preservation and what is the scientific basis of this need ? How can the productivity of communities be measured in such a way that it will truly reflect the value of these communities to mankind, and will show what measures should be taken to increase productivity ? Are existing schemes for synthesizing new communities of animals and plants biologically sound ? In particular: What is the practicability, from the biological point of view, of building up pastoral communities of animals and plants in which game animals, either native or introduced, will be managed for food in place of or in company with domestic cattle and sheep ?

Studies of this nature have been begun in various parts of Africa, as reviewed by Sir Julian Huxley in a recent article in *Endeavour*. In the United States the use of African antelopes as an additional source of food is being studied by research workers on the King Ranch in Texas. Much of the work of this sub-committee will follow up leads developed by the two sub-committees already mentioned. These three sub-committees, which are planning research on terrestrial communities, form a natural unit, and will regularly exchange information.

Research on the productivity of communities cannot be confined to land areas; productivity of fresh water and marine communities must also be studied. Since specialists on these types of communities were not present at the meeting of the Planning Committee at Morges, sub-committees for research in these fields have been slower in organizing their plans than have the other four. Nevertheless, the sub-committee on fresh-water communities, under the direction of Prof. W. Rodhe, was able to meet in Madison, Wisconsin, in connexion with the fifteenth International Congress of Limnology. They have sent me a report of their meeting, the most important parts of which are the following.

The programme should be orientated towards increasing knowledge of a kind which reveals fundamental reasons for biological productivity and which at the same time points the way to obtaining more food (by increasing productivity) or more clean water (by reducing productivity and pollution). In order to provide a yardstick against which productivity and energy turn-over can be measured, it is necessary to conserve and maintain for permanent study examples of the different types of freshwater ecosystems distributed through the climatic and biological zones. This activity will be closely related to others already described for the study of land ecosystems.

The productivity of freshwaters can be divided into: (*a*) primary production of organic matter through the conversion of energy, usually light; (*b*) secondary production through the turn-over of grazers, usually zooplankters but sometimes fish; (*c*) terminal production, that is usually of animal species which offer an exploitable source of food. These three aspects will need elaboration in a programme which is based on the different climatic zones, using where possible established centres of research. There should perhaps be two or three main centres of work selected in the arctic zone, four or five in the northern temperate, three or four in the tropics, perhaps two in the southern temperate and probably not more than one in the antarctic. Since the distribution of centres is not uniform in relation to the distribution of waters in these various zones, it may be necessary to fill in certain gaps by the organization of expeditions.

Among particular items for inclusion in the programmes would be methods of testing primary production, including comparison between the behaviour and metabolic rates of young and old cells both in unicellular and multicellular organisms, the effect of bacteria and of pollution on the energy flow through aquatic ecosystems, especially in the return of nutrients to circulation, the interchange of energy between aquatic and land systems. In the last-mentioned there are included, for example, fæces deposited in water by hippopotami, food derived from water by land animals and birds, and the emergence of aquatic insects. An over-riding fact is that much more is known at present about productivity and energy exchange in standing water than in rivers, so that special investigations must be arranged for rivers, including their impoundments, for such purposes as hydropower and water supply.

Research on marine communities will probably be the hardest of all to organize on an international scale. At Morges, Dr. S. J. Holt of the Fisheries Division of FAO warned the members of the Planning Committee of the difficulties involved. Ocean-going ships are far larger and more costly than any equipment needed for studying terrestrial communities. Although some biological research can be done in connexion with large-scale oceanographic projects, such as the International Indian Ocean Expedition, the needs of marine biologists are so different from those of physical oceanographers that adequate estimates of the biological productivity of the sea and the factors which influence it can be made only by expeditions organized especially for biological research. At the present time, however, ships for international expeditions of this sort will be both costly and difficult to obtain.

Nevertheless, the biological productivity of marine communities is, perhaps, the field in which research co-ordinated on an international scale can potentially accomplish more in comparison to the efforts of individual institutions than can any other form of international biological research. The oceans are truly international, and the fishes which inhabit them can migrate freely from the fishing grounds of one nation to those of another. Furthermore, oceanic communities of organisms are less influenced by local climatic differences than are terrestrial and fresh-water communities. Consequently, co-ordinated data taken in various parts of the Earth at the same time by different workers using the same methods should have a particularly high value in interpreting the productivity of such communities.

For these reasons, the sub-committee on marine communities will have a particularly difficult and critical task in formulating their plans. As yet, this sub-committee has not been fully organized.

A programme on the Biological Basis of Productivity and Human Welfare would be incomplete without co-ordinated research on the human populations which might benefit from increased production. Consequently, the final one of the six sub-committees established at Morges is planning research on the biological basis of human adaptability, both physiological and genetical. Dr. J. S. Weiner (Oxford), the chairman of this sub-committee, is familiar with numerous research projects now being conducted in this field in various parts of the Earth, and has already made contact with their leaders. Efforts will be made to co-ordinate on a world-wide scale data on human growth at various ages, skeletal and dental development; physique, body composition and skin colour. Similar correlations will be sought of data on diurnal rhythms, as well as tolerance of cold, heat and high altitude.

Co-ordinated data will be obtained on such genetic characteristics as blood groups, characteristics of urine and saliva, and the distribution of genes for hereditary diseases. From such data geneticists could make more accurate estimates of the intensity of natural selection in human populations of spontaneous mutation-rates, and of the effects of radiation on human genes.

In enumerating the various types of research which we hope to co-ordinate and stimulate during the International Biological Programme, I realize that much has been left out. This does not mean that the Committee considers these other topics unworthy of its attention. Either we are unaware of their importance or have not yet learned of practicable, worth-while and well-defined projects which can be carried out in areas other than those so far mentioned. At this early stage of planning, all the chairmen of the sub-committees, as well as Prof. Montalenti and myself, are much interested in learning of additional projects related to our general theme of biological productivity and human welfare. Any of you who have such projects in mind are urged to tell or write us about them.

The calendar of progress for the International Biological Programme has not yet been firmly fixed. The six sub-committees are expected to complete their plans early in 1963, after which they will be carefully reviewed by both the Planning Committee as a whole, and by other interested biologists. A minimum of two years will be required to secure funds, establish teams of research workers, and find places for them to work. Consequently, a reasonable estimate of the actual time during which the major research effort will be carried out is a three- to five-year period beginning in 1965. This estimate may, however, be modified as plans become more definite.

Training and Education in Field Biology

A fourth activity which now needs to be developed on an international scale is education and training in biology. Two goals must be kept in mind. One is preparing a larger number of able young men and women for careers in biology and applied fields related to it, and the other is educating the public of all countries to the great contributions which biological knowledge is making to their welfare.

One way in which professional training in biology may be stimulated is through the International Biological Programme. If enough financial support for this programme can be obtained to activate all of the research projects contemplated, the next bottleneck will be personnel. Consequently, the Public Relations and Training Sub-committee for the Programme, of which I am chairman, hopes to establish a number of fellowships, both pre-doctoral and post-doctoral, by means of which young biologists with adequate background and superior ability can be given an opportunity to do research of the types visualized for the Programme, and in a laboratory or field unit directly associated with it. We are already formulating our programme for these fellowships, and expect to request financial support for them at the same time that support is requested for the research projects themselves. Such fellowships should both increase the availability of manpower for the IBP itself and provide a firmer basis for continuing into the future the types of research begun or co-ordinated under the IBP. Furthermore, they should increase the opportunities for young biologists in the less-developed countries to obtain postgraduate training and higher degrees in fields of biology closely related to the needs of their countries.

Dr. V. Kovda, director of the Division of Natural Sciences of UNESCO, has expressed great interest in this proposal, and his group will co-operate closely with our committee in working out the fellowship programme. Education in biology at the undergraduate university- and school-level is being effectively stimulated and aided by UNESCO, so that we of the IBP and IUBS do not feel the need of independent efforts in this direction. We are, however, sending representatives to symposia on education in science which UNESCO is organizing from time to time in various parts of the world, and have agreed to supply trained biologists for their working committees when such experience is needed.

The Planning Committee for IBP, and particularly its sub-committee on Public Relations and Training, recognize fully the possibilities which our programme will offer for making the general public more completely aware of the valuable contributions which research in biology makes to their daily lives. We expect to exploit these possibilities as much as possible. We hope that when research teams enter a new area, or when working units of biological laboratories become associated with the International Biological Programme, we can make statements in the public Press as well as organized radio and television programmes to inform the public of the presence and objectives of these groups. As results of the research become well enough developed to warrant it, we hope to organize both technical and semi-popular symposia for discussing them, and to hold these symposia in localities as close as possible to the places where the bulk of the research has been done. At the same time, public lectures on particular phases of the work by biologists having a world-wide reputation would aid both the IBP and the recognition of biology on the part of the general public. More definite plans for this phase of the programme cannot be made until its research phase is more fully planned, but in the long run this type of activity may become one of the most valuable contributions of the programme as a whole.

Standardizing the language of biologists; increasing the opportunities for direct contacts between biologists of different countries, co-ordinated biological research on an international scale, and world-wide stimulation of training and education in biology; these objectives are all broad, timely and worthwhile. Though its means are modest, the International Union of Biological Sciences is doing what it can to fulfil these needs. We are most anxious to welcome into our organization groups of biologists who feel that at this critical period of the Earth's history such objectives are worthy of their attention.

CHEMISTRY AND MENTAL DISEASE

A JOINT symposium organized by the Royal Institute of Chemistry, the Chemical Society and the Society of Chemical Industry was held on September 26 in the University of Manchester. The purpose of the symposium was to review and discuss the more recent advances, as they affect the chemist, that have been made in the treatment, and in understanding the cause, of mental disease.

During the morning session the chairman was Dr. F. L. Rose (Imperial Chemical Industries, Ltd., Pharmaceuticals Division). He welcomed those present, and proceeded to contrast the half-day devoted to the topic during a similar symposium in 1957 with the present symposium occupying a whole day. This was an indication of the increased tempo of research and of the increasing interest in this particular field of medicine. With the flood of novel chemotherapeutic agents and new biochemical information had come a battery of new tests and indeed almost a new language. While listening to the accounts of the recent developments in the treatment of mental disease, we should not forget the debt owed to earlier workers, nor should we omit to recognize how many of the more recent discoveries were based on earlier drugs and observation of their side-effects.

The opening address was delivered by Dr. A. Todrick (Crichton Royal, Dumfries) who dealt with biochemical processes in relation to mental disease. Since the audience was largely composed of chemists he began his talk by describing a classification of mental disease in which a distinction was drawn between psychosis and neurosis, and between mental deficiency involving an anatomical or biochemical defect present at birth which prevents normal development, and mental illness which is understood to imply a disturbance in previously normal individuals. In recent years a greatly increased research effort, much of the credit for which goes to the pharmaceutical firms, has produced many valuable drugs. On the biochemical side the research effort has been aimed at discovering a biochemical lesion or a biochemical consequence of the lesion which might assist in diagnosis or in control of treatment. A great deal of work has been done on schizophrenia but much less on the manic, depressive psychosis, probably because the latter disease is more capable of being effectively treated. Many hypotheses as to the cause of schizophrenia have been proposed, but most of them involving enzyme or endocrine imbalance, or defects of the liver, central nervous, homeostatic or vascular systems, it was never clear whether the abnormality was a cause of, or a result of, schizophrenia. The body fluids of schizophrenic patients have been subjected to intensive examination both before and after drug administration. Many of the earlier published results were suspect because all too frequently the investigators concerned paid insufficient attention to the effects of drugs being administered at the time the experiments were being performed. The endogenous toxin explanation for the cause of schizophrenia has been popular for a long time, and results both in support of and against the hypothesis have been forthcoming from different laboratories.

Recently, highly significant findings have been obtained by Roman and Malcolm in Aberdeen, who have shown that the induced potentials produced in rat brain by the application of schizophrenic plasmas are clearly distinguishable from those produced by normal plasmas. The next step in the investigation would be to attempt the isolation of the compound responsible for the effect. Based on the views of Brodie that noradrenaline is the hormone responsible for the ergotrophic or activating system in the central nervous system, and that 5-hydroxytryptamine is the hormone responsible for the trophotropic or inhibitory system, the levels of these two agents and their metabolic products in the blood and urine of both normal and schizophrenic patients have been frequently measured and compared. The study of metabolites has been made easier latterly by increased knowledge on the metabolic pathways for the two hormones. Recent work with radioactive serotonin and also estimations of the urinary level of 3-methoxy-4-hydroxymandelic acid (a metabolite of noradrenaline), has indicated that there is no significant difference between the way and extent to which these two hormones are metabolized by schizophrenic and normal human subjects. The biochemical lesion present in schizophrenia, if indeed such a lesion exists, remains to be identified. Clinical studies with drugs which produce changes in behaviour are also shedding much new light on the problem of mental illness, but the biochemical processes initiated by these agents are only beginning to be understood.

Dr. L. I. Woolf (Department of the Regius Professor of Medicine, Radcliffe Infirmary, Oxford), then spoke about inborn metabolic errors and mental disease, with special reference to the genetic metabolic disorder, phenylketonuria. He was primarily concerned with mental deficiency, which was usually sharply distinguished, particularly by psychologists, from mental illness. The difference was perhaps not so great as sometimes supposed, being at least partly related to the age at which the disability becomes apparent. In the mentally deficient the damage to the brain usually commences shortly after birth, and normal intelligence has no time to develop, whereas in the psychotic individual normal intelligence is evident until late childhood or adult life, and is then followed by progressive decline and increasing dementia.

Phenylketonurics are mentally defective, usually having an intelligence quotient of less than 25, and they are often markedly disturbed. The disease, which become detectable at six months of age and which is pronounced at two years of age, is due to the absence of the pair of genes responsible for synthesizing the enzyme, phenylalanine hydroxylase. Absence of this enzyme results in an accumulation of dietary phenylalanine due to non-conversion to tyrosine. Some of the phenylalanine, which is not readily excreted, is converted to abnormal metabolites which are known to inhibit several decarboxylase enzymes that are important to the normal functioning of the brain. There is also evidence of the failure to lay down myelin in the brain nerve fibres of phenylketonurics, and/or the destruction of myelin already formed. Treatment consists of a diet free from phenylalanine, which to be effective must be initiated at an early stage in the development of the disease before extensive irreversible damage has occurred. This can now be assured by routine examination of the urine of all new-born babies for the presence of phenyl pyruvic acid. Analytical control is essential and the concentration of phenylalanine in the blood must be measured and adjusted to retain the minimum required for maintenance of life. Other metabolic errors affecting the mind which are due to the congenital absence of essential enzymes are cretinism caused by an absence of thyroid hormone, maple syrup urine disease (leucinosis) caused by incomplete amino-acid metabolism, and galactosaemia caused by inability to convert galactose to glycogen.

A paper entitled "The Pharmacological Assessment of Psychotropic Activity", presented by Dr. M. W. Parkes (Roche Products, Ltd.), completed the morning's programme. The relative ease of devising an animal test for bacterial and parasite infections, or for organic diseases such as hypertension, in which the animal test bore some relevance to the disease state in man, was contrasted with the difficulties of devising an animal test that in any way resembled human mental illness. It was the development of a higher mental life that distinguished man from the comparatively elementary mental state of all other animals. Yet it is the higher functions of the brain that are affected most seriously by mental illness. Nevertheless, psychiatric conditions such as anxiety, aggressiveness, hostility, rage and fear have been set up in animals (following the lines of Pavlov's classical experiments) by the alternate application of reward and punishment. Other techniques involve the electrical stimulation of the brain by electrodes implanted in the hypothalamus, or by exposure to fearful experiences.

The effects of drugs on these conditions have then been investigated. An entirely empirical approach has been to screen new compounds and to select those showing similar biochemical or pharmacological properties to those of clinically useful drugs even though there may be no apparent connexion between these properties and a property relevant for psychotherapeutic action. Measurement of the locomotor activity of small rodents has been extensively utilized and a wide variety of methods has been developed for recording and assaying the results. Biochemical techniques have also been invoked, as, for example, the development of a test for antidepressant drugs based on the inhibition *in vitro* and *in vivo* of the enzyme monoamine-oxidase. The antagonism between stimulant and sedative drugs has formed the basis of a number of screening procedures, and is almost the only way of selecting drugs likely to be antidepressants of the imipramine and amitryptyline class. The use of hallucinogenic agents or psychomimetics to produce an artificial psychosis has been utilized in human volunteers, but it is of doubtful application in animals because of the difficulty of measuring the effects produced. Furthermore, in both man and animals it is questionable whether the psychosis produced is in any way related to schizophrenia. All means of looking for psychotropic activity are undoubtedly empirical to a greater or lesser extent, even where a basis of relevance is apparent. Tranquillizing or antipsychotic properties appear to be best detected by tests depending on the emotional components of animal behaviour.

The chair was occupied during the afternoon session by Prof. R. N. Haszeldine (Manchester College of Science and Technology). The first speaker, Dr. A. F. Crowther (Imperial Chemical Industries, Ltd., Pharmaceuticals Division) dealt with structure activity relationships in the tranquillizer group of drugs. Tranquillizers were divided into two types, designated 'major' and 'minor'. Since the milder-acting minor tranquillizers, which are used in the treatment of neuroses, are the most difficult to evaluate clinically—the placebo effect being most marked in their case—structure-activity relationships based on clinical trials are less meaningful than those obtained with the major tranquillizers. The latter, which are used in the treatment of psychotic states can be classified into two main groups depending on whether they are based on the phenothiazine molecule or the *Rauwolfia* alkaloids. The phenothiazines, which comprise a large compact group, were developed from the early antihistamine drug promethazine. Most of the variations of the original structure which have resulted in clinically useful drugs consist of changes in the length. the branching and the terminal groups of the so-called 'basic side-chain' attached to the nitrogen atom of the phenothiazine nucleus, and also in the nature of the substituent in the two-position of the nucleus. Replacement of the nuclear nitrogen and/or sulphur atoms by carbon atoms has given the three other valuable drugs chlorprothixene, imipramine and amitryptyline. The two last-named are not, however, used as tranquillizers, although pharmacologically they have chlorpromazine-like properties, but as antidepressants. Norchlorpromazine and chlorpromazine *N*-oxide, two metabolites of chlorpromazine, resemble the parent agent in activity in animal behavioural tests. In the *Rauwolfia* alkaloid series the best-known compound is reserpine, which has both sedative and hypotensive properties. Many attempts have been made to dissociate these two actions, and the activity of derivatives of reserpine without the 11-methoxy group, with variation in the 16-ester group and 18-acyloxy group, were discussed by Dr. Crowther. The drugs tetrabenazine and benzquinamide were somewhat related in structure to reserpine. The former was claimed as being superior to reserpine and chlorpromazine in the treatment of psychoses. A series of piperidinobutyrophenones, of which halopiperidol is a member, falls into this category of major tranquillizers. Of the many minor tranquillizers, some like methaminodiazepoxide have unusual chemical structures, but the majority are glycols, and the related carbamoyl derivatives, amino alcohols and amides.

Psychostimulants, which for this paper at least were defined as those drugs used in the treatment of depression, were the subject of the next paper, presented by Dr. E. H. P. Young (Imperial Chemical Industries, Ltd., Pharmaceuticals Division). The structures of some of the earlier phenylethylamine derivatives and the way in which this molecule may be visualized as a component of more recent drugs such as methylphenidate and pipradrol were illustrated. The earlier drugs have now been almost completely superseded by compounds characterized by their ability to inhibit the enzyme monoamine oxidase, and by a group of drugs related to imipramine. The first and most important members of the former class were derivatives of hydrazine, of which some hundreds have been prepared, and which have readily lent themselves to work on structure-activity relationships Other differently constituted monoamine enzyme inhibitors were certain harmine derivatives, phenyl*cyclo*propylamines, α-alkyltryptamines and benzylamines. The most potent and clinically useful of the antidepressant drugs were the compounds imipramine and amitryptyline, which as indicated earlier were derived from phenothiazine. Their metabolites, the desmethyl compounds, have been found to be more rapidly effective than the parent drugs. Among other chemical types which have produced antidepressant properties are certain esters of 3-piperidol, but side-effects have limited their clinical usefulness.

The final paper of the symposium was given by Dr. F. A. Jenner (Department of Psychiatry, University of Sheffield). In it he described some of the problems arising from the use of new modern drugs in psychiatric practice. The new drugs presented a great challenge to the clinician if only because of the rate at which they appeared. The need for sophisticated methods of assay, backed by precise statistical assessment, was essential to overcome the placebo response and other extraneous factors such as the environment of the patient and the doctor's own outlook. Many psychiatric patients are talking about their own private world, and it is the task of the psychiatrist to express this personal experience in psychoanalytical language. However, the use of this specialized language itself imposes its own limitations. The diagnostic terms of psychiatry are words not things, and they cannot be reduced to understandable chemical terms. Nevertheless, it cannot be doubted that the chemical architecture of the brain is relevant to psychology, and most interesting is the finding that the metabolism of monoamines, which are localized in specific areas of this organ, is influenced by drugs of known value in psychiatry. Most of the drugs commonly used treat symptoms and not diseases, but yet they can be shown to be effective in 'double-blind' controlled trials. The phenothiazines have been of great value in the management of schizophrenics, while imipramine, its metabolite and monoamineoxidase inhibitors are established as anti-depressive agents. Anxiety is often alleviated by chlordiazepoxide or diazepam.

The value of drugs in the treatment of neurosis is often more difficult to assess. They may give patients sufficient support to recover spontaneously, since they can certainly induce increased tranquillity, but too often they are used by doctors as an alternative to listening to the patient's own story. Psychotherapy might often produce the same or better results than drugs. The psychomimetic drugs are important tools for psychopharmacology but are doubtful additions to the drug cupboard of hospital wards. The most reliable group of psychotropic drugs are the hypnosedatives, of which the barbiturates are best known; but the great contribution of the major tranquillizers or neuroleptics has been their ability to produce tranquillity without sleep. The major tranquillizers frequently produce extra-pyramidal side-effects which can, however, be treated with effective anti-Parkinsonian drugs. The tranquillo-sedatives such as meprobamate, chlordiazepoxide and diazepam tend to be muscle relaxants and are of particular value in allaying anxiety. For the treatment of depression imipramine, amitryptyline and their metabolites are recommended. The monoamine oxidase inhibitors are also effective, but are often toxic. All these new drugs have made the wards quieter, but their overall effects on patients' ability to return to a normal life are more difficult to assess. Psychopharmacology is here to stay, and what has been so far achieved is probably only a prelude to more effective treatment for the mentally disturbed.

The interest shown by the audience present at this symposium was demonstrated by the number of questions put to the speakers at the end of the morning and afternoon sessions. E. H. P. YOUNG

NEWS and VIEWS

The Royal Society: Medal Awards

THE following medal awards of the Royal Society have recently been announced:

Copley Medal to Sir Cyril Hinshelwood, Dr. Lee's professor of chemistry in the University of Oxford, for his distinguished researches in the field of chemical kinetics, including the study of biological reaction mechanisms, and for his outstanding contributions to natural philosophy.

Rumford Medal to Prof. D. M. Newitt, emeritus professor of chemical engineering in the University of London, for his distinguished contributions to chemical engineering.

Davy Medal to Prof. H. J. Emeléus, professor of inorganic chemistry in the University of Cambridge, for his distinguished researches in inorganic chemistry and the discovery and examination of a wide range of new compounds.

Darwin Medal to Prof. G. G. Simpson, Alexander Agassiz professor of vertebrate palæontology, Museum of Comparative Zoology, Harvard University, for his distinguished contributions to general evolutionary theory, based on a profound study of palæontology, particularly of vertebrates.

Buchanan Medal to Sir Landsborough Thomson, formerly second secretary of the Medical Research

Council, for his long and distinguished services to the support and administration of medical and biological research.

Hughes Medal to Prof. B. Bleaney, Dr. Lee's professor of experimental philosophy and head of the Clarendon Laboratory, Oxford, for his distinguished studies of electrical and magnetic phenomena and their correlation with atomic and molecular properties.

Instrument and Meter Department of A.E.I. Instrumentation Division: D. R. Fenwick

MR. D. R. FENWICK has been appointed chief engineer of the Instrument and Meter Department of Associated Electrical Industries Instrumentation Division. Mr. Fenwick received his technical education at Sunderland Technical College and Manchester College of Technology, and after preliminary training at Messrs. Richardsons-Westgarth and Co., Ltd., Hartlepool, joined Metropolitan-Vickers Electrical Co., Ltd., as a school apprentice in 1937. After the War he became an engineer in the Company's Instrument and Meter Engineering Department, being particularly associated with the development of automatic voltage regulation schemes, many of which are associated with some of the world's largest hydroelectric and thermal generating plant. In 1956 he was appointed section leader of the Department's Automatic Control and Regulation Section, and after the formation of the A.E.I. Instrumentation Division became assistant chief engineer (control systems) in the Instrument and Meter Department. He serves on the Measurement and Control Section Committee of the North-west Centre of the Institution of Electrical Engineers.

Biochemistry at Hull: Prof. E. A. Dawes

DR. E. A. DAWES, of the University of Glasgow, has been appointed to the newly created Reckitt chair of biochemistry at Hull as from July 1, 1963. Dr. Dawes, who is thirty-seven, was born in Goole in Yorkshire and attended Goole Grammar School. He took his first degree in chemistry at the University of Leeds in 1946. He then carried out research in the Biochemistry Department under Prof. F. C. Happold before being appointed assistant lecturer in biochemistry in 1947. He was awarded his Ph.D. in 1948 and was promoted to a lectureship in 1950. In 1951 he left Leeds to take up a lectureship in biochemistry in the University of Glasgow and in 1961 was promoted to a senior lectureship; in the same year he was awarded a D.Sc. by the University of Leeds. During October–November 1959, Dr. Dawes was visiting lecturer to the Memorial University of Newfoundland and to Dalhousie University, Halifax, Nova Scotia, and during August–September 1960 he conducted courses in microbiological biochemistry at the University of Brazil at Rio de Janeiro. Recently he has participated in and served as chairman of a symposium on "Endogenous Metabolism of Bacteria", sponsored by the New York Academy of Sciences held in New York City. Dr. Dawes's research work has been mainly concerned with enzymology and with various aspects of the biochemistry of micro-organisms with particular reference to their metabolism. His well-known book, *Quantitative Problems in Biochemistry*, has now been translated into Spanish and Japanese; a revised second edition is in the press. Since April 1958 Dr. Dawes has been an editor of the *Biochemical Journal*.

Botany at Osmania University: Prof. M. R. Suxena

AFTER the death of Prof. M. Sayeeduddin, the chair of botany at the Osmania University, Hyderabad-Deccan, remained vacant for a couple of years and it is only now that it has been filled by the appointment of Dr. M. R. Suxena. Born at Hyderabad on January 18, 1914, Suxena took his M.Sc. degree from the Osmania University in 1937, gaining a first class and a gold medal. His first research experience was with Dr. N. L. Bor (now at the Royal Botanic Gardens, Kew), under whose guidance he studied the grasses of Andhra Pradesh. Then he directed his attention to the anatomy of some members of the Asclepiadaceae, Compositae, and other families found in Hyderabad. Later he went to London, where he came under the influence of Prof. F. C. O. Jane and Prof. W. H Pearsall, and obtained a Ph D. of the University of London in 1950. This gave him considerable experience of the ecology and taxonomy of algae, and on his return to India he set up a school of research at Hyderabad, in which the Desmidiaceae, Euglenineae and Cryptophyceae have been the principal objects of investigations. At present he is engaged in writing a monograph on the Bacillariophyceae of India and guiding research on various aspects of algology. Dr. Suxena is a man of wide interests and his warm and generous nature will attract many students and research workers to Hyderabad, where he has already served for several years, first as a lecturer and then as a reader.

University and College Entrance in the United Kingdom

THE fourth edition of *University and College Entrance: The Basic Facts*, to which Sir Ronald Gould contributes a brief introduction, presents the basic facts of entrance requirements for the universities and colleges of advanced technology of the United Kingdom, including requirements to teacher training courses and some information on grants, scholarships and living costs (Pp. 72. London: The National Union of Teachers, 1962. 4*s*.). There is a note on the operation of the Universities Central Council on Admissions, and, under the title "The Changing Scene", higher education in Britain to-day is briefly surveyed. This survey makes it clear that no decrease in the demand for places can be expected and that competition for entry is likely to increase rather than diminish over the next decade, varying, however, with different universities and different subjects. The extension of the guide this year to cover entry to the colleges of advanced technology and to teacher training colleges by giving a broad picture of the opportunities and facilities may assist in spreading the demand. The National Union of Teachers is to be congratulated on an excellent piece of work, as timely as it is useful and succinct.

The Soddy Research Fellowship in Geographical Sociology

A SODDY research fellowship in geographical sociology has been founded at the University of Sussex. The endowment of the fellowship—for a period of three years in the first instance—has been made possible by a gift from the Frederick Soddy Trust. The Trust was set up by the will of the late Prof. Frederick Soddy, who was professor of chemistry at the University of Oxford from 1919 until 1936, and who was awarded a Nobel Prize in chemistry in 1921 for his prediction of the existence of isotopes.

The object of the Frederick Soddy Trust is to develop the study of the social, economic and cultural life of specific geographical regions on the general lines developed by the Le Play Society (see *Nature*, 191, 1144; 1961). Mr. B. T. Wood, formerly assistant lecturer in geography in the University College of Wales, Aberystwyth, has been appointed the first Soddy Research Fellow.

Industrial Support for School Science in Britain

In the latter part of 1955 an appeal was launched to British industry for a fund to improve the facilities for teaching the physical sciences in those schools which could not look for support to the Ministry of Education or the Local Education Authorities. A sum of more than £3,000,000 was raised and by 1957 most of the resources had been allocated and building operations were well under way. The grants for building thus provided enabled the number of rooms for teaching physics and chemistry to be increased by nearly 50 per cent. By 1959 most, if not all, of the buildings were in full operation, and in 1960 the funds then seen to be available enabled grants to be made to ten schools not previously helped. All schools receiving building grants also received help toward the purchase of apparatus, and in addition, other schools not receiving building grants were given help to purchase apparatus; altogether, 326 schools were helped. In July 1962, grants were made to twelve schools taking part in a 'pilot scheme' for teaching physics on more modern lines, to enable them to purchase the necessary special apparatus, and a grant was made to the Science Masters' Association to assist in the setting up of their headquarters at Cambridge. A sum has also been set aside for the issue of a brochure on the design and equipment of science buildings to be published shortly. During the winding-up operation, the secretary will be Sir Graham Savage (Barbary, Maresfield Park, Near Uckfield, Sussex), to whom correspondence should now be addressed. All the funds at the disposal of the Executive Committee of the Fund have been committed and no further requests can now be entertained.

National Nature Week

The Council for Nature is sponsoring a National Nature Week in order to direct the attention of the public to the pleasure that can be obtained from studying Britain's wildlife and to the need for active conservation. It is hoped that the majority of the 300 member bodies of the Council will arrange special events during the week and that every sizeable town will have a programme of local events, including competitions for school children, film shows, popular lectures and exhibitions. Such activities will be arranged by local natural history societies and county naturalists' trusts. Many museums are arranging exhibitions, often with the collaboration of local naturalists, and it is expected that most public libraries will organize displays of natural history books. Open-air activities will play a large part in the week: guided rambles and tours, labelled nature trails and visits to nature reserves, including some of those under the Nature Conservancy, are being planned. The Council itself is holding a competition for natural history photographs and arranging exhibitions in London and Edinburgh on Britain's wildlife intended to attract large numbers of the general public. Further information can be obtained from the General Secretary, Council of Nature, 41 Queen's Gate, London, S.W.7.

Analytical Methods for Meteorites

An invitation conference on analytical methods for meteorites was held at the British Museum (Natural History) during September 5–6. Of the twenty participants, ten came from overseas; a communication on analytical methods used in the U.S.S.R. was received. The four sessions of the conference were devoted to: determination of the principal constituents of 'irons' (iron, nickel, cobalt, sulphur and phosphorus), and the separation of the metal, sulphide and silicate parts of stones; determination of the principal constituents of stones, with especial reference to the determination of Al_2O_3, CaO, Na_2O and K_2O; determination of trace elements in irons and in stones; and the use of the Castaing micro-analyser. The selection and preparation of material for analysis were also discussed. A report on the conference is available from the Department of Mineralogy, British Museum (Natural History), Cromwell Road, London, S.W.7.

Peaceful Uses of Nuclear Power

Atom, No. 70, August 1962, includes, besides papers by Dr. R. Roberts on uses of nuclear radiation in the textile industry, and by F. R. Farmer on the evaluation of power reactor sites, Sir Roger Makins's speech to the British Nuclear Energy Society on May 30, in which he suggested that 1962 would inaugurate a third period in the peaceful application of nuclear energy: the years of fresh advance. He attributed present disillusionment about nuclear power to over-optimistic economic forecasts and to underestimates of the technical problems involved in bringing civil nuclear power stations into being. It seemed likely that by the early 1970's nuclear stations would be generating power on base load as cheaply as the best coal-fired stations situated near to the East Midlands or Yorkshire coalfields, and when towards the end of the century the cheaper known uranium deposits might begin to run out, nuclear energy would be at a stage which would permit the mining of uranium from plentiful but higher-cost low-grade deposits. Sir Roger recognized that there were political as well as economic considerations to be taken into account, but he was satisfied that the structure of the industry and the relations of the Authority with industry and the Government was appropriate. He briefly reviewed the reactor systems which the Authority was developing or assessing, noting the importance of the use of pre-stressed concrete pressure vessels for the magnox system and the potentialities of the steam generating heavy-water reactor and the work at Culham on the fusion of the lighter elements.

Cultural Problems in Pictorial Perception

Pictorial perception depends on artistic convention; unacculturated groups do not comprehend this convention and misperceive the cues. Tests of perception of pictorial depth carried out by Dr. W. Hudson on European, coloured Bantu and Indian samples gave that descending order of three-dimensional pictorial perception with educated samples (*South African Journal of Science*, 58, No. 7; July 1962). Evidence from school-going Bantu groups and from European and Bantu occupational samples warranted the hypothesis that cultural experience and not educational standard was the determining factor in dimensional perception of picture material. Misperceptions of symbolism in the Western style picture were reported. The need for further research and a new orientation into the use of didactic illustration for unacculturated groups is indicated.

University News : Edinburgh

The following appointments have been announced: *Readerships*, Dr. P. S. Farago (physics); Dr. B. Meltzer (electrical engineering); Dr. P. D. Mitchell (zoology). *Lectureships*, Dr. E. J. Williams (senior lecturer in biophysics); Dr. R. A. Wall (chemistry); Dr. O. L. R. Jacobs and A. R. Dinnis (electrical engineering); Dr. B. G. J. Upton (geology); T. Sprinks and E. J. Squires (mathematical physics); Dr. R. B. Galloway, R. C. Dougal and A. S. McKirdy (natural philosophy); Dr. J. R. Beloff (psychology); Dr. A. Huitson (senior lecturer in public health and social medicine); R. C. Burgess (public health and social medicine). Miss H. C. Nibbet has been appointed curator of the Geology Museum and Departmental Collections.

London

Mr. Seton Lloyd, formerly director of the British Institute of Archæology at Ankara, has been appointed to the chair of Western Asiatic archæology tenable at the Institute of Archæology. Dr. J. B. Jepson, senior lecturer in biochemistry at Middlesex Hospital Medical School, has been appointed to the readership in biochemistry tenable at that School. Dr. Konrad Singer, senior lecturer at Royal Holloway College, has been appointed to the readership in chemistry tenable at that College.

Oxford

The following appointments to readerships have been announced: Dr. J. L. Harley (plant nutrition); Dr. J. W. Linnett (inorganic chemistry); Dr. R. E. Richards (physical chemistry); Dr. L. E. Sutton (physical chemistry). L. Castillejo has been appointed university lecturer in theoretical physics.

The Night Sky in December

Full moon occurs on Dec. 11d. 09h. 28m. U.T. and new moon on Dec. 26d. 22h. 59m. The following conjunctions with the Moon take place: Dec. 2d. 03h., Saturn 0·2° S.; Dec. 4d. 03h., Jupiter 2° N.; Dec. 16d. 07h., Mars 0·9° N.; Dec. 23d. 07h., Venus 2° S.; Dec. 29d. 14h., Saturn 0·2° N.; Dec. 31d. 15h., Jupiter 2° N. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 5h. 15m., 4h. 30m. and 4h. 10m. on Dec. 1, 15 and 31, respectively. Its stellar magnitude is −4·3; its distance increases during the month from 29 to 47 million miles and the visible portion of the apparent disk increases from 0·100 to 0·365. Mars rises at 21h. 20m., 20h. 40m. and 19h. 40m. on Dec. 1, 15 and 31, respectively. It is at a distance of 84 million miles on December 15, when its stellar magnitude is 0·0; conditions are now favourable for observation. Jupiter sets at 22h. 50m., 22h. 05m. and 21h. 20m. at the beginning, middle and end of the month, respectively. It is in Aquarius, its stellar magnitude is −1·9 and its distance from the Earth in the middle of the month is 480 million miles. Saturn is in Capricornus, setting at 20h. 15m., 19h. 25m. and 18h. 35m. on Dec. 1, 15 and 31, respectively. Its stellar magnitude is +0·9 and its distance from the Earth on December 15 is 985 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Dec. 8d. 19h. 13·8m., μ Cet. (*D*); Dec. 13d. 05h. 51·3m., ζ Gem. (*D*); Dec. 13d. 06h. 51·8m., ζ Gem. (*R*); Dec. 14d. 03h. 39·4m., 85 Gem. (*R*); Dec. 14d. 22h. 45·4m., δ Cnc. (*D*); Dec. 14d. 23h. 41·4m., δ Cnc. (*R*); Dec 31d. 18h. 54·0m., 74 Aqr. (*D*). *D* and *R* refer to disappearance and reappearance, respectively. The Geminid meteors are active during December 9–14, but conditions for observation are unfavourable. The Ursids are active during December 20–22, and conditions for observation are favourable: the radiant is near R.A. 14h. 28m., Dec. +76°. The winter solstice occurs on Dec. 22d. 08h.

Announcements

Dr. C. N. D. Cruickshank, formerly assistant director of the Medical Research Council Unit for Research on the Experimental Pathology of the Skin in the Medical School, University of Birmingham, has recently been appointed director in succession to Prof. J. R. Squire.

The Medical Research Council has recently agreed to establish a Neuroendocrinology Research Unit under the honorary direction of Prof. G. W. Harris, in the Department of Human Anatomy, University of Oxford. The unit will be primarily engaged in investigating the relationships between hormones and the brain.

The Medical Research Council has recently appointed a Committee on Steroid Sex Hormones under the chairmanship of Sir Charles Dodds. The terms of reference of this Committee are: "To advise and assist the Council in promoting research into the possible long-term effects of administration of steroid sex hormones".

The tenth International Botanical Congress will be held in Edinburgh during August 1964. Further information can be obtained from the Secretary of the Congress at 5 Hope Park Square, Edinburgh 8, Scotland. Copies of the first congress circular and a preliminary registration form are already available.

The tenth annual conference of the Western Spectroscopy Association will be held in Asilomar, California, during January 24–25. The programme will include sessions on: energy transfer in laser materials; solid state; high resolution; planetary atmospheres and extragalactic nebulæ. Further information can be obtained from R. A. Satten, Department of Physics, University of California, Los Angeles 24, California.

A symposium on "Education in Vacuum Science and Technology", arranged by the Institute of Physics and the Physical Society on behalf of the Joint British Committee for Vacuum Science and Technology, will be held at Brunel College, Acton, on May 29, 1963. Further information can be obtained from the Administration Assistant, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

Erratum. In the communication by Dr. R. A. Anderson and S. Sardo Infirri entitled "Effect of Electric and Magnetic Fields on the Boiling Point of Alcohol", which appeared on p. 267 of the October 20 issue of *Nature*, lines 10–16 of the second paragraph should read: we used the following frequencies and root mean square (r.m.s.) field strengths:

Frequency (c.p.s.)	Field strength (kV/cm)
0	2·7
50	3·5
250	2·2

D.S.I.R. SUPPORT FOR UNIVERSITIES AND COLLEGES

*D.S.I.R., Universities and Colleges, 1956–60** reviews the support given by the Department of Scientific and Industrial Research to research and postgraduate training in universities and colleges from August 1956 to December 1960. An introductory chapter outlines the functions and organization of the Department and summarizes the growth of support of this kind from 1929–30 to 1959–60. The two main chapters of the report, covering the training awards, studentships and fellowships, and the research grants are discussed on p. 605; a separate chapter reviews the support given to the human sciences, particularly the work of the Human Sciences Committee, and its sub-committees, which in 1958–60 considered 75 applications and by July 1960 had recommended more than 38 awards at an estimated cost of £193,000. Besides the figures given in the report itself appendixes, numbering some 100 pages, include, besides particulars of membership of the Council and its Committees and Sub-committees, statistics of studentships, fellowships and of research grants for the period.

In 1960 the Department awarded 942 research studentships, 274 advanced course studentships, 77 first-year studentships and 39 fellowships. Of the research studentships 13·3 per cent were in biology and biochemistry; 7 per cent in chemical engineering and metallurgy; 25·7 per cent in chemistry; 4·2 per cent in electrical engineering; 7·6 per cent in other engineering; 6·6 per cent in geology; 2·4 per cent in human sciences; 11·6 per cent in mathematics; and 21·5 per cent in physics. Over the period 1957–60 the corresponding percentage figures for new advanced course studentships are: 8·8; 9·9; 1·5 ; 7·3 ; 15·0; 10·2; 7·7; 22·6; 17·2. Only 20 students and 17 Fellows from the United Kingdom held their awards abroad in 1959–60 and 26 and 30, respectively, in 1960–61, but over the period 1957–60 there was a slight but steady increase in migration of research students between universities and in 1960, 19 per cent of research students and first year students migrated on taking up an award in Britain. There was a marked movement away from the colleges of advanced technology and the technical colleges and the likelihood of migration varied with the subject of research. Of 467 research students whose awards terminated in the year ended September 30, 1959, 99 took up overseas posts; 91 posts in industry, 79 research posts and 57 teaching posts in universities, 25 posts in the Scientific Civil Service and 18 in technical colleges.

Of research grants awarded during October 1956–September 1960, £409,783 was in astronomy, £400,139 in biology, £750,820 in chemistry, £326,267 in geology, £640,169 in low-temperature and solid-state physics, £10,125 in mathematics, £2,655,108 in nuclear physics, £288,501 in other physics, £627,812 in technology, £225,326 in human sciences and £297,543 in space research. Of the total of £6,631,943 all except £189,493 was to universities.

A section of the chapter in the report dealing with research grants reviews some of the results obtained from the Department's support of research in the various fields during the past four years.

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* Department of Scientific and Industrial Research. *D.S.I.R., Universities and Colleges, 1956–60· a Report on D.S.I R. Support for Research and Training in Universities and Colleges, 1956–60.* Pp. viii+232+15 plates. (London. H M.S.O.. 1962.) 15s. net. (see also p. 605 of this issue.)

REPORT OF THE GOVERNMENT CHEMIST FOR 1961

IN his recently published report*, Dr. D. T. Lewis, the Government Chemist, describes the wide range of work done in his Laboratory during 1961. During that period an average of more than 1,000 samples were analysed or otherwise examined every working day. These included such materials as manufactured fabrics, tobaccos, wines, spirits, drugs, foodstuffs, and a wide variety of inorganic and organic chemicals, which might, for example, occur in chemical formulations of pesticides, fertilizers and animal feeding stuffs. Almost all the work has been done at the request of various Government Departments in the United Kingdom, but some Commonwealth countries and Colonial Administrations have also availed themselves of the services offered by the Laboratory.

A great deal of the Laboratory's time is spent on various statutory duties, principally in collaboration with H.M. Customs and Excise; this is a reminder that the Laboratory was originally created by the Board of Inland Revenue in 1842 for the purpose of aiding the revenue authorities to detect and prevent infringements of the various Customs and Excise regulations which deal with dutiable commodities such as tobacco, beers, wines and spirits, and sugar. Although this basic work of the Laboratory changes little from year to year, nevertheless alterations and additions to its general work have to be made in accordance with the changing laws of the country. The Laboratory has constantly to be on the look-out for new forms of tax evasion, intentional or otherwise, on materials where the duty liability, particularly to purchase tax, is more readily avoided. Now fiscal measures can, of course, create new problems for the Laboratory which must be ready to help with the implementation of the new laws by providing suitably accurate and reliable methods of analysis for the materials in question. An example of this is given in the Finance Act of 1960, which requires certain marker substances to be added to gas oils sold for purposes other than road use. The Laboratory has had to develop analytical methods for the detection and determination of these additives (furfural and quinalizarin) in this type of fuel oil, and satisfactory procedures have recently been published.

A small but increasing amount of forensic work is done principally for the General Post Office and mainly involves the examination of questioned documents, of which British Postal Orders suspected of alteration are by far the most numerous. Betting letters have also been examined for reasons which Dr. Lewis gives as follows.

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* Department of Scientific and Industrial Research Laboratory of the Government Chemist. Report of the Government Chemist, 1961. Pp. v+89+3 plates (London· H.M S.O., 1962.) 6s 6*d*. net.

"Every year disillusioned punters conclude that betting would be profitable if conducted after results of races were known and the rule that postal bookmakers accept bets postmarked before starting times tends to spur some misguided individuals to ingenious action". Most of this forensic work involves examination of documents by ultra-violet radiation and often requires that members of the Laboratory staff must act as witnesses in those cases which come before the law courts.

Certain matters of public health are also referred to the Laboratory, which undertakes examinations of water and sewage for various authorities, but principally the Ministries of Health, and Housing and Local Government. One particular national problem concerns the disposal of some 11 million tons of household refuse each year in England and Wales—equivalent to 5 cwt. per person per year. The tipping of this refuse in disused gravel pits and other dumping grounds can lead to pollution of underground and surface water supplies. The Laboratory has made careful studies of the degrees of pollution which can occur and its results have been passed to the Ministry of Housing and Local Government, with recommendations for a continued study of the problem in collaboration with local authorities and water undertakings.

An increasing part of the work of the Laboratory is concerned with long-term research work on specialized matters such as radioactive fall-out and effluents from nuclear power plants. The development of new analytical methods using physical techniques such as X-ray diffraction and X-ray fluorescent spectroscopy is also continuing. These physical methods have advantages where a non-destructive examination is required.

There has, however, been a need in the Laboratory for a group to undertake research on problems of a more general nature. With this in mind, a new division of the General Methods Research Committee has been formed the purposes of which are to review existing methods of analysis and to develop new ones for the variety of materials with which the Laboratory is concerned in its day-to-day work. In this connexion, excellent work has been done on the detection and identification of a large number of organic pesticide chemicals using mainly the techniques of paper chromatography and infra-red spectroscopy. Each year the Laboratory has to undertake an appreciable amount of investigatory work connected with the possible hazards not only to humans but also to wild-life which arise from the extensive use of pesticide chemicals in agriculture; the wide range of these chemicals now commercially available must be readily identifiable in all sorts of animal and vegetable matter.

The reports of the Government Chemist are always of considerable interest and the present one is no exception. For sheer diversity of content there is little with which to compare it; for where else could one find an account of the detection of the pesticide 'Aldrin' in a sample of rice pudding, or the involved radiochemical investigations arising from nuclear fall-out. The Laboratory exists to safeguard the public; this report leaves no doubt that it is work well done.

W. I. Stephen

FIFTH INTER-AMERICAN SYMPOSIUM ON COSMIC RADIATION

It was perhaps rather fitting that the staff of the world's highest cosmic ray laboratory should play host to the experts of the fourteen nations who contributed to the Symposium on Cosmic Radiation in La Paz, Bolivia, during July 17–27. This laboratory, situated at Mt. Chacaltaya some 35 km from La Paz, is rapidly becoming one of the foremost centres of high-altitude research. The altitude of the laboratory is 17,600 ft., and as such affords an ideal site for recording cosmic radiation and observing the night sky. Among its equipment is a cosmic ray air shower-recording array the central detector of which has an area of 60 m^2 and is shielded by 400 tons of lead ore.

After the formal opening by the President of Bolivia, whose speech stressed the contribution that this scientific endeavour was making in combating the conditions of under-development in the country, inaugural speeches by Prof. Vallarta from Mexico, Dr. Establier of Unesco and the rector of the University of La Paz, emphasized the amount of international co-operation involved in the work going on in Bolivia. Scientists from Brazil, Bolivia, Great Britain, India, Japan and the United States are now working in the Chacaltaya Observatory. Thanks to aid from Unesco, the U.S. Office of Scientific Research, and the International Union of Pure and Applied Physics, it was possible to gather in La Paz some of the foremost authorities on cosmic radiation for the Fifth Inter-American Symposium.

It is not possible in such a short summary to do justice to the enormous amount of interesting and significant contributions made by the various speakers in the symposium. Sixty-four papers were presented, and covered such fields as the measurement of the prehistoric cosmic radiation flux to the latest satellite measurements made by the U.S. National Aeronautical and Space Administration. Russian speakers described experiments going on in the Crimea for the detection of Čerenkov radiation produced by γ-rays coming from other galaxies. The subject of γ-ray astronomy was well covered by the Massachusetts Institute of Technology groups working in New Mexico and their associates working in Chacaltaya in conjunction with colleagues from the Universities of Tokyo and La Paz. The subject of time variations was well covered by the speakers, and in this field, as well as geomagnetism, one appreciated the tremendous activity now going on in Latin America in the realm of geophysics. South America to-day supports a number of flourishing research organizations, all of which made significant contributions to the Symposium; among them may be mentioned the time-variation groups in Rio de Janeiro, Buenos Aires, geomagnetic observatory in Huancayo, Peru, and the emulsion work at São Paulo, Brazil, and Tucúman, Argentina. The Symposium illustrated the enormous increase that has been made in our knowledge of the conditions in the space surrounding the Earth as well as the conception of the universe as a whole.

In addition to the scientific activities, a number of interesting social events and excursions was arranged which included a trip to the Inca ruins at Tihuanaco and an airborne excursion to the headwaters of the Amazon, by courtesy of the Bolivian Air Force.

No such gathering would have been possible but for the existence of the Laboratorio de Física Cósmica at Chacaltaya, to the director of which, Prof. Ismael Escobar, goes the credit for its foundation and expansion into its now pre-eminent position as a high-altitude observatory, where many nations co-operate to discover some of the characteristics of the space in which we move.

C. J. BLAND

NEW NATURE RESERVES AND EXTENSIONS IN ENGLAND AND SCOTLAND

THE Nature Conservancy declared the following new National Nature Reserves and extensions on September 28:

ENGLAND

Colt Park Wood, Yorkshire

This Reserve, at Ribblehead in the Craven Pennines, has been purchased by the Nature Conservancy and is a 21-acre strip of ash wood growing on a narrow, mile-long scar on the lower slopes of the north-east face of Ingleborough, at a height of 1,100 ft. It is only about 1½ miles from Ling Gill National Nature Reserve which was declared in 1958. The Reserve, which is in the Yorkshire Dales National Park, contains one of the few remaining examples of high-level ash wood on limestone pavement. The northern part is dominated by ash varying in height from 25 to 40 ft. Other tree species include bird cherry, silver birch, rowan and hazel. The trees grow directly on the limestone, rooting in the fissures or 'grikes' which dissect the pavement deeply. Farther south along the scar the wood thins out and the clints are devoid of plant cover although a 'woodland' ground flora persists in the shelter of the grikes. The ground flora throughout the Reserve is most striking and a number of rare species is present. For a long time the wood has been valued for its shelter in an otherwise almost tree-less landscape, a fact which may explain its survival; but it has suffered extensively from grazing and little tree regeneration is taking place. The first step in management, therefore, will be to fence the Reserve to encourage fresh tree growth to replace the present ageing canopy.

Access is by permit only. Applications to visit or for permission to undertake research or to collect specimens of plants or animals should be addressed to the Regional Officer for the North Merlewood Research Station, Grange-over-Sands, Lancashire.

Blean Woods (Grimshill Wood Extension) Kent

The first part of this Reserve was declared in 1953, and a small portion added in 1959 brought the Reserve up to 81 acres. A further 84 acres, known as Grimshill Wood, have been purchased. The Reserve consists almost entirely of oak woodland with a variety of coppice species, but there is considerable diversity in the area owing to the different types of management to which each part has been subjected. That part previously declared had been under management for coppice, whereas the newly acquired portion has been cut much less frequently and the coppice is therefore larger and older. The new area will be suitable for relatively quick conversion to high-canopy woodland, and will thus enable some of the previously declared part of the Reserve to be released for short rotation coppice management for which it is better suited.

Application for permits to visit parts of the Reserve away from public footpaths should be addressed to the Regional Officer for the South-East, the Nature Conservancy, 19 Belgrave Square, London, S.W.1.

SCOTLAND

St. Cyrus, Angus and Kincardineshire

The St. Cyrus National Nature Reserve occupies 227 acres of coastal land between the estuary of the River North Esk northward to the mouth of the Woodston Burn. It consists of a sandy foreshore, with rocks at the north end, a line of dunes, a salt marsh (a former channel of the North Esk), dune pasture and cliffs. The cliffs reach a height of 200 ft.; southwards of St. Cyrus village they lie inland behind the dunes, but at the north end of the Reserve they rise directly from a rocky foreshore. The primary interest of the Reserve is botanical. More than 300 species of plants are recorded for the area, many of these being near the northern limits of their range in Britain. The physical features, good soil, and climate combine to favour the establishment of a variety of plant communities remarkably rich in species for such a coastal strip in north-east Scotland. The cliffs support a particularly attractive and interesting flora, with more than 170 species of flowering plants, including clustered campanula, great mullein, hemp agrimony, henbane, viper's bugloss, Nottingham catchfly, marjoram, hairy violet and maiden pink.

The Reserve was declared by nature reserve agreements with the owners, the Tay Salmon Fisheries Co., Ltd., and Joseph Johnston and Sons.

Beinn Eighe (Extension), Ross and Cromarty

This fifty-seven acre strip of pinewood and moorland between the main Kinlochewe–Gairloch road (*A*.832) and the shore of Loch Maree runs from the east end of the Loch to the estuary of the Grudie River. The Beinn Eighe Reserve, the first in Britain, was established in 1951 primarily for the protection and rehabilitation of Coille na Glas Leitire, the relict pinewood on the west side of the main road from which, by historical accident, the present area had been severed under different ownership. The new addition is ecologically an integral part of the main woodland, and contains some fine mature Scots pine with flora and fauna characteristic of western pinewoods, and of the loch margin. Included in the fauna are the pine marten, wild cat and crossbill.

The extension was declared under a nature reserve agreement with the owner, Colonel W. H. Whitbread.

Inverpolly (Knockan Cliff Extension), Western Ross

The impressive crag of Knockan Cliff runs for just more than a mile along the Ullapool–Elphin road and its southern end has been acquired as an extension to the east side of the Inverpolly National Nature Reserve. This small extension of 36 acres includes one of the most important and frequently visited geological localities in Britain. The west-facing cliff rises about 250 ft. above the road and exposes a sequence of relatively undisturbed Cambrian sediments overlain by Moine schists brought westwards for a distance of several miles by the Moine Thrust—one of the principal structural features of north-western Europe. The seemingly anomalous relationship of the sedimentary and metamorphic rocks has been investigated by geologists for more than a century and the discovery of the true explanation (suggested by Nicol as long ago as 1861) played a vital part in elucidating the structure of the Scottish Highlands and in the development of the science of tectonics.

The area has been purchased by the Nature Conservancy.

Loch Druidibeg (Extension), Inverness-shire

The extension comprises 1,568 acres of enclosed ground, *machair*, sand-dunes and sandy shore with a complex of lochs and watercourses in the crofting townships of Stilligarry and Dremisdale, and part of Drimore Farm, South Uist. It is an extension to the Loch Druidibeg National Nature Reserve (hitherto 2,577 acres) which is owned by the Conservancy. The Reserve was established in 1958 to protect the most important breeding ground in Britain of the native grey lag goose, but the ground then acquired covered only half the area used by the geese which nest by moorland waters in the Reserve and take their broods for rearing to the richer *machair* lochs of the extension. Throughout the year the geese use the original Reserve for roosting and as a refuge; the extension, which is part of a private estate, safeguards their feeding grounds.

In addition to containing this unique population of geese, Loch Druidibeg with its extension is a comprehensive Outer Hebridean nature reserve containing moorland, in-bye croftland, *machair*, dunes and shore. Loch Druidibeg and the neighbouring *machair* and lochs within the extension constitute one of the finest and most varied samples of this Outer Hebridean system remaining in a comparatively unspoilt state. The extension has a rich flora and fauna characteristic of the *machair* grasslands of the Outer Hebrides, which contrast with the very different flora and fauna of Loch Druidibeg, its islands and surrounding moorland. The whole area presents fine research opportunities for studying the ecology and management of grey lag geese and other wildfowl, *machair* and dune communities, and the physiography of sand-dunes.

The Warden of the Loch Druidibeg Nature Reserve and extension is Mr. M. MacRury. He is a local crofter and his duties include patrolling, fire prevention, prevention of damage to crops by geese, reception of visitors, liaison with crofters and co-operation with the staff of the South Uist Estates.

A nature reserve agreement in respect of the extension was concluded between the Conservancy and the owners of South Uist Estates. The crofters of the Stilligarry and Dremisdale townships and the tenants of Drimore Farm were included in the consultations.

Tentsmuir Point (Extension), Fife

The extension to the Tentsmuir Point Nature Reserve increases the area from 92 acres to 1,249 acres. The original Reserve declared in 1954 consisted only of land above the high-water mark. The interest here is chiefly physiographical and botanical, for exceptionally rapid accretion has resulted in a considerable area being won from the sea by natural processes of dune formation and their subsequent stabilization by sand-fixing grasses. Behind the seaward dunes are other dunes showing more advanced stages of plant colonization, and between them are wet slacks, some in process of colonization by alder and birch. The new extension consists mainly of foreshore brought within the Reserve by agreement with the Crown Estate Commissioners; within this area lie the Abertay Sands, a winter roost for wildfowl, including geese, so that Tentsmuir Point Nature Reserve is now both a nature reserve and a wildfowl refuge in which shooting will be prohibited. The extension also includes an area of 24 acres at the southern end containing samples of alder and willow slacks not represented in the existing reserve. It has been purchased from the Forestry Commission, together with a narrow strip of 12 acres of marshy ground along the western boundary.

RADIO TELESCOPE ENGINEERING

A RECENTLY published article by Mr. Jacob Feld* describes some of the engineering problems concerned in the design of radio telescope structures and where these problems differ from those encountered in normal structures. It is thus of value not only to the engineer but also to the scientist, who is able to appreciate more fully the difficulties which have to be overcome in satisfying the somewhat exacting requirements demanded by this relatively new science.

The introductory chapter outlines the new radio astronomy techniques, its purposes and possibilities, and indicates that requests for reflector surfaces of even greater accuracy are inevitable as the performance of receiving equipment and amplifiers continues to improve.

The application and adaptation of radar reflectors for radio astronomical purposes is described in the succeeding chapter and the need for new designs emphasized because of the unprecedented size of new structures and the extreme precision required in rates of motion and angular accuracy of control.

Some of the problems associated with wind loading and the differences in approach from normal structural design are dealt with later. It is made clear that the shape of radio telescope reflectors must be maintained under operating wind and temperature conditions: the required tolerance being about one-tenth of the received wave-length measured from a geometric paraboloid. If part of the reflector is beyond such shape tolerances, the efficiency of the instrument is impaired. The shorter the wave-length of operation, the smaller the tolerances permitted. Thus, for 10-cm wave-lengths the reflector surface may not be out of shape by more than ± 1 cm.

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* *Annals of the New York Academy of Sciences*. Vol. 93, Article 10: Radio Telescope Structures. By Jacob Feld. Pp. 351–456. (New York: New York Academy of Sciences, 1962.)

The accuracy of pointing and tracking required for these very large masses presents entirely new problems. Whereas optical telescopes are mounted on equatorial axes and it would be desirable for radio telescopes to be similarly mounted, the high resulting torques and the necessity for counterweights that practically double the mass to be moved, increase the costs above the more practical alt-azimuth mounting.

Mechanical power transmissions and gear reduction assemblies are easily available items, but the accuracy of control requires some unusual tolerances in machining, while speeds of motion are so slow that new concepts of bearing designs and frictional values are required.

Two somewhat longer chapters discuss design studies for extremely large telescopes. Brief mention is made of some types of fixed reflectors, while the specifications and design procedure for a 600-ft. fully steerable paraboloid reflector are described in greater detail. Mr. Feld summarizes his own complete design study for a 600-ft. instrument and suggests that his findings indicate certain trends which should be further examined and evaluated. In a later chapter, describing some instruments in the United States, mention is made of the 600-ft. telescope which has been under construction at the U.S. Navy Observatory at Sugar Grove, West Virginia. Construction started early in 1959 and completion at an estimated cost of 79 million dollars was scheduled for 1962. However, the design was completely revised, resulting in indications pointing to a much larger weight to obtain the desired stiffness and accuracy of shape. Corresponding modifications to undercarriage, track and foundations, together with considerable increased cost estimates, would have delayed completion until about 1964. Since publication of this paper, cancellation of the whole project has been announced.

Comparative studies of the design of the 140-ft. telescope now being constructed at Green Bank, West Virginia, are described in the latter part of this paper, which concludes with a chapter discussing a number of fixed types of reflector, planned or already in use.

A full list of references, together with a number of illustrations and drawings, complete an interesting and valuable contribution to the limited reading matter available on radio telescopes. R. F. TOLSON

GENERAL STUDIES IN TECHNICAL COLLEGES

GUIDANCE to technical colleges on the selection and treatment of the content of 'general studies' for young craftsmen and technicians are contained in a report published by the Ministry of Education *.

The report has been prepared by an advisory committee set up after the publication of the White Paper, "Better Opportunities in Technical Education". The White Paper stated that, when courses were lengthened to the 330 hours a year recommended by the Crowther Committee, the time given to English and general subjects, including physical education, should be increased. The report recalls the "four strands" of the Crowther Report which outlined the wider social purposes of further education. These were to help young workers to find their way successfully about the world both as consumers and citizens, to form standards of moral values by which they can live in the new world in which they find themselves, to continue and develop the pursuits and activities which they have begun at school and to improve their basic education. The report points out that the first need for the students in both the technical and general elements of their course is to develop their communication skills. They must be able to make themselves understood in speech and writing, and, through listening and reading, to understand other people. Success in their technical subjects directly depends on mastery of these skills; they are essential, too, for the student's development as an individual and as a member of society. It is for these reasons that the teaching of English and general studies should be regarded as a single operation.

The report outlines five broad areas of knowledge and experience from which the subject-matter for general studies might be selected. Some of the many suggestions made, under these main headings, are:

(1) *The student and the college*—nature and organization of the college; the student's place in it; his relations with staff and fellow students.

(2) *The student and his job*—training facilities; prospects and ambitions; the student's firm; relations on the shop floor; work, wages, costs and profits; health and safety at work.

(3) *The student, his home, his family and his friends*—the significance of the human family group in society; relations between the sexes; love, courtship marriage.

(4) *The student and the wider community*—the local community, its history, industry and places of interest; national Government; the welfare state; discipline and the law; present-day economic problems.

(5) *The widening of horizons and the development of personal interests*—the universe and man's place in it; concepts of space and time; the practice, study and appreciation of the arts; opportunities to practise handicrafts; physical education and recreation.

The report indicates that this broad scheme is not exhaustive nor is it intended to be a syllabus. It is more a storehouse for which topics might be drawn. Not all the subjects would be covered in class time. Many of the activities and interests should spill over into societies and informal groups with a vigorous life of their own.

An appendix suggests how the general lines of approach may be applied to a specific theme. Four 'theme treatments' are given, all of them examples of courses which have been planned and carried out with day-release students.

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* Ministry of Education. *General Studies in Technical Colleges.* Pp. ii+23. (London: H.M.S.O., 1962.) 2s. net.

TEST OF THE GENERAL THEORY OF RELATIVITY

By DR. M. SUBOTOWICZ

Lublin, Poland

WITH the help of artificial satellite techniques there are new experimental possibilities of testing two of the three effects predicted by the general theory of relatively, namely, the perihelium advance and the shift of spectral lines of the electromagnetic monochromatic radiation along the gravitational gradient as a result of the difference in the energy-levels of an atom when it is placed at different gravitational potentials[1–6]. To avoid the linear Doppler effect in 'red' shift experiment Singer[7] proposed measuring the influence of the gravitational field on the rate of atomic clocks, one of which was placed on the surface of the Earth, the other in the satellite. Ginzburg[8] and Badessa *et al.*[9] suggested other methods of cancelling the first-order Doppler term.

In this connexion we might refer to Schiff's results[10] that the precession of the line of apsides provides the only real test of general relativity[11–13].

From the experimental point of view, the most difficult to investigate is the relativistic effect of deflexion of light; it will be discussed more extensively in this article, especially its experimental aspect.

Light deflexion. The deflexion ψ_r of light passing the massive celestial body of mass M_p and radius R_p, from its straight trajectory is given by the equation:

$$\psi_r = 4kM_p/(c^2R_p)$$

This effect was tested by measuring the apparent position of stars near the Sun during eclipse. (It seems also possible to verify the relativistic light deflexion by taking photographs of the sky from the surface of the Moon during an eclipse of the Moon.) The value predicted by the theory is 1·75″, the measured values were 1·61″–2·01″ sec of arc[14,15]. The most recently measured value is 1·70″ (v. Biesbroek, 1952).

An artificial satellite circling around a celestial body in a well-defined orbit and emitting electromagnetic radiation can be used to measure the deflexion of light in the gravitational field. But instead of measuring the deflexion of the emitted radiation as in the photographic method[16] we can measure the time of the covering of the satellite by the celestial body (Fig. 1). As a result of the deflexion of the radiation emitted by the satellite the covering time will appear to be shorter than if there were no deflexion. This makes it possible to measure the time instead of measuring the angles, where precision is smaller. We describe (without taking into account the relative motion of the Earth and Sun) the motion of the satellite S around the central body P in the geocentric reference system E (Earth) (Fig. 1). The deflexion angle of the light-beam is ψ, the part of the trajectory of S unobserved from the Earth is D_{nd} if the ray is undeflected and D_d for the case of the deflected ray. The ratio:

$$\Delta n = (D_{nd} - D_d)/D_{nd} = d_s r/[R_p(R_p + r + h_s)]$$

can be the measure of the required precision of the clock at the point of observation (Table 1).

Using this same satellite, we could perform the tests of the other two relativistic effects: the 'red' shift and the perihelium advance. We must assume the possibility of change of the satellite's orbit owing to additional engine and fuel; then the satellite could transfer on to the required orbit. This will be possible owing to the development of advanced space research by using nuclear space power systems[15,17]. To fulfil all this programme the cosmic probe should perform some flights around the Sun; then the deflexion experiments could be carried out. After that the cosmic probe should transfer on the Earth's satellite orbit in order to measure the last two relativistic effects.

Possibilities of the laser transmitters. The verification of the relativistic light deflexion seems to be possible by using artificial satellites with the maser and laser radiation sources. They could be used as the electromagnetic radiation amplifiers, detectors and generators. The light which has passed close to the limb of the Sun, emitted by the Sun's artificial satellite flying around the Sun at the distance 10^{13} cm, can be distinguished from the background radiation of the Sun. The total radiation of the Sun at all wave-lengths is 7 kW/cm^2. But in the narrow spectral region of 5000 Å the Sun emits only 10^{-5} W/cm^2. The beam of short bursts of pink ruby radiation, less than 1 cm^2 in cross-section, can reach

Table 1. DATA CONNECTED WITH THE VERIFICATION OF THE GENERAL RELATIVITY THEORY IN PROPOSED EXPERIMENTS

Symbol	Units	Earth	Moon	Satellites of Mars	Jupiter	Sun
r_{EP}	cm	$3·84 \times 10^{10}$	$3·84 \times 10^{10}$	$7·83 \times 10^{12}$	$6·29 \times 10^{13}$	$1·5 \times 10^{13}$
h_s	cm	$3·69 \times 10^{9}$	$1·00 \times 10^{9}$	$1·00 \times 10^{10}$	$1·00 \times 10^{11}$	$1·0 \times 10^{13}$
d_s	cm	12 00	$1·5 \times 10^{-1}$	6	$8·83 \times 10^{3}$	$8·5 \times 10^{7}$
v_s	cm/sec	$3·07 \times 10^{5}$	$6·4 \times 10^{4}$	$6·5 \times 10^{4}$	$1·14 \times 10^{6}$	$3·6 \times 10^{6}$
φ	sec of arc	$5·80 \times 10^{-4}$	$2·6 \times 10^{-5}$	$1·19 \times 10^{-4}$	$1·60 \times 10^{-6}$	1·75
χ	erg/g	$6·28 \times 10^{11}$	$2·82 \times 10^{10}$	$1·30 \times 10^{11}$	$1·82 \times 10^{13}$	$1·9 \times 10^{15}$
Δn	—	$7·80 \times 10^{-9}$	$8·30 \times 10^{-10}$	$1·78 \times 10^{-10}$	$1·29 \times 10^{-6}$	$7·4 \times 10^{-4}$
$(\chi_2-\chi_1)/c^2$	—	$5·94 \times 10^{-10}$	$3·16 \times 10^{-11}$	$1·40 \times 10^{-10}$	$1·89 \times 10^{-8}$	$1·9 \times 10^{-6}$
$v_s^2/2c^2$	—	$5·23 \times 10^{-11}$	$2·16 \times 10^{-12}$	$2·35 \times 10^{-12}$	$0·07 \times 10^{-8}$	$0·7 \times 10^{-8}$
$\Delta\tau/\tau$	—	$5·40 \times 10^{-10}$	$2·94 \times 10^{-11}$	$1·38 \times 10^{-10}$	$1\ 82 \times 10^{-8}$	$1·9 \times 10^{-6}$
$\left.\begin{matrix} s = 0·035 \\ P = h_s;\ \varphi \end{matrix}\right\}$	sec of arc	16·8	0·52	0·06	32·8	10·8
$\left.s = 0\ \overset{\varphi}{058}\right\}$	sec of arc	1263 $P = 228$ km	87·8 $P = 100$ km			

Here $\Delta\tau/\tau$—the relative difference between the rates of two atomic clocks placed: one on the central body, the second in its satellite, circling with the velocity v_s, on the height h_s.

χ—the gravitational potential.

φ—the perihelium advance in sec of arc per century.

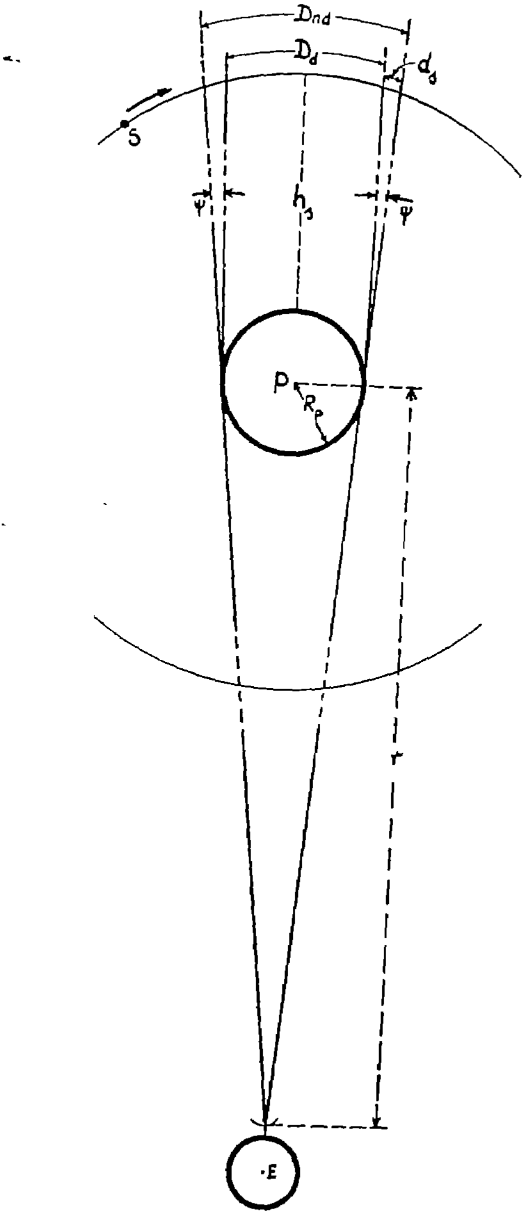

Fig. 1

up to 10^4 and 10^5 W. The angular spread of the beam is so small that it enables it to project a spot of light only two miles in diameter from the Earth on the Moon. The power of the gas maser output per kilocycle of band-width is about 10^8 times that of 1 cm^2 of the Sun's surface[18,19].

The monochromaticity of the electromagnetic radiation is defined as brightness per unit wave-length. For lasers the monochromaticity is 10^{12} times the spectral brightness per unit wave-length of the Sun[10]. The problem of detection of the laser radiation against the background of the radiation of the Sun is partly solved owing to the narrow frequency of the output of the laser. Further discrimination could be achieved by narrowing the band-width to less than 1 Mc/s. Discussing the interstellar and interplanetary communication, Schwartz and Townes[21] proposed to use a 'system (*a*)' of a laser coupled with a reflector of a diameter $D = 5 \times 10^2$ cm. In such a system with the laser producing continuous frequency $\lambda \simeq 5000$ Å, band-width 1 Mc/s, power-level 10 kW one could get the beam-width B equal $B = \lambda/D = 10^{-7}$ rad $= 0{\cdot}02$ sec of arc. Another 'system (*b*)' represents a group of 25 lasers, each having the same laser characteristics as system (*a*), but with an effective system aperture $D = 10$ cm. Here we achieve a beam-width of 1 sec of arc or 5×10^{-6} rad. The entire group points in the same direction within the accuracy of the beam-width. Supposing that system (*a*) is radiating a beam of light towards the Earth from the distance $2{\cdot}5 \times 10^{13}$ cm (system (*a*) is orbiting the Sun at the distance of 10^{13} cm), we should get on the Earth a spot of light about 25 km in diameter. For system (*b*) this spot would be 1,250 km in diameter. A sufficient number of photons should reach the Earth's surface to detect this radiation by using proper receivers; or our detecting system should distinguish this radiation from the background of the solar radiation.

Suppose that the two systems (*a*) and (*b*) are orbiting the Sun at the distance of 100 million km, the distance from the Earth being 250 million km. The system (*a*) could produce an intensity 27 times that of the Sun in a spectral region of $\lambda = 5000$ Å, and system (*b*) four times. This relative intensity could probably be increased by reducing the 1 Mc/s band-width chosen arbitrarily in above considerations, and by raising the peak power generated in short pulsed beams of radiation up to, for example, 100 kW. All the advantages of maser and laser generators can be better utilized by placing the detectors on the platform of the Earth's satellite or, even better, on the surface of the natural Moon.

To determine the orbit of the laser satellite of the Sun exactly we should use telescopes of a very high angular resolution up to 1/10 sec of arc[21,22]. The present accuracy of the determination of the star position is 0·05 sec of arc[23]. Using the atomic frequency standards the precision of the short-term tracking of the space probes can be increased to about 1 : 10^7. The long-term tracking can be increased to 1 : 10^8 (ref. 24). That means that the position of our laser-satellite of the Sun at the distance of $2{\cdot}5 \times 10^{13}$ cm could be established with a precision of about 2·5 km. Such an accuracy would be sufficient to discover the deflexion of light in the gravitational field not only of the Sun but also of Jupiter (see the data for Δn in Table 1). (The method given by Badessa *et al.*[9] enables[1] very precise measurement (1 : 10^9) of the satellite's radial velocity.) All the given data show that it is possible to determine the orbit elements of the satellite with enough precision, at least within the state of the art.

One could argue that the solar atmosphere—the corona—could effect the laser-light seriously, deflecting it still more. Mikhailow[25] showed that the corona of the Sun would give the additional deflexion of only 0·1 sec of arc assuming an inadmissibly large density of the corona. The stars which are at the very limb of the Sun's disk cannot be observed in Sun-photographing experiments even during a solar eclipse, because their light is totally obliterated by the inner corona. As it has been shown here, it is not true in the case of laser radiation.

It can be shown that a similar experiment in detecting light deflexion can also be performed in microwave range[19]. Let us suppose that the power transmitted from the satellite of the Sun is P_{tr} 100 = W, the signal-to-noise ratio is $S/N = 10$ db., the temperature of the antenna is 70° K and its efficiency is $\varepsilon = 0{\cdot}5$. Then the diameter of the Earth-borne antenna[26] should be 76–91 m if the distance of the satellite from the Earth is 250×10^6 km (as it is in our example). The range of the feasible communication in space depends on the ratio of received power P_r to interference power in the band-width of interest, $\Delta\nu$. The most important interference power in this case is the noise power P_n, proportional to the temperature of circuitry, T. Here T means the weighted sum of all different sources of temperature, where the weighting factors depend on how the source is seen by the receiving antenna. $P_n = kT\Delta\nu$ where $\Delta\nu$ is measured in c/s. The distance d at which communication is feasible ($P_r/P_n = 1$) is defined as 'threshold reception range'. The Sun is the main source of noise. For the Sun in the first side-lobes of a typical antenna for the frequency 500 Mc/s the noise temperature[27] would be 10^3 °K, and for 5 kMc/s—only 10^2 °K.

$$d = \sqrt{P_t A_t G_t / 4\pi k T \Delta\nu}$$

where P_t is the transmitted power by the antenna of the area A_t and gain G_t. For the effective reception of the signals from the distance $d = 2{\cdot}5 \times 10^{13}$ cm, using the vehicle antenna of the surface 10 m², gain $G_t = 100$, band-width $\Delta\nu = 10$ cps and $P_t = 100$ W, we should expect as the largest possible source temperature $T = 10^4$ °K. Those data are acceptable for the Sun in the first side-lobes of the typical antenna. According to the calculations[28] for transmitted power 100 W, using the maser detector of temperature 40° K in the ground antenna 27 m in diameter and of the $G_r = 4 \times 10^4$, we can get the range 5×10^{14} cm, it means—all the solar system for the band-width 30 c/s and signal to noise ratio $S/N = 10$ db.

The numerical values given in Table 1 were calculated according to the formula given in this article or given in the quoted bibliography. One supposes that all mentioned satellites are observed from the Earth or from a platform on the satellite orbit close to the Earth. Only the Earth's satellite used for relativistic experiments is supposed to be observed from the Moon. In all cases it is assumed that the satellite has a circular orbit except in the case when the perihelium advance is investigated. For this case the excentricity ε and the height of the perihelium P, above the Earth's surface, were given. The velocity of the satellite v_s given in Table 1 is measured relatively to the central body.

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ATMOSPHERIC CONDUCTIVITIES OVER WHITE SANDS, NEW MEXICO

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IT is well known from the atmospheric dynamo theory that the daily geomagnetic variations observed at the ground is mainly due to the induced current systems in the upper atmosphere.

To investigate the induced current systems in the upper atmosphere quantitatively, it is necessary to know the various atmospheric conductivities.

Since the International Geophysical Year substantially more accurate data on the atmospheric structure have been accumulated from the rocket and satellite measurements of the atmosphere. For example, atmospheric densities calculated from observed orbit perturbations of artificial satellites differed from the densities given in the *ARDC Model Atmosphere* (1956) by as much as a factor of 20 (ref. 1). For this reason it is interesting to calculate the atmospheric conductivities using the recent more accurate data.

Conductivity formulæ. The ionosphere contains equal numbers of positive ions and negative electrons embedded in a neutral gas. When electric field $\vec{E}$ is applied to the ionosphere there will be the flow of current. The ionosphere is a non-isotropic conductor under the action of the Earth's magnetic field and has a tensor conductivity.

In the general case applied electric field $\vec{E}$ may be resolved into components $\vec{E}_\parallel$ and $\vec{E}_\perp$ respectively parallel and perpendicular to the Earth's magnetic field, $\vec{F}$. If $\vec{f}$ is a unit vector parallel to $\vec{F}$, the current density $\vec{j}$ is given by:

$$\vec{j} = k_0\vec{E}_\parallel + k_1\vec{E}_\perp + k_2(\vec{f} \times \vec{E}) \qquad (1)$$

where k_0, k_1 and k_2 are longitudinal, Pedersen, and Hall conductivities respectively. In deriving the formulæ for the conductivities some workers have used the mean-free-path method, and others the more exact 'velocity-distribution' method, originally developed by Chapman and Enskog. The formulæ derived by the mean-free-path method are simple and illuminating but their accuracy cannot always be assured. On the other hand, the formulæ derived by the velocity-distribution method are very accurate but are too complicated for practical applications unless certain approximations are made.

For the major part of the ionosphere the simplest model is a neutral ternary mixture consisting of particles of three kinds: (1) singly ionized positive ions; (2) electrons; (3) neutral particles.

Cowling[2] applied the Chapman–Enskog methods to the problem of a neutral ternary partially ionized gas and found the conductivities to a first approximation. Later, Chapman[3] discussed the conductivity formulæ in his review article on the subject. The results obtained by Cowling and Chapman will be summarized here.

Let the gases 1, 2 and 3 be the singly ionized positive ions, electrons and neutral molecules respectively. The mass and charge (in E.M.U.) of a particle of the rth gas are denoted by m_r, e_r; the number density and mass density for the rth gas are n_r, ρ_r; mass density and temperature of the gas as a whole are ρ and T; average collision frequency of a particle of kind q with particles of kind r is ν_{qr}; the coefficient of mutual diffusion of the two constituent gases, q and r, in the mixture is D_{qr}.

Cowling gave the conductivities k_1 and k_2, to a first approximation, as:

$$[k_1 + ik_2]_1 = i\rho L'/FL \tag{2}$$

where:

$$L = F^2\rho_3^2 n_1 n_2 e_1 e_2 - iF\rho\{\theta_{23} n_1 e_1(\rho - 2\rho_1) + \theta_{13} n_2 e_2 (\rho - 2\rho_2)\} - \rho^2 (\theta_{12}\theta_{13} + \theta_{21}\theta_{23} + \theta_{31}\theta_{32}) \tag{3}$$

$$i\rho L'/F = -\rho^2 n_1^2 e_1^2 (\theta_{13} + \theta_{23}) \tag{4}$$

$$\theta_{qr} = \frac{\rho_q \rho_r}{\rho_q + \rho_r}[\nu_{qr}]_1 = \frac{n_q n_r}{n_q + n_r}\frac{kT}{[D_{qr}]_1} = \theta_{rq} \tag{5}$$

In these formulæ, enclosure in square brackets with exterior suffix 1 denotes the first approximation.

The laws of interaction force between the neutral particles and the ions, or the electrons, are not known in the ionosphere. Chapman assumed that particles involved behave like rigid elastic spheres during the interactions. In this case:

$$[D_{qr}]_1 = \frac{3}{8(n_q + n_r)\sigma_{qr}}\left\{\frac{kT(m_q + m_r)}{2\pi\, m_q m_r}\right\}^{1/2} \tag{6}$$

where σ_{qr} is the equivalent diameter at collision and k denotes Boltzmann's constant.

For numerical discussion of the ionospheric conductivities, Cowling[2] adopted the value:

$$\sigma_{13} = 5{\cdot}9 \times 10^{-8}\,(300/T)^{1/4} \tag{7}$$

Nicolet[4] took:

$$\sigma_{23} = 1{\cdot}44 \times 10^{-8} \tag{8}$$

By substituting (6) into (5), it follows from (7) that:

$$\nu_{12} = 2{\cdot}6 \times 10^{-9}\,(n_1 + n_2)W^{-1/2} \tag{9}$$

and from (8) that:

$$\nu_{23} = 5{\cdot}4 \times 10^{-10} n_3 T^{1/2} \tag{10}$$

W denotes the molecular weights of the ions and neutral particles (taking them to be the same).

Ions and electrons do not interact as rigid elastic spheres; they interact according to the inverse square law of attraction.

In this case:

$$[D_{12}]_1 = \frac{3}{8(n_1 + n_2)A_1(2)}\left\{\frac{kT(m_1 + m_2)}{2\pi m_1 m_2}\right\}^{1/2}\left(\frac{2kTC^2}{e_1 e_2}\right)^2 \tag{11}$$

where $A_1(2)$ is a pure number and based on the Debye cut-off, and $C = 3 \times 10^{10}$. By substituting (11) into (5), and by adopting the value for $A_1(2)$ given by Nicolet[4], it follows that:

$$\nu_{12} = \{34 + 4{\cdot}18 \log (T^3/n_2)\}\, n_2/T^{3/2} \tag{12}$$

Now taking $n_1 = n_2$, and ignoring n_1/n_3:

$$\theta_{23}/\theta_{13} = 1{\cdot}13 \times 10^{-4}(T/W)^{1/2} \tag{13}$$

so that θ_{23} can be omitted from (3) and (4).

Omitting θ_{23} from (3) and (4), (2) becomes:

$$[k_1 + ik_2]_1 = \frac{n_2 e}{F}\Big/\left\{\frac{\rho_3\omega_1}{\rho\nu_{13}} - \frac{(\nu_{12} + \nu_{23})}{\omega_2} - i\right\} \tag{14}$$

where $\omega_1 = \dfrac{e_1 F}{m_1}$ and $\omega_2 = \dfrac{e_2 F}{m_2}$. When n_1/n_3 is small, so that ρ_3/ρ can be replaced by unity, (14) may be expressed in the form:

$$[k_1 + ik_2]_1 = \frac{n_2 e}{F}\Big/\left(\frac{1}{X_i} + X_e - i\right) \tag{15}$$

where:

$$X_i = \frac{\nu_{13}}{\omega_1} \tag{16}$$

and:

$$X_e = -\frac{\nu_{12} + \nu_{23}}{\omega_2} \tag{17}$$

When $F = 0$:

$$[k_0]_1 = [k_1 + ik_2]_1 = \frac{n_2 e^2}{m_2(\nu_{12} + \nu_{23})} \tag{18}$$

When Hall current is prevented from flowing, another conductivity may be defined as:

$$k_3 = k_1 + k_2^2/k_1 \tag{19}$$

k_3 is sometimes called the Cowling conductivity.

The conductivities k_0 to k_3 vary markedly with height. To estimate the current systems of the ionosphere it is necessary to know the values of the height-integrated conductivities rather than their particular values at each height.

If h is the height above the base of the ionosphere, the height-integrated conductivities will be given by:

$$K_i = \int k_i dh \qquad i = 0, 1, 2, 3 \tag{20}$$

Model of Upper Atmosphere over White Sands. To calculate the conductivities k_0–k_3 it is necessary to know the height distributions of T (temperature), W (mean molecular weight) $n_{1,2}$ (electron or ion density), n_3 (density of neutral molecules), and F (magnetic field intensity).

Although recent rocket measurements, and more recently, satellite measurements of upper atmosphere have revealed the reasonably accurate picture of the atmospheric structure, all five are still uncertain. Any distribution of these quantities considered, therefore, will be subject to certain criticism.

Height distributions of T, W and n_3 were adopted from the *ARDC Model Atmosphere* (1959)[1], which is based on the best curve drawn through plotted rocket and satellite density points. Height distributions of T and W are plotted in Fig. 1. T is taken at the constant value of 165·7 °K between 80 and 90 km. From 90 km T mounts slowly until it attains 221·8° K at 106 km. Above this level T mounts, very rapidly but almost linearly, with the average gradient to 16·5° per km until it reaches 1,359° K at 175 km. From 175 km T mounts again but more slowly, the average mount-rate being 0·9° per km. At 700 km T reaches 1,812° K.

The mean molecular weight, W, lapses monotonously with the height although the lapse-rate is different from one height to another, as shown in Fig. 1. W is taken as 28·97 from the ground-level to 90 km where molecular oxygen begins to dissociate because of the solar radiation. Above this level W begins to lapse. W lapses rapidly to 28·04 at 160 km and then lapses again almost uniformly, but more slowly to 17·03 at 700 km. In W-distribution the value 23·03 at 270 km corresponds to the complete dissociation of molecular oxygen, while the value 18·01 at 530 km corresponds to the 50 per cent dissociation of molecular nitrogen. In the work reported here the mean molecular weights of the positive ions and neutral molecules are taken to be the same. This assumption is reasonable since only singly ionized positive ions are considered here.

Fig. 2 shows the height distributions of n_2 and n_3. n_3 decreases very rapidly to $3 \cdot 748 \times 10^{10}$ per c.c. at 150 km. From 150 to 200 km it decreases again but more slowly. Above 200 km n_3 decreases almost exponentially. n_3-distribution is very different from those used by other workers[3,5]. This is due to the fact that the adopted n_3-distribution is based on the values of density recently obtained by use of rockets and satellites. The recent atmospheric densities calculated from observed orbit perturbations of satellites due to atmospheric drag, differed from the densities obtained previously by as much as a factor of 20.

The adopted distribution of n_2 from 300 to 700 km is that given in a graph of n_2 as a function of height by Townsend[6]. n_2-distribution from 130 to 300 km was adopted from the daytime electron density model of *ARDC Model Atmosphere* (1959)[1]. Below 130 km the adopted n_2-distribution is that given in a graph of n_2 as a function of height by Seddon and Jackson[7], which is essentially the summary of U.S. Naval Research Laboratory rocket measurements of the ionosphere over White Sands. n_2-distribution considered here corresponds to that of summer noon in sunspot maximum year. It should be noted here that the ratio $n_3/n_2 = 100$ at 480 km. This means that n_2 is negligible in comparison to n_3 below 480 km but not above this level.

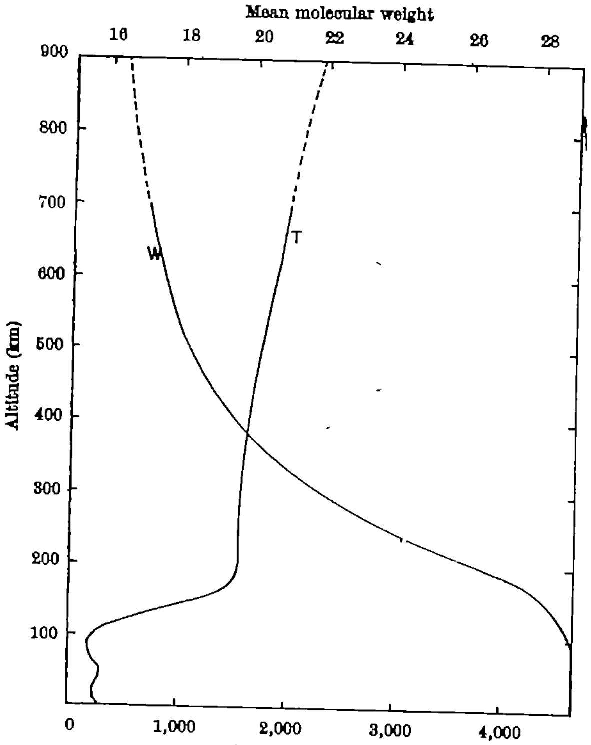

Fig. 1. Height distributions of temperature and mean molecular weight, *ARDC Model Atmosphere* (1959)

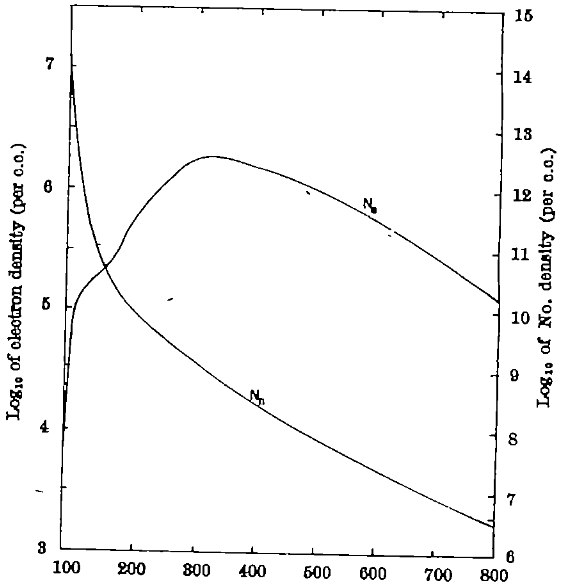

Fig. 2. Height distributions of neutral molecule and electron densities over White Sands

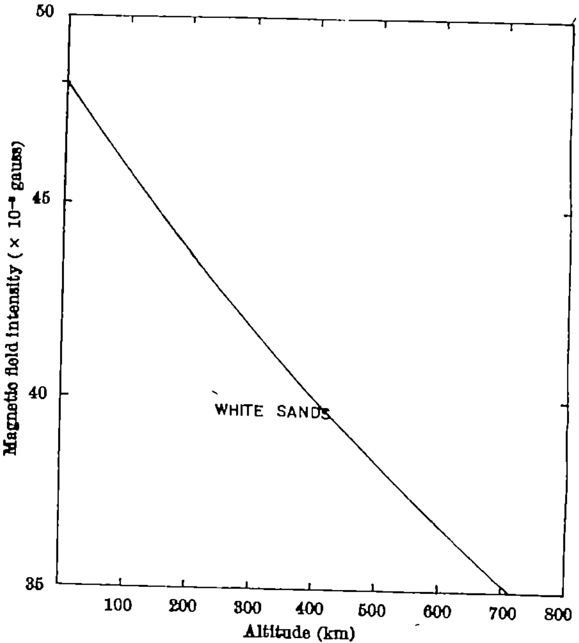

Fig. 3. Height distribution of magnetic intensity over White Sands

The distribution of the magnetic field intensity F was computed from equations (21) and (22), by assuming the Earth to be a dipole of magnetic moment $8 \cdot 19 \times 10^{25}$ c.g.s. unit:

Table 1. HEIGHT DISTRIBUTIONS OF CONDUCTIVITIES, k_0–k_3 AT WHITE SANDS

No. of row	Height (km) 80	90	100	110	130	150	200	250	300	350	500	700
1. n_3 (cm^{-3})	$3{\cdot}37^{3}$ *	$2{\cdot}92^{4}$	$1{\cdot}33^{5}$	1·46	1·79	2·17	$5{\cdot}96^{5}$	$1{\cdot}07^{6}$	1·83	1·73	1·00	$3{\cdot}00^{5}$
2. n_2 (cm^{-3})	$4{\cdot}410^{14}$	$5{\cdot}918^{13}$	$7{\cdot}783^{12}$	1·240	$1{\cdot}254^{11}$	$3{\cdot}748^{10}$	$6{\cdot}406^{9}$	$3{\cdot}145^{9}$	1·306	$5{\cdot}834^{8}$	$7{\cdot}290^{7}$	$8{\cdot}143^{6}$
3. W	28·97	28·97	28·90	28·82	28·59	28·27	26·32	23·87	21·95	20·55	18·28	17·03
4. m_2 (gm)	$4{\cdot}81^{-23}$	4·81	4·80	4·78	4·75	4·69	$4{\cdot}37^{-23}$	3·96	3·64	3·41	3·03	2·83
5. F (gauss)	$4{\cdot}64^{-1}$	4·60	4·59	4·57	4·52	4·49	$4{\cdot}37^{-1}$	$4{\cdot}28^{-1}$	4·19	4·10	3·83	3·52
6. T (°K)	165·7	165·7	199·0	286·7	664·9	1031·0	1,404	1,415	1,423	1,445	1,576	1,812
7. ν_{12} (sec^{-1})	$7{\cdot}44^{1}$	$5{\cdot}91^{2}$	$1{\cdot}96^{2}$	1·30	$4{\cdot}95^{2}$	3·24	$5{\cdot}55^{2}$	$9{\cdot}71^{1}$	$1{\cdot}61^{1}$	1·50	$7{\cdot}83^{2}$	2·02
8. ν_{23} (sec^{-1})	$3{\cdot}07^{6}$	$4{\cdot}11^{5}$	$5{\cdot}93^{4}$	1·13	$1{\cdot}75^{3}$	$6{\cdot}50^{2}$	$1{\cdot}70^{2}$	$6{\cdot}39^{1}$	2·66	1·20	$1{\cdot}56^{0}$	$1{\cdot}87^{-1}$
9. $\nu_{12}+\nu_{23}$ (sec^{-1})	$3{\cdot}07^{6}$	$4{\cdot}12^{5}$	$6{\cdot}13^{4}$	1·26	$2{\cdot}25^{3}$	$9{\cdot}74^{2}$	$7{\cdot}25^{2}$	$1{\cdot}04^{2}$	1·64	1·51	—	—
10. ν_{13} (sec^{-1})	$2{\cdot}13^{5}$	$2{\cdot}86^{4}$	$3{\cdot}76^{3}$	$6{\cdot}01^{2}$	$6{\cdot}10^{1}$	1·83	$4{\cdot}26^{0}$	1·67	$7{\cdot}25^{-1}$	3·35	$4{\cdot}43^{-3}$	$5{\cdot}13^{-3}$
11. X_e	$3{\cdot}76^{1}$	$5{\cdot}09^{-2}$	$7{\cdot}60^{-3}$	1·57	$2{\cdot}83^{-4}$	1·23	$9{\cdot}43^{-5}$	$1{\cdot}38^{-4}$	2·23	2·09	—	—
12. $1/X_i$	$7{\cdot}25^{-4}$	$5{\cdot}35^{-3}$	$4{\cdot}07^{-2}$	$2{\cdot}55^{-1}$	$2{\cdot}50^{0}$	8·40	$3{\cdot}76^{1}$	$1{\cdot}04^{2}$	2·54	5·75	—	—
13. k_0 (E.M.U. cm)	$3{\cdot}09^{-16}$	$2{\cdot}00^{-14}$	$6{\cdot}12^{-12}$	$3{\cdot}27^{-11}$	$2{\cdot}24^{-11}$	6·28	$2{\cdot}32^{-10}$	$2{\cdot}94^{-10}$	3·32	3·62	5·50	9·49
14. k_1 (E.M.U. cm)	$3{\cdot}83^{-17}$	5·71	$2{\cdot}24^{-16}$	$1{\cdot}23^{-15}$	2·19	$9{\cdot}09^{-16}$	$5{\cdot}82^{-18}$	$3{\cdot}86^{-18}$	2·76	1·18	$9{\cdot}22^{-19}$	$3{\cdot}65^{-19}$
15. k_2 (E.M.U. cm)	$1{\cdot}02^{-16}$	$1{\cdot}02^{-15}$	4·63	4·80	$8{\cdot}74^{-16}$	1·08	$1{\cdot}55^{-17}$	$3{\cdot}71^{-18}$	1·08	$2{\cdot}04^{-19}$	$2{\cdot}03^{-21}$	$9{\cdot}77^{-24}$
16. k_3 (E.M.U. cm)	$3{\cdot}07^{-16}$	$1{\cdot}82^{-14}$	9·59	2·00	$2{\cdot}54^{-15}$	$9{\cdot}12^{-16}$	$5{\cdot}82^{-18}$	$3{\cdot}86^{-18}$	2·76	1·18	$9{\cdot}22^{-19}$	$3{\cdot}65^{-19}$

* The forms of $N\times10^{+m}$ or $N\times10^{-m}$ are given in the forms of N^{m} or N^{-m}.

$$F_r = -\frac{2M\cos\theta}{r^3} \tag{21}$$

$$F_\theta = -\frac{M\sin\theta}{r^3} \tag{22}$$

where M is the magnetic moment of the Earth.

F_r and F_θ are r and θ components of the magnetic field intensity F at a point (r, θ), θ being measured from the geomagnetic north pole and r from the centre of the Earth. At White Sands θ and r at ground-level are taken as 48·7° and 6,370 km respectively. The computed F-distribution is plotted in Fig. 3.

Results at White Sands. (*a*) *Calculation of Conductivities, k_0–k_3.* For the calculation of the conductivities, k_0–k_3, the height range of 80–700 km is considered. Conductivities below 80 km and above 700 km are so small that they need not be considered. From 80 to 400 km conductivities, k_0–k_3, were calculated for each height at 10 km intervals and at 20 km intervals from 400 to 700 km.

By substitution of the atmospheric data given in the model of atmosphere into appropriate formulæ, the collision frequencies ν_{12}, ν_{13}, ν_{23} and the ratios X_e, $1/X_i$ were calculated for each height. Then conductivities, k_1 and k_2, were calculated for each height from either equation (15) or equation (2), depending on whether n_3/n_2 is greater than 100 or less than 100. k_0 and k_3 were calculated from equation (18) and equation (19) respectively. The results were plotted in Fig. 4. To save space only the results for the selected heights were tabulated in Table 1.

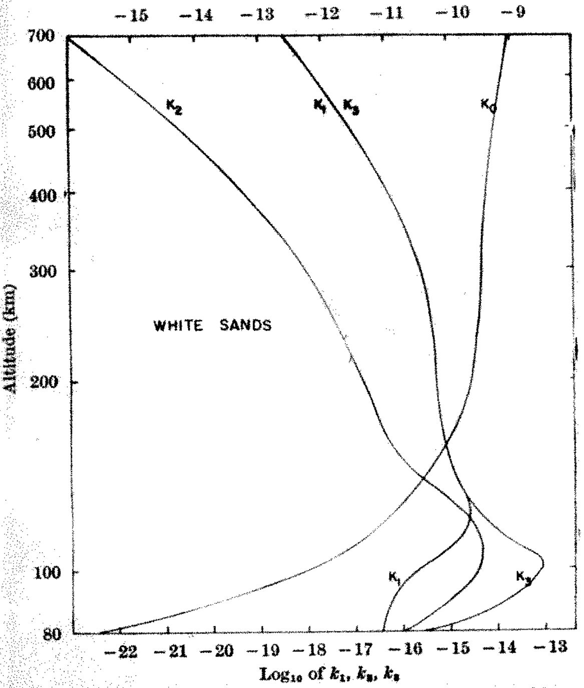

Fig. 4. Height distributions of conductivities, k_0–k_3 over White Sands (E.M.U. cm)

k_0 increases upwards over the range considered, though with a varying mount-rate as seen in Fig. 4.

The conductivities, k_1, k_2, k_3, each have two maxima in the range considered. In each case maxima corresponding to the lower altitude are more pronounced than those corresponding to the higher altitude. To determine the heights at which peak values of k_1, k_2, k_3 occur, additional calculations were made at appropriate intervening heights. The peak values of k_1, k_2, k_3, thus obtained, are tabulated in Table 2.

Table 2. PEAK VALUES OF ELECTRIC CONDUCTIVITIES AT WHITE SANDS

Conductivity	Peak level (km)	Peak value (E.M.U. cm)
k_1	121	$2{\cdot}93\times10^{-15}$
k_2	107	$4{\cdot}88\times10^{-15}$
k_3	98	$1{\cdot}01\times10^{-13}$

k_2 is larger than k_1 in the lower region of atmosphere, indicating that Hall current is dominant. At 118 km k_2 is equal to k_1. Above 118 km k_1 is greater than k_2 indicating that the currents are predominatingly Pedersen currents. Below 110 km Cowling conductivity k_3 is much greater than either k_1 or k_2. Above this level k_3 approaches k_1 and they coincide at 200 km as seen in Fig. 4. It should be noted from Fig. 4 that k_3 rises very sharply from 80 to 98 km, where its peak value occurs, and falls off very rapidly above 98 km. The layer in which Cowling conductivity k_3 exceeds a tenth of its maximum value is much thinner than the corresponding layers for k_1 and k_2.

(*b*) *Height-integrated conductivities, K_1–K_3.* The height-integrated conductivities K_1, K_2, K_3 were computed by integrating the conductivities, k_1, k_2, k_3 over the whole range considered. The total integrated conductivities, K_1, K_2, K_3 thus obtained are 17·4 × 10^{-9}, 14·9 × 10^{-9}, and 15·4 × 10^{-8} E.M.U. cm, respectively.

The integrated conductivities in the ranges between the heights in Table 1, and their percentage contributions to the total integrated conductivities, K_1, K_2, K_3 were calculated and tabulated in Table 3.

As shown in Table 3, the integrated conductivity of k_1 from 110 to 150 km accounts for 44·1 per cent of K_1. The contributions to K_1 from the regions below 110 km and above 250 km are negligible. 91·1 per cent of K_2 comes from the region of 90–130 km, indicating that the flow of Hall current is restricted in this region. For K_3, the major contribution

Table 3. CONTRIBUTIONS OF DIFFERENT LAYERS OF THE IONOSPHERE TO THE INTEGRATED CONDUCTIVITIES AT WHITE SANDS

Limit of intervals (km)	80–90	90–100	100–110	110–130	130–150	150–200	200–250	250–300	300–350	350–400	400–500	500–600	600–700
$10^9 \times \int k_1 dh$ (E.M.U. cm)	0·06	0·11	0·58	4·89	2·90	3·40	2·36	1·73	0·94	0·39	0·22	0·06	0·01
Percentage	0·3	0·6	3·3	27·7	16·4	19·3	13·4	9·8	5·3	2·2	1·3	0·4	0·1
$10^9 \times \int k_2 dh$ (E.M.U. cm)	0·47	2·75	4·79	5·83	0·71	0·07	0·04	0·01	—	—	—	—	—
Percentage	3·2	18·7	32·7	39·7	4·8	0·5	0·3	0·1	—	—	—	—	—
$10^9 \times \int k_3 dh$ (E.M.U. cm)	0·51	7·08	5·14	1·48	0·31	0·34	0·24	0·17	0·09	0·04	0·02	0·01	—
Percentage	3·3	45·9	33·3	9·6	2·0	2·2	1·5	1·1	0·6	0·3	0·1	0·0	—

comes from the region of 90–110 km, the percentage contribution of this region to K_3 being 79·2 per cent. This result indicates that the effective layer for the Cowling conductivity, k_3, is thin and located in the region of 90–110 km. This confirms the conclusions drawn by Baker and Martyn[5] and Chapman[3]. Table 3 also shows that the ionospheric conditions above 150 km have practically no influence on the results of the total integrated conductivities, K_2 and K_3.

(c) *Comparison.* The total integrated conductivities, K_1, K_2, K_3, obtained for the ionosphere over White Sands, are given in Table 4. The values obtained by Baker and Martyn[5], Maeda[8], Fejer[9], together with the two sets of values calculated by Chapman[3] for his two model ionospheres, Model *h* and Model *c*, are also given in Table 4 for comparison.

Table 4. COMPARISON OF DIFFERENT VALUES OBTAINED FOR HEIGHT-INTEGRATED CONDUCTIVITIES

Model	$10^9 \times K_1$ (E.M.U. cm)	$10^9 \times K_2$ (E.M.U. cm)	$10^9 \times K_3$ (E.M.U. cm)
Kim and Kim	17·64	14·67	15·43
Chapman—Model *h*	14·4	22·5	20·8
Chapman—Model *c*	9·9	17·8	19·3
Baker and Martyn	6·4	13·6	16·4
H. Maeda	6·7	9·8	7·75
J. A Fejer (equator)	6	21	18

These values, in general, show good agreement considering that different assumptions concerning ionospheric conditions were made for their calculations of conductivities in each case. However, it is contrary to the present results that the values of K_2 obtained by other workers are always greater than the corresponding values of K_1. The main reason for the discrepancy is that they overestimated the densities of neutral molecules in the region of 100–150 km, approximately by the factor of 10. The recent rocket and satellite measurements revealed that densities of atmosphere in this region were quite overestimated in the past.

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CRISTOBALITE IN THE CARBO IRON METEORITE

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CRISTOBALITE, SiO_2, has been identified by optical and X-ray determinations as an accessory mineral in a troilite nodule of the Carbo iron meteorite. Cristobalite has been reported in only two other meteorites: Abee, an enstatite chondrite[1], and Nuevo Laredo, a brecciated eucrite[2]. In both stones cristobalite and quartz occur together in very small traces.

In Carbo the cristobalite forms dendritic, microcrystalline aggregates within a droplet of green glass embedded in troilite. The droplet is irregular in shape with a maximum length of about 0·5 mm (Fig. 1). The cristobalite is clearly a devitrification product of the glass.

The Carbo iron meteorite. Carbo is a medium octahedrite, weighing about 1,000 lb., which was found in Sonora, Mexico, in 1923. It contains 90·64 per cent iron and 8·68 per cent nickel[3]. The absence of argon-39 indicates that it fell to the Earth more than 1,500 years ago[4].

Etched sections through the main mass show that the meteorite is essentially a single crystal of exsolved kamacite and taenite. Nodules of troilite, most of which are enveloped by a thin shell of schreibersite and numerous small, spherical nodules of graphite, occur in the metal without distortion of the structure.

The glass containing the cristobalite was embedded against the wall of a troilite nodule, 1 cm in diameter, in a section of the metal taken from about 9 cm beneath the crust. No cracks or veinlets connected the nodule to the outer surface of the meteorite. Within a few millimetres of the glass, the troilite contained two small grains of a dark red, transparent variety of chromium–iron spinel.

X-ray data. The cristobalite was identified by means of X-ray powder films made on minute shards of the glass. After 24 h of exposure in filtered copper radiation the films registered the four main lines of low-cristobalite. The strongest line is intense and

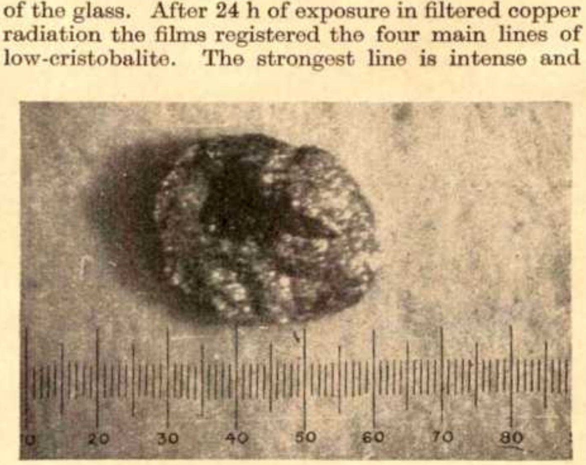

Fig. 1. Fragment of troilite with embedded glass, 10 divisions on scale equal 200μ. The dark, irregular patch of glass measures about 0·48 mm from its left-hand edge to the tip of the lobe at the right

sharply defined, the others are faint but distinct. The measured spacings are as follows:

Low-Cristobalite, Carbo		Low-Cristobalite, ASTM No. 4–0379		
I	*d*	*I*	*d*	*hkl*
100	4·05	100	4·04	101
10	3·14	12	3·138	111
20	2·83	14	2·845	102
20	2·48	18	2·489	200

Copper radiation, nickel filter
Camera: 57·3 mm radius

(Plus 33 more lines of intensity less than 6)

Optical properties. The fine-grained dendrites of cristobalite are faintly birefringent with a mean refractive index of 1·485. This value is identical with that reported for the mean refractive index of numerous natural and synthetic samples of low-cristobalite. No measurement was possible of the axial angle or the optic sign.

The glassy matrix has conchoidal fracture and is pale green and isotropic with refractive index of 1·590–1·600. This index is too high for silica glass (1·459). It does, however, correspond to that of some basaltic glasses[5] which contain about 50 per cent silica and range in index from 1·580 to 1·620.

Chemical composition. Qualitative analyses were made of one fragment of the glass with inclusions of cristobalite and one of the surrounding troilite on an electron probe X-ray microanalyser by Dr. F. Behn Riggs, jun., of the Smithsonian Institution. The *K* spectra of elements magnesium through rubidium and the *L* spectra of elements arsenic through tin were scanned on each fragment. The elements detected in the glass are silicon, chromium, iron, titanium, calcium and potassium. Of these, only silicon is abundant. Chromium is approximately 3 per cent or less, and the other four elements are present in amounts of less than 1 per cent.

The troilite contains iron, sulphur, and a small percentage of chromium.

The pulse height analyser was used to detect the presence of magnesium and aluminium in the glass, but neither element was found although test runs on standards and other materials indicated that the equipment would register as little as 0·10 per cent of magnesium and 0·06 per cent of aluminium.

The chemical composition of the glass differs significantly from that of other natural glasses. Although, as noted here, the refractive index resembles that of basaltic glass, the lack of magnesium and aluminium indicates that it cannot have been derived from any combination of the basaltic minerals that are common in meteorites: enstatite, $Mg_2(Si_2O_6)$; forsterite, $Mg_2(SiO_4)$; or calcic plagioclase, $CaAl_2Si_2O_8$.

No specific mineralogical composition is evident for this glass unless it represents a grain of one of the silica minerals, containing traces of calcium and potassium as impurities, that has taken chromium, iron, and titanium from the troilite to form a droplet of green silicate slag. The high refractive index of the glass matrix may result from depletion in silica following the crystallization of cristobalite.

Re-examination of 'Cristobalite' in the Kendall County iron. The first suggestion that cristobalite might occur in meteorites was made by Cohen[6], who reported finding tiny crystals of a cristobalite-like silicic acid ('cristobalitartige kieselsaure') in the black veins and nests of Kendall County, a brecciated hexahedrite containing 5·64 per cent nickel. To check on this occurrence, I removed a small portion of the soft black material from Kendall County and isolated some of the clear 'cubic and cubo-octahedral' crystals described by Cohen. These crystals are not truly isotropic, as reported by Cohen, but are faintly birefringent, complexly twinned, biaxial positive with 2 *V* medium to small, and a mean refractive index of 1·470. Their optical properties and X-ray powder pattern show that these crystals are not cristobalite but tridymite. Tridymite has long been known as an occasional accessory mineral in stony meteorites and stony irons. This occurrence in Kendall County establishes it as a constituent of an iron meteorite.

Significance of silica minerals in meteorites. Three of the polymorphic forms of silica—cristobalite, tridymite, and quartz—have been identified in meteorites, and although they occur as very minor accessories they are important for the indications they give of the conditions of meteorite petrogenesis.

In terrestrial rocks the predominant form of free silica is quartz, which is stable under most temperatures and pressures in the crust. Tridymite and cristobalite are rare, low-pressure polymorphs which are limited to near-surface deposits. Tridymite cannot withstand more than 3,000, or cristobalite more than 5,000 atmospheres of pressure without inverting to high quartz (Fig. 2, after Boyd and England[7]). This inversion takes place regardless of temperature or the buttressing effect of foreign ions in the lattices. Thus, the presence of either mineral sets severe upper limits to the pressures sustained by any host rock or meteorite.

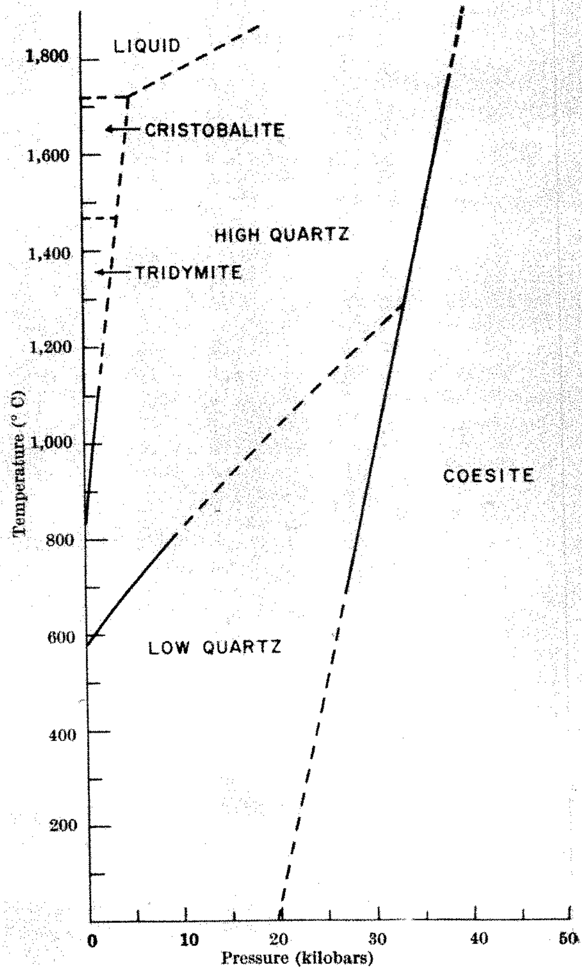

Fig. 2. Stability fields of five of the polymorphic forms of silica (after Boyd and England)

As temperature indicators cristobalite and tridymite are less reliable. They are stable only at high temperatures, but both minerals often form and persist for long periods as metastable phases at much lower temperatures.

In igneous rocks, such as lavas and tuffs, they crystallize in discrete grains which are generally interpreted as meaning a 'high' temperature of formation followed by rapid cooling. Cristobalite, however, also occurs in siliceous clays, gels and opals, and as a devitrification product of volcanic glass. In occurrences of this type it forms leafy, fine-grained aggregates at medium to low temperatures. Thus, although both minerals are dependable indicators of low pressures, the environment and mode of crystallization of each occurrence, particularly of cristobalite, must be considered before its significance can be determined with respect to temperature.

The tridymite in the Kendall County iron, and that described in numerous other meteorites, occurs in euhedral crystals or interstitial grains resembling the igneous tridymite of terrestrial volcanics. Such tridymite probably indicates high temperatures and clearly signifies crystallization under pressures of less than 3,000 atmospheres. This suggests that the iron meteorites as well as the stony irons and stones containing it may have originated in small parent bodies of the type postulated by Fish, Goles and Anders[8], with diameters of 1,000 km or less and core pressures of below 4,500 atmospheres.

The cristobalite in the Carbo iron is of a different nature, and probably bears no relationship to the origin and early development of the meteorite. It is the microcrystalline dendritic type of material which forms as a metastable phase at low temperatures and pressures. It crystallized during the cooling of the glass at a time when the interior of Carbo was under a pressure of less than 5,000 atmospheres. This might have occurred after the meteorite had separated from its parent body or, as a remote possibility, during the 1,500 years or more which have elapsed since it fell on the Earth.

Cristobalite is a normal devitrification product of silica-bearing glass. The presence of the glassy droplet itself within the troilite nodule is more difficult to explain. The formation of glass generally requires high temperatures followed by rapid cooling. For this reason the glass seems unlikely to be a primary constituent that survived millions of years of slow cooling while the metal assumed the structure of a gigantic single crystal. The glass must, therefore, be a relatively late product formed by some selective process of sudden re-heating or shock which melted one mineral grain to a droplet of silicate slag, but did not destroy the Widmanstatten patterns of the metal on the margin of the nodule, or melt the neighbouring troilite, schreibersite, or spinels.

Carbo shows only slight evidence of re-heating on one small area of its outer surface. However, Neumann-lines are abundant in the kamacite, and these indicate that it has been subjected to shock since the formation of the Widmanstatten patterns. Furthermore, the distribution of helium-3, as determined by Fireman[9], shows that the former core of the meteorite is now exposed on one surface, and that its present shape is the result of asymmetrical splitting or ablation relatively late in its history.

Carbo probably sustained shock, which caused the melting of the glass droplet as well as the modification of its shape and the Neumann-lines, by collision with another body in space, or on impact with the Earth. Of the two possibilities, the former seems more likely because Carbo is small enough to have landed on the Earth under conditions of free fall.

I thank Prof. Clifford Frondel for the use of specimens of Carbo and Kendall County meteorites, and for a critical reading of the manuscript; Dr. F. Behn Riggs, jun., for the electron probe X-ray microanalysis; and Dr. E. L. Fireman for helpful discussions.

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A HIGH-FREQUENCY AERIAL ARRAY WITH SURFACE WAVE LAUNCHING TO ATTAIN LOW ANGLES OF PROPAGATION

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THE maximum frequency permitted for oblique incidence, high-frequency, reflexion from the ionospheric F layer is assumed traditionally to vary as the function $f_c \sec\theta_i$ where f_c is the vertical incidence penetration frequency and θ_i the angle of incidence (measured to the normal) at the point of reflexion. Ray path mode plots[1] deduced on this basis predict maximum ranges and upper limits of useful frequency which are often exceeded in practice[2-4]. To the body of data depicting anomalous results based on observed modes, and also to the reports of the extreme ranges detected in trans-equatorial ionospheric propagation by point-to-point and back-scatter techniques, can be added the observations of antipodal propagation from satellites[6,7] carrying high-frequency transmitters.

There appears to be sufficient experimental evidence to indicate that for large values of θ_i the secant law is replaced by an exponential expression which can explain the focusing[8], or in the limit the 'trapped' type radiation. Although, for the normal F layer, of virtual height, say 400 km, the largest value for θ_i attainable for illumination from the Earth's surface is about 74°, there appear to be excellent reasons for approaching in practice as closely as possible to this limit. For the disturbed ionosphere, near-grazing incidence, leading to unusual propagation effects, can be experienced sporadically as reported anomalies indicate. Since most high-frequency aerial arrays in use at present have major lobes with maxima at angles between 7° and 14° above the Earth's tangent, conditions are far from those necessary to achieve optimum reflectivity at the ionosphere. There is strong evidence[5] to indicate that if modes of ionospheric propagation can be excited for which both the angles of fire and the angles of reception in the vertical plane are reduced to 2° or less, then higher operational frequencies and improved propagation should result. Such a possibility is of particular importance during the ensuing years of near-minimum sunspot activity with the associated depressed maximum usable frequencies.

In order to transmit and receive radiation of metre wave-lengths effectively at these extremely low angles in the vertical plane, the only method available is to use vertically polarized radiation launched above an earth plane for which the pseudo-Brewster angle, considered as measured with respect to the tangent plane, is itself less than the angle desired for the main ray. Then, theoretically, the aerial and its image are correctly phased to give enhancement along the tangent to the Earth's surface, and loss at vanishingly small angles is due to absorption rather than to ray interference. For the angular limits already discussed this requires propagation over a near perfect Earth, and in practice this means either over salt water or over an extensive, perfectly conducting metallic earth mat.

Work has been in progress in Melbourne for two years on the design of a suitable aerial array to achieve, in the high-frequency region of the radio spectrum, the desired polar patterns in the vertical and the horizontal planes. Laboratory experiments on models at 2,000 Mc/s, 800 Mc/s and 156 Mc/s led to the design of a full-scale prototype for operation at 25 Mc/s, and this was erected on the aerial test site of the Australian Post Office Research Laboratories at Tullamarine, near Melbourne, in 1961. It was used thereafter in long-range back-scatter experiments for a period of six months until early 1962[5]. Field data thus gathered indicated that propagation enhancements were observable and as a result, at present at the University of Melbourne, a larger, optimized array is being designed from model experiments scaled to 750 Mc/s. The performance to be expected, however, for radiation at high-frequency over an actual Earth cannot be simulated satisfactorily with such a model.

The prototype of 1961 was based on the end-fire principle with surface wave excitation, described for microwave frequencies by Elliott[9] and Simon and Biggi[10]. The theory of this was discussed by Hurd[11], by Robieux[12,13] and by Felsen[14], while model data, relevant in part, were given by Ehrenspeck and Poehler[15] and Ehrenspeck and Kearns[16]. The excitation of the slow wave—or artificial dielectric—structure was achieved by means of a vertical corner, 0·5λ high, included angle 110°, side-length 1·0λ, illuminated from an impedance transforming, double monopole element of height about 0·25λ but made variable for tuning purposes. The eighteen 'thick' monopole elements, 0·048λ in diameter, 0·16λ in height, were arranged in two domains with element spacings of 0·2λ and 0·3λ respectively extending over a distance of approximately 4·2λ. The complete configuration was erected above a copper mesh earth plane of average width 2λ which extended longitudinally beyond the array itself for 2λ, thereafter tapering to a width of 0·3λ at a range of 7λ from the excitation point, to merge finally with the actual earth which was estimated to have average electrical properties. The change in element spacing yielded a discontinuity for radiation, but achieved some measure of gradation between the slow wave phase velocities of 0·93 c. and 0·97 c. and that of free space c. As constructed, therefore, the array exhibited in an approximate fashion properties discussed for microwave frequencies by both Robieux[13] and Ehrenspeck and Poehler[15].

The unbalanced input impedance of the structure could be varied by the choice of feed point near the current anti-node region of the double monopole. For non-reactive conditions the input resistance was 175 ohms and was shown during construction to vary in a damped, periodic fashion as additional elements were added to the slow wave structure. Perturbations due to any particular element out to the fifteenth could be detected in this process. The useful bandwidth was approximately ± 1 Mc/s.

The gain of the array with respect to a quarter wave monopole over an equivalent Earth was approximately 8 db. at an elevation of 1°—a non-optimum condition for this particular array—with the front-to-back ratio greater than 24 db. for the same vertical angle. The gain with respect to the monopole for a constant elevation angle of 1° was found to decrease with range. Commencing with an estimate of + 10 db. in the Fresnel region, the mean of several measurements at 12λ was + 8·0 db.; at 25λ, + 7·2 db.; at 130λ, + 5·3 db.; at 800λ, + 5·0 db. and at 1,170λ, + 3 db. Near field and far field *E* plane and *H* plane polar diagrams were measured, and balloon-borne transmitters and aircraft flights were used to explore for the vertical plane the polar diagram in the far field with the existing conditions of an average earth. Some care was taken to establish in detail the minor lobe configurations in the azimuthal and the zenithal planes. The main results are depicted in the curves of Figs. 1 and 2: Fig. 3 is an illustration of portion of the actual array. The lobe structure for the horizontal plane as measured is narrower than theory predicts, probably due partly to the

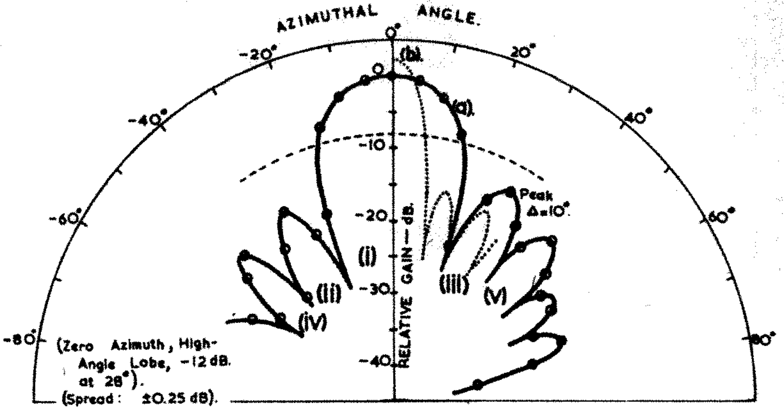

Fig. 1. *H*-plane polar pattern at elevation angle 1°, range 10λ. (*a*) ——, array (18 elements); (*b*)...., predicted for interferometry pair; – – –, reference monopole

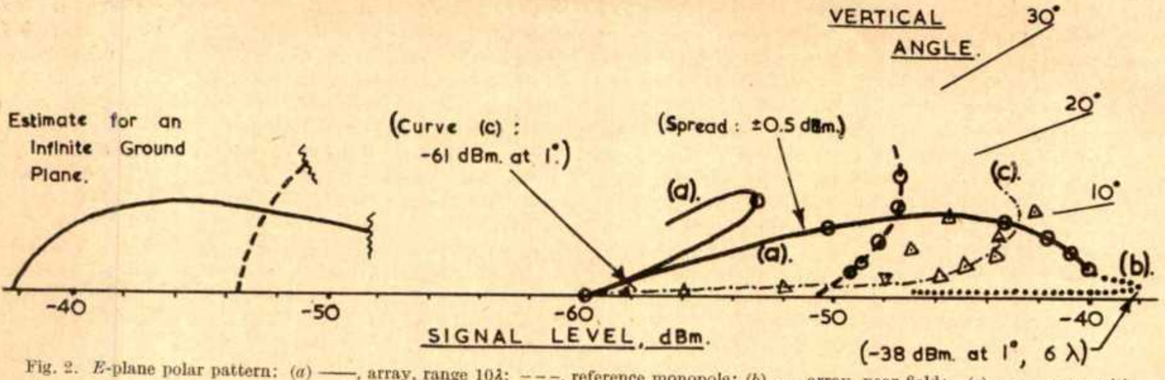

Fig. 2. *E*-plane polar pattern: (*a*) ——, array, range 10λ; – – –, reference monopole; (*b*) ....., array, near field; (*c*) —·—·—, array with aircraft at 7 miles

effect of the corner illuminated by a distributed source, and also to the elevation of the peak of the beam in the vertical as a result of the limitations of the earth plane. It is not considered, however, that the beam-width measured to the nulls is greatly in error.

For the vertical polar pattern (Fig. 2) the transition from the favourable near-field conditions, curve *a*, where the earth plane is effective, to the less satisfactory far-field performance over the actual Earth, curve *c*, is notable. The fine detail exhibited by curve *b* for the near field above an effective earth, but at extremely small vertical angles, emphasizes the complex situation which exists where surface wave and freely radiated electromagnetic field components co-exist. An extrapolation of what might be expected in the far field for perfect earth conditions is shown in the left-hand quadrant.

The degree of absorption at low vertical angles is controlled by the physical extent of the earth mat; accordingly, a low loss at say 1° elevation appears to be a practical aim. The unique feature which becomes clear from the present measurements is that the minor lobes numbered II, III, IV and V in Fig. 1 can be shown to have peaks at significantly higher elevation angles than applies for the major lobe I. This means that, by operating at the m.u.f. for the small angle of elevation of the main lobe, all spurious radiation from minor lobes will penetrate the ionospheric *F* layer and, in effect, a pencil beam type of array performance should result. This desirable property is enhanced if reciprocal transmitting and receiving array configurations are arranged to excite a single hop mode over a 4,000-km path. With a low, well-defined beam thus attained the azimuthal interferometry pattern of two such arrays, Fig. 1, curve *b*, will yield a new pair of minor lobes at low elevation angles. Within the near field of the structure, however, it is believed that these can be diminished by means of diffraction screens of low height and of moderate extent in the horizontal plane. For example, in the main beam of the prototype, at 25 Mc/s, some control of pattern between elevations of 1·5° and 1·25° was evident using a screen of width 2·5λ, height 0·1λ at a range of 4λ. This appears to be an occasion when a diffraction screen technique[17], successful at microwaves, has become applicable to a high-frequency array.

In the improved array derived from the model experiments at present in progress, the basic element will be a surface wave structure of length 5λ, with a configuration calculated from the Anger function analysis of Ishimaru and Bernard[18] suitably modified for the corner illumination by an extended source. A pair of such elements will be used in an interferometry arrangement of broadside spacing approximately 4λ, with perhaps some displacement, of the order of a few wave-lengths, along the line of fire to enable adjustment of the pattern in the vertical plane for high-elevation angles. Provision has been made for the addition, if radio-frequency correlation measurements prove favourable, of a second set of arrays similar to those just described to behave as a further interferometry pair, spaced perhaps fifty to one hundred wave-lengths distant along the great circle propagation path.

The effect of an extensive periodic structure of the type discussed is to produce a region over which the leaky wave gives rise to free radiation to create a virtual aperture in both the azimuthal and zenithal planes. Elliott[9] has shown clearly that this virtual aperture must be enabled to develop its full image in order to achieve effective radiation at the desired vertical angle. If this aperture extends for,

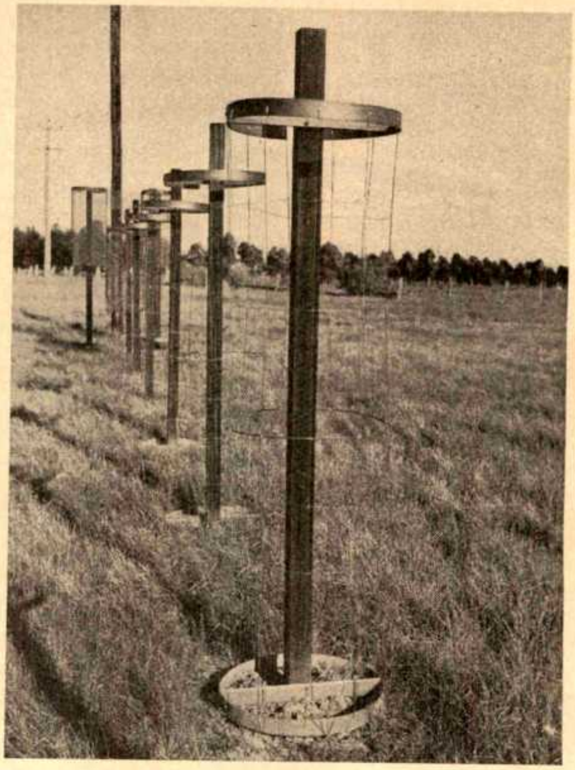

Fig. 3. Portion of slow wave structure, 25 Mc/s array, Victoria, Australia, Sept. 1961. (*Photo:* P.M.G.'s Department, Australia)

say, 3λ above the Earth's surface, and an angle of fire of 2° is required, allowing for some measure of Fresnel clearance, then a perfect earth should be provided for greater than 80λ outwards in front of each array group. Such earth plane dimensions have been specified for the full-scale array. Azimuth and zenithal slewing by radiofrequency phasing has been envisaged for a later stage. It is planned to construct this array, for operation at about 18 Mc/s, during the coming Australian summer.

The initial facilities and encouragement for this work were given by the Research Laboratories of the Australian Post Office, and the first prototype, partly shown in Fig. 3, was constructed by those Laboratories. Since then design has continued at the University of Melbourne and has received support from the Australian Radio Research Board. The co-operation of the Test Flight of the R.A.A.F. (A.R.D.U.) in the preparation of the far-field, vertical polar diagram of the 1961 prototype is acknowledged.

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PLEISTOCENE CLIMATES AND CULTURES IN NORTH-EASTERN ANGOLA

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THE pluvial/interpluvial framework of fluctuating Pleistocene climates in Africa, based on interpretation of geological morphology and sedimentation units, has, from the recent work of Flint[1], Cooke[2] and others, been shown to be still unproved for those cycles postulated before the Upper Pleistocene. The last interpluvial and last, or Gamblian, pluvial and later cycles are, however, reasonably well attested in the type area from terraces in the closed basins of the lakes in the East African Rift and also from river valley deposits in southern and central Africa where cultural remains are often associated. As yet, however, no precise means of estimating the extent of humidity and temperature fluctuations from this sedimentary evidence is available.

Confirmation of the geological evidence for the fluctuations which occurred since the beginning of the last pluvial is now being provided by pollen investigations. These not only reflect significant changes in the vegetation patterns but also provide a much better basis for estimating the extent and nature of these changes. Comparison of the pollen diagrams from widely separated localities (Kaisungor, Cherangani Hills, north-western Kenya[3]; the Kalambo Falls south of Lake Tanganyika[4] and Florisbad, near Bloemfontein[5] with those obtained from the Lunda region of north-eastern Angola described here show that these changes, which are synchronous at such widely separated places, can only have been brought about by climatic change.

In several cases the pollen spectra have been dated by radiocarbon and in the last three instances are directly related to prehistoric cultural material. The Angola radiocarbon-dates obtained recently by the University of California Laboratory at Los Angeles (UCLA) provide good supplementary evidence for a general correlation between the Last Pluvial in sub-Saharan Africa and the Wurm glaciation in Europe. Thus there now exists the basis for a more exact method of correlating fluctuations in the tropical humidity and temperature pattern of the past 60,000 years with the glacial and post-glacial cycles in the temperate zone of the northern hemisphere. These results can also provide the prehistorian with the ecological evidence he requires for estimating the effect of environment on the culture pattern and for suggesting the causes of variation in population density.

The northern half of Angola is drained by the southern tributaries of the Congo. These have cut deep valleys through the sands of the Kalahari System and through the later redistributions of these sands which overlie erosion surfaces now preserved as the interfluves between the main rivers. The rivers in the north-east all run parallel in a northerly direction and have their origin on the watershed between the Kasai and the Zambezi about 370 km south of Dundo. The valleys in which the sites recorded here are found lie between 700 and 800 m above sea-level while the sands of the interfluves stand at altitudes of 900–1,000 m. Opencast mining operations in the north-east corner of Angola round Dundo have yielded much prehistoric cultural material which, sometimes, where the deposits have remained waterlogged, is found in association with organic remains.

The vegetation of the Province of Malange, which forms the north-eastern part of Angola, can, according to Gossweiler and Mendonça[6], briefly be described as follows:

The province consists of two districts, the smaller Malange district in the west and the much bigger eastern Lunda district. On its north and east sides this province borders the Congo and on the south is

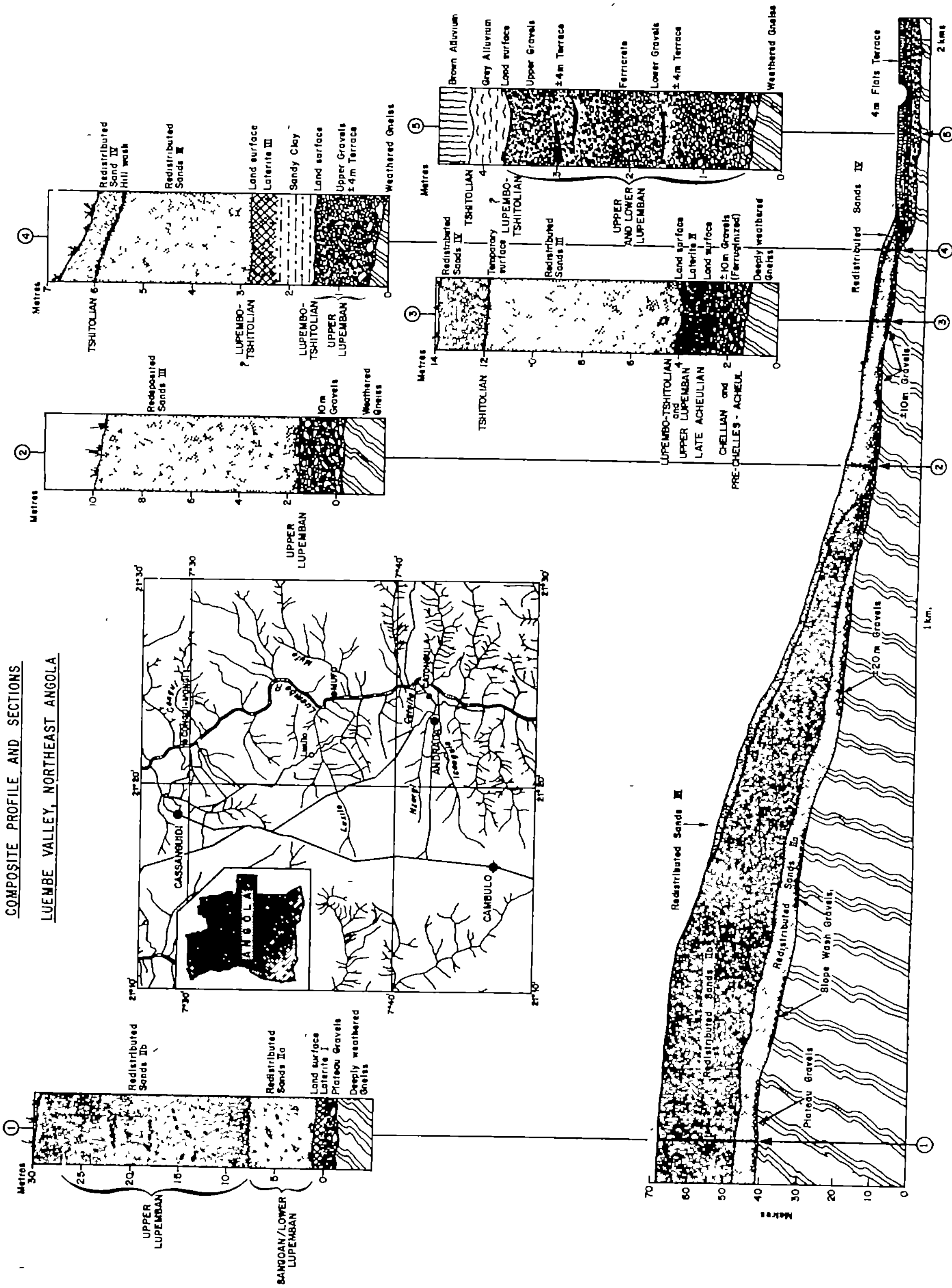
COMPOSITE PROFILE AND SECTIONS
LUEMBE VALLEY, NORTHEAST ANGOLA
① Metres 30 25 20 15 10 5 0
Redistributed Sands IIb
Redistributed Sands IIa
Land surface Laterite I Plateau Gravels
Deeply weathered Gneiss
UPPER LUPEMBAN
SANGOAN/LOWER LUPEMBAN
② Metres 10 8 6 4 2 0
Redeposited Sands III
10m Gravels
Weathered Gneiss
UPPER LUPEMBAN
③ Metres 14 12 10 8 6 4 2 0
Redistributed Sands IV
Temporary ? surface
Redistributed Sands III
Land surface Laterite II Land surface ±10m Gravels (Ferruginized)
Deeply weathered Gneiss
TSHITOLIAN
LUPEMBO-TSHITOLIAN and UPPER LUPEMBAN
LATE ACHEULIAN
CHELLIAN and PRE-CHELLES - ACHEUL
④ Metres 7 6 5 4 3 2 1 0
Redistributed Sand IV Hill wash
Redistributed Sands III
Land surface Laterite III
Sandy Clay
Land surface Upper Gravels ±4m Terrace
Weathered Gneiss
TSHITOLIAN
? LUPEMBO-TSHITOLIAN
LUPEMBO-TSHITOLIAN
UPPER LUPEMBAN
⑤ Metres 4 3 2 1 0
Brown Alluvium
Grey Alluvium
Land surface
Upper Gravels
±4m Terrace
Ferricrete
Lower Gravels
±4m Terrace
Weathered Gneiss
TSHITOLIAN
? LUPEMBO-TSHITOLIAN
UPPER AND LOWER LUPEMBAN
21°10' 21°20' 21°30'
7°30' 7°40'
CASSANGUIDI
CAMBULO
ANDRADA
ANGOLA
Luembe R.
Lexilo
Cassonso
Metres 70 60 50 40 30 20 10 0
Redistributed Sands III
Redistributed Sands IIb
Redistributed Sands IIa
Slope Wash Gravels
Plateau Gravels
±20m Gravels
±10m Gravels
Redistributed Sands IV
4m Flats Terrace
1 km.
2 kms
① ② ③ ④ ⑤

Fig. 1

practically delimited by the Kasai/Zambezi watershed.

The Malange district is covered with a high grass–low tree savannah in which *Canarium*, *Combretum* and *Diplorhynchus* play an important part. The Lunda district, as most of the eastern half of Angola, is covered with deciduous woodlands (Hiemilignosa). This woodland of the plateau (Hiemisilva do planalto) is closely related to the *Brachystegia–Isoberlinia* woodland of the Rhodesias. On the plateau areas covered with grass undershrubs are also found. Along the rivers we find smaller and bigger stands of evergreen fringing forest which show affinities with the rain-forest of lower altitudes. In the rivers Podostemonaceae occur up to an altitude of 1,300 m.

In the southern districts of Moxico, Bié, Benguela and Huila relics of an evergreen montane forest rich in *Podocarpus* are found at altitudes of 1,600–2,200 m. This forest type at present only occurs at least 320 km south of Dundo.

Investigations in 1959 and 1960 enabled the prehistoric cultural succession to be more closely related to the morphological history of the region and the phases of alluvial deposition to the later redistributions of the sands of Kalahari type. The general succession in the main rivers (Luembe, Chiumbe, Luachimo and Tshikapa) is seen in Fig. 1. The sites yielding cultural material in association with pollen spectra are situated at Mufo and Malumba Mines (7° 36′ S., 21° 25′ E.) in the valley of the Luembe River; at Furi I Mine on the stream of that name draining into the Chiumbe River and at Calunda 3 Mine situated on the upper reaches of a tributary stream draining to the Tshikapa River. The last site lies some 80 km to the south-west of the others.

River Valleys

At Mufo and Malumba the later alluvial sediments fill a buried channel and rise to form a low aggradation terrace (Flats Terrace) some 3–4 m above the present channel of the river. Typical sections at these mines can be seen at 4 and 5 in Fig. 1.

These sediments are divisible into two series—an earlier and lower series comprising gravels, sometimes, as at Mufo, divisible into two phases, and an overlying, or later, alluvial series of current bedded sands and dark clays. Series I can be dated to the Last Pluvial and Series II is post-Pleistocene in age. Two accumulations of sands (Redistributed Sands III and IV), partly colluvial and partly aeolian in origin, cover the gravels and alluvium respectively (Fig. 1; 2–4). The absolute dating, cultural associations and vegetation patterns pertaining when these sediments were laid down are given below:

I. Lower Gravels of the Flats Terrace (Series Ia): Early Gamblian (Fig. 1; 5).

Absolute Age. UCLA-168, 38,000 ± 2,500 years B.P. UCLA-169 > 34,000; obtained from peat and wood.

Culture. The most advanced artefacts present belong to an early Middle Stone Age (Lower Lupemban) industry.

Pollen Spectrum. Indicates a climate probably slightly less warm than to-day.

II. Upper Gravels of the Flats Terrace (Series Ib) Later Gamblian (Fig. 1, 4 *and* 5).

Absolute Age. Not yet dated, but wood from the topmost 50 cm at another section at Mufo was dated (Chicago–581) to 14,503 ± 560 years B.P.[7]. An industry of similar cultural stage belonging to similar pollen spectra at the Kalambo Falls is dated (Lamont, *L*-399I) to between 30,500 ± 2,000 and 27,000 ± 2,300 years B.P.[8].

Culture. Later Middle Stone Age (Upper Lupemban) tools.

Pollen Spectra. The two spectra counted both represent a vegetation characteristic of a climate definitely cooler and wetter than to-day. The *Brachystegia* woodland was replaced by a montane forest vegetation.

III. Land Surface at the top of the Flats Gravels and overlain by Redistributed Sands III on the valley flanks: Terminal Gamblian (Fig. 1; 4).

Absolute Age. A date of 11,189 ± 490 years B.P. was obtained by Chicago in 1951 (*C*-580) from wood lying on the surface of the Flats Gravel at Mufo and overlain by 2·5 m of redistributed sand[7]. (See also IV).

Culture. A worked blade of Second Intermediate (Lupembo–Tshitolian) or Later Stone Age (Lower Tshitolian) type was found by Janmart[9] in association with this wood.

Pollen Spectrum. Not known.

IV. Peaty Clay from the Series II deposits overlying the Upper Flats Terrace in the centre of the valley at Mufo and Malumba: Makalian Wet Phase.

Absolute Age. UCLA-167 6,830 ± 120 years B.P. from sample from Mufo.

Culture. Sterile in the immediate layer dated but at Malumba Later Stone Age (Tshitolian) artefacts are buried by these deposits.

Pollen Spectra. Two pollen spectra indicate warm and moist conditions.

Stream Valleys

The foregoing results are supplemented by those obtained from peaty clays and wood from gravels exposed in sections in the beds of tributary streams. These 'Lower Stream Gravels' are of Post-Gamblian age. They must be divided into an original depositional phase equivalent in time to the upper alluvial series of sediments composing the Flats Terrace in the main rivers and a later, resorted phase belonging to the present cycle, though it is often difficult to distinguish the two apart.

V. Lower Levels of Redistributed Sands III overlying the youngest aggradations of the Upper Stream Gravels at Calunda 3 *Mine: Terminal Gamblian.*

Absolute Age. UCLA-172 12,970 ± 250 years B.P. obtained from charcoals.

Culture. Same as III.

Pollen Spectrum. Not known.

VI. Lower Stream Gravels exposed just below water level at Calunda 3 *Mine: Makalian Wet Phase.*

Absolute Age. UCLA-171 4,700 ± 100 years B.P. obtained from wood.

Culture. None at this particular site but, elsewhere, Tshitolian.

Pollen Spectrum. Indicates a climate resembling that of the present day though slightly warmer and wetter.

VII. Lower Stream Gravels exposed just below water level at Furi I Mine: resorted gravels of the present channel.

Absolute Age. UCLA-170 1,880 ± 80 years B.P. obtained from wood.

Culture. Early Iron Age potsherds are reputed to have been recovered from the gravels adjacent to the section from which the dated sample was taken.

Pollen Spectrum. Much the same as the present-day vegetation.

Discussion

Tabulating the evidence and correlating it with that obtained from the Kalambo Falls excavation (8° 35′ S., 31° 15′ E.)[10] the results set out in Table 1 are obtained.

The fossil pollen spectra show that the temperature decreases from the drier Gamblian Interval to the Main Stage of the Gamblian. This fall in temperature was apparently quite distinct and can have been equivalent to a rise in altitude of at least 500 m.

Table 1. TENTATIVE CHRONOLOGY OF CULTURES, VEGETATION AND CLIMATES IN LATE AND POST PLEISTOCENE TIMES IN SOUTH-CENTRAL AFRICA

Climatic stages	Angola vegetation and climate	Main cultural division	Kalambo Falls cultures and C-14 dates B.P.	Angola cultures and C-14 dates B.P.
Present phase	Present vegetation and climate	Iron Age	±875	Iron Age (±1800)
Makalian wet phase	Gallery forest and Brachystegia woodland (warm and wetter)	Later Stone Age		Tshitolian (±4,700; ±6,830)
		Second intermediate	Magosian (±9,550)	Lower Tshitolian (±11,189) Lupembo-Tshitolian (±12,970)
Main Gamblian Stage	Montane forest and Brachystegia woodland of higher altitudes (wetter and cooler)	Middle Stone Age	Upper Lupemban (30,500–25,500)	Upper Lupemban (±14,503)
Drier interval	Dry open Brachystegia woodland (present day climate)			Lower Lupemban (±38,000)
Early Gamblian stage		First intermediate	Sangoan (41–43,000)	
Pre-Gamblian Non-Pluvial		Earlier Stone Age	Late Acheulian (±57,300)	

Good proof has further been found for a climatic optimum during the Makalian Wet Phase which was marked by a high humidity and a raised temperature. Since this climatic optimum, temperature and humidity must have declined.

Making comparison between the prehistoric cultures of Africa and of Europe it is apparent from the Angola and Kalambo Falls radiocarbon-dates that the Sangoan is in large part contemporary with the Cold Mousterian.

Some appreciation of the duration of each of the main cultural stages can also be attempted, but, owing to the discontinuities in the time scale represented by the erosion phases of the stratigraphical record, it is not yet possible to obtain any accurate estimate. It seems apparent, however, that the rate of culture change was not constant and was accelerated during the 'intermediate phases'. The pollen evidence lends support to the belief that such times of more rapid cultural evolution were also times of readjustment to changed environmental conditions.

We thank the Companhia de Diamantes de Angola for its generous support of the archæological investigation in that territory and Dr. Willard F. Libby and his colleagues of the Radiocarbon Laboratory of the University of California at Los Angeles for dating the samples. The South African Council for Scientific and Industrial Research and the Nuffield Foundation financially assisted one of us (E. M. v. Z. B.) with his pollen research.

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ETHYLENE MALEIC ANHYDRIDE COPOLYMERS AS VIRAL INHIBITORS

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POLYMERS, macromolecules of both natural and synthetic origin, have been shown to be inhibitory to a number of biological systems, including enzymes[1-3], tumours[2,6,7], bacteria[8,9] and viruses[10,11]. In 1942, Cohen was able to concentrate tobacco mosaic virus and various proteins with anionic polymeric hydrophilic colloids such as heparin and hyaluronic acid[10]. More recently, Burger and Stahmann[12] and others[13-17] inactivated tobacco mosaic virus, mumps, Type *B* influenza, and Newcastle disease virus with cationic macromolecules, such as polylysine and polyvinylamine. Neher and Kradolfer[18] inhibited influenza *B* and Newcastle disease with aromatic polysulphonates, and Stahmann and Gothoskar[19] prevented tobacco mosaic virus infection with polyglutamate, polyacrylate, and polypectate polymers. In 1958, Heymann *et al.*[20] examined the effect of a series of polymeric anionic ribonuclease inhibitors on a number of viruses. Young and Mowra[11] have shown inhibition of *E. coli* bacteriophage (*T*-2) with a polyglucose sulphuric acid polyanion.

We have been working with anionic polyelectrolytes (that is polysulphonates, polyphosphates, and polycarboxylates) which produce significant inhibition of transplanted tumours, as well as *in vitro* inhibition of deoxyribonuclease II, the intracellular deoxyribonuclease[4,5]. The biological activity of the ethylene maleic anhydride copolymers suggested the possibility that they might exert inhibitory effects on animal viruses. The polymers examined have included polycarboxylates derived from ethylene maleic anhydride[6]. *In vitro* prevention of viral cytopathogenicity in monkey and hamster kidney cells in tissue culture was used as the index of effect.

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The viruses used in this investigation were Echo 9 Hill strain (through the courtesy of Dr. David Karzon, University of Buffalo School of Medicine), and Semliki Forest (through the courtesy of Dr. Hilary Koprowski, Wistar Institute, Philadelphia, Pennsylvania).

Monkey and hamster kidney fibroblasts (provided by Microbiological Associates, Inc., Bethesda) were maintained in medium No. 199 at 37° C. No additional nutrient supplements such as serum or chick embryo extract were added.

Hydrolysed ethylene maleic anhydride and ammoniated ethylene maleic anhydride copolymers of molecular weights ranging from 2,000 to 120,000 were obtained from the Monsanto Chemical Corporation, Dayton, Ohio. Before use, these polymers were filtered through ('015 *VFA* Selas') sintered glass filters, and were frozen at − 4° C. All polymers tested were readily dissolved in saline and adjusted to a *p*H of 7 with N sodium hydroxide.

The concentration of ammoniated ethylene maleic anhydride or hydrolysed ethylene maleic anhydride copolymers used in this work was below that which produced apparent toxicity to the tissue culture test systems, as evidenced by the absence of *p*H and morphological changes and the unimpaired ability of the cells to proliferate on sub-culture in the presence of polymer-containing medium.

Echo 9 was assayed in monkey kidney cell cultures, and Semliki Forest in hamster kidney cell cultures. Cytopathogenicity (CPE) was recorded semi-quantitatively: negative (−), equivocal or early (±) or positive (+). Negative (−) cultures showed no alteration of the normal tissue culture morphology and growth pattern. Equivocal or early CPE (±) was shown by initial cytopathogenic effect, with some rounding of cells and some isolated areas of CPE. Positive CPE (+) was indicated by the complete destruction of the tissue culture sheet.

Experiments were designed to determine the effect of timed addition and removal of copolymers before and after inoculation of Echo 9 on monkey kidney cells. One-minute exposure of monkey cultures to undiluted Echo 9 virus, which had a tissue culture infective dose$_{50}$ TCID$_{50}$ of 10^6/0·1 ml., followed by 5–6 washings with fresh 5 c.c. amounts of media number 199, or isotonic saline, was sufficient to produce complete cytopathogenic effect in four days.

For each experiment, at each dilution, cytopathogenic effect was ascertained with 3 tissue culture tubes, and results were usually uniform for all three tubes. For presentation of results in Tables 2 and 3, when an odd tube appeared, results are a summary of two of three tubes, or reported as + when a ± and + appeared simultaneously. At zero time, 0·1 ml. of undiluted Echo 9 virus was inoculated into replicate monkey kidney tissue culture tubes. Maintenance medium number 199 was decanted 2, 4, 6, 11, 15, 21, and 26 h later; the cells were washed 5 times with 5 c.c. of isotonic saline for each wash, and fresh medium number 199 was added. To the first, second, and third tube in each group of 3 was added 0·1 ml. of hydrolysed ethylene maleic anhydride 214 (mol. wt. 20–30,000) diluted with isotonic saline 1:1, 1:10, and 1:100 respectively. Cytopathogenic effect was determined by daily examination of tissue culture tubes during the 6-day observation period. (Table 1. Results obtained at 4 and 11 h are omitted because they are essentially the same as those obtained at the immediately preceding observation times.)

When hydrolysed ethylene maleic anhydride 214 was added to the medium at concentrations of 1×10^{-4}, 10^{-5}, and 10^{-6} M after 2–6 h exposure to Echo 9 virus, complete protection was afforded for up to 6 days (Table 1). Similar protection occurred when hydrolysed ethylene maleic anhydride 214 was added as late as 15–26 h after viral inoculation. However, with exposure to virus for this period, the 2 days immediately following addition of copolymer to the medium were characterized by some degeneration (±) of the tissue culture, which repaired itself by the third day.

The following method was developed for comparing the viral inhibitions activity of the different copolymers.

In this method, the tissue culture passage virus was used in 10-fold saline dilutions from 10^{-1} to 10^{-6}. To each 1 ml. of a virus dilution was added 1 ml. (10 or 25 mg) of the polymer test compound. This mixture was allowed to incubate for 1 h at 22° C. After the incubation period, 0·1 ml. of each dilution of virus–copolymer was transferred to each of 3 tissue culture tubes which were then incubated at 37° C and observed daily for cytopathogenic effect.

The results of one experiment comparing the activities of ammoniated ethylene maleic anhydride copolymers and hydrolysed ethylene maleic anhydride copolymers of varied molecular weight on pre-incubation with 10^{-1}–10^{-6} dilutions of Echo 9 virus are presented in Table 3. The results indicate that the copolymers of high molecular weight showed the best inhibition of viral cytopathogenicity. Ammoniated ethylene maleic anhydride copolymers and hydrolysed ethylene maleic anhydride copolymers of molecular weights approximating 120,000 completely prevented cytopathogenicity at all virus concentrations (10^{-1}–10^{-5}).

A summary of experiments with ethylene maleic anhydride copolymers is listed in Table 3. All

Table 1. PROTECTION BY HYDROLYSED ETHYLENE MALEIC ANHYDRIDE (mol. wt. 20–30,000) AFTER VIRAL (Echo 9) INOCULATION

Time of pre-polymer viral exposure	2 h				6 h				15 h				21 h				26 h			
		Polymer dilutions																		
Days post viral inoc.	Saline control (S.C.)	1×10^{-4} 1:1	1×10^{-5} 1:10	1×10^{-6} 1:100	S.C.	1:1	1:10	1:100	S.C.	1:1	1:10	1·100	S.C.	1·1	1.10	1:100	S.C.	1:1	1·10	1·100
1	±	−	−	−	±	−	−	−	±	±	±	±	±	±	±	±	±	±	±	±
2	+	−	−	−	+	−	−	−	+	±	±	±	+	±	±	±	+	±	±	±
3	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−
4	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−
6	+	−	−	+	+	−	−	±	+	−	−	−	+	+	−	−	+	−	−	−

0·1 c.c. of undiluted viral suspension to each tissue culture tube. Each experimental group consists of 3 tubes. Virus in medium number 199 is washed from tissue culture, 5 times with an equal volume of saline, and 1·0 c.c. of fresh medium number 199 is added.
Protection by hydrolysed ethylene maleic anhydride copolymer added to tissue culture 2–26 h after viral exposure.

Table 2. PROTECTION AGAINST ECHO 9 VIRUS CYTOPATHOGENICITY TO MONKEY KIDNEY CELL CULTURES

	Polymer No. 1	Polymer No. 2	Polymer No. 3	Polymer No. 4	Polymer No. 5	Saline control
Specific viscosity	0·1	0·1	0·67	1·8	1·8	
Approx. mol. wt.	2–3,000	2–3,000	20–30,000	120,000	120,000	
Approx. mol. conc. polymer on pre-incubation with virus	2×10^{-3}	2×10^{-3}	4×10^{-5}	4×10^{-6}	4×10^{-6}	
Pre-incubation virus conc.						
10^{-1}	+	+	+	−	−	+
10^{-2}	+	+	±	−	−	+
10^{-3}	±	+	−	−	−	+
10^{-4}	−	+	−	−	−	+
10^{-5}	−	−	−	−	−	+
10^{-6}	−	−	−	−	−	−

Results of a typical experiment showing protection afforded by ethylene maleic anhydride copolymers of higher molecular weight.

Polymer No. 1. Ammoniated ethylene maleic anhydride 732
Polymer No. 2. Hydrolysed ethylene maleic anhydride 213
Polymer No. 3. Ammoniated ethylene maleic anhydride 750
Polymer No. 4. Ammoniated ethylene maleic anhydride 735
Polymer No. 5. Hydrolysed ethylene maleic anhydride 781

copolymers tested, having molecular weights ranging from 2,000 to 120,000, prevented cytopathogenicity by a factor of at least two tenfold dilutions. Where complete protection from cytopathogenic effect in 6 days did not occur, cytopathogenic effect was often delayed 24–48 h. The most active viral inhibitors were those of highest molecular weight. The ammoniated ethylene maleic anhydride copolymers 750 and hydrolysed ethylene maleic anhydride 731 copolymers also prevented Semliki Forest virus cytocidal activity. In addition, virus–copolymer mixtures were pre-incubated and afterwards diluted immediately before introduction into the tissue culture. It was found that the copolymers could be diluted out so that they would no longer protect against viral cytopathogenicity at dilutions around 10^{-2}.

Incubation of undiluted Echo 9 virus and Semliki Forest virus suspensions for 1 h at 37° C, 22° C, and 4° C, prior to tissue culture inoculation, resulted in a progressive increase in cytopathogenic potency with decreased incubation temperature. Despite the increase in cytopathogenic effect seen for Semliki Forest virus at the lower incubation temperatures, similar protection was afforded by hydrolysed ethylene maleic anhydride 214, 215, 731 (mol. wt. 20,000–120,000) at both 4° C and 22° C incubation temperatures.

The cytopathogenic effect produced in monkey and hamster kidney cell cultures by Echo 9 virus and Semliki Forest virus was prevented by exposure of the viruses to the amide-acid and hydrolysed derivatives of ethylene maleic anhydride copolymers. Cytopathogenicity could be prevented or delayed by the direct addition of the polyelectrolyte copolymers during the initial incubation of virus in the tissue culture cells (that is up to 26 h after inoculation of Echo 9 virus).

A method has been developed for comparing the anti-viral activity of the copolymers. The results indicate that the ethylene maleic anhydride copolymers of higher molecular weight are the most potent inhibitors of viral CPE (Tables 2 and 3).

Results suggest that cytocidal activity of the virus cannot be prevented in the absence of an optimal concentration of copolymer in the tissue culture media. Pre-incubation of virus with copolymer alone apparently does not inactivate undiluted virus prior to its addition to the tissue culture system.

The ability of the ethylene maleic anhydride copolymers to inhibit cytopathogenicity completely up to 26 h following exposure to Echo 9 virus implies that the presence of the copolymers in the medium impeded some phase of the viral life-cycle in relation to the cell.

There is no apparent alteration of the tissue culture cells as established by viability, alterations in pH, and microscopic morphology at the concentration of copolymers used.

Both cationic and anionic polyelectrolytes have shown *in vitro* virus-inhibitory activities. The action of the polycationic viral inhibitors such as polylysine resides in the ionic bond between the charged virus particles and the positively charged polypeptide-molecule[12]. One might liken the action of the soluble anionic polyelectrolytes to certain exchange resins which have been used to isolate and characterize viruses[21–23]. Although the ethylene maleic anhydride copolymers are soluble, they can form gels with intact cells. This may affect their ability to bind and inactivate viruses, as in the case of ion-exchange resins. The gels may also serve as three-dimensional networks, or as macromolecular sieves, impeding the entrance or release of virus at the cell surface.

In the latter case, viral inactivation would be independent of pH and salt concentration, and would depend, as Laurent and Pietruszykiewicz[24] have shown for hyaluronic acid, on the size of the virus particle and the pore size of the gel. Support for this may be seen in the difference in salt dependency between anionic and cationic polyelectrolytic viral inhibitiors.

In contrast to cationic polylysine[12], the virus-copolymer interaction produced by the macroanions studied by Cohen[10] and by Young and Mowra[11] was not salt-dependent, and took place in a pH range where virus and polymer possessed the same negative charge. Similarly, Heymann *et al.*[20] found that salt concentrations did not always affect the inhibition of vaccinia and Type *B* influenza by aromatic polycarboxylates and sulphonates.

Burger and Stahmann[12] have shown that the greater the chain length of the polylysine polypeptide, the more potent the inhibition of the virus. According to these authors, lengthening of the polymeric chain seems to facilitate the formation of ionic bonds. That the chain-length of anionic copolymers can also be a factor was observed by Young and Mowra[11], who showed the inactivation of $T2$ phage

Table 3. VIRAL INHIBITION DATA FOR ETHYLENE MALEIC ANHYDRIDE DERIVATIVES

Pre-incubation of copolymer-virus for 1 h at 27° or 4° C

Specific viscosity	0·1	0·1	0·67	0·6	1·4	1·8	1·8
Approx. mol. wt.	2–3,000	2–3,000	20–30,000	20–30,000	80–100,000	120,000	120,000
Approx. mol. conc. in tissue culture	2×10^{-3}	2×10^{-3}	2×10^{-5}	2×10^{-5}	5×10^{-6}	4×10^{-6}	4×10^{-6}
Viruses							
Echo 9 *TC*	P(2) *P*	P(3) *P*	P(5)	P(2)	P(1)	P(2)	P(2)
Semliki forest virus *TC*	NT	NT	P(1)	NT	NT	NT	P(3)

P, An effect of polymer preventing cytopathogenic effect by a factor of at least two ten-fold dilutions of virus as compared with control.
P, A delay in cytopathogenic effect by at least 48 h as compared with control.
NT, Not tested.
No. of experiments in brackets.

produced by polyglucose sulphuric acid polymers was enhanced at higher molecular weights.

The precipitation of various protein macromolecules by heparin, hyaluronic acid, and other polyelectrolytes, and its application to the technique of isolation and purification of tobacco mosaic virus and tomato bushy stunt virus, have been described by Cohen[10], and for other viruses by Philipson *et al.*[22] and Frick[23]. Here, too, the macromolecular character of the anionic polyelectrolytes was a factor. We have seen in similar fashion that the most active inhibition of Echo 9 virus was produced by the ethylene maleic anhydride copolymers of highest molecular weight (120,000).

Unlike the inhibitory action of polyglucose sulphuric acid polymers, the inhibition of viral cytopathogenicity by ethylene maleic anhydride copolymers could take place in the presence of cells. The inhibition of cytopathogenic effect was similar to that seen for Type *B* influenza virus and Newcastle disease virus which could be accomplished with aromatic polysulphonates before cell penetration or during the latent phase, as reported by Neher and Kradolfer[18]. Similar inhibitory activity has been reported for polylysine[13-16].

The inhibitory action of the anionic ethylene maleic anhydride copolymers may be mediated through their action on receptor sites, as has been reported for sodium polypectate and for polyglutamic or polyacrylic acid. These compounds inhibit tobacco mosaic virus by blocking receptor sites on the cell surface[19]. Polyacrylic acid is structurally similar to the polycarboxylic hydrolysed ethylene maleic anhydride copolymers, and has similar antitumour[5] and deoxyribonuclease inhibitory activity[25].

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INHIBITORY PHASING OF NEURONAL DISCHARGE

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SEVERAL large aggregations of neurones in the central nervous system exhibit a tendency to synchronize responses which recur at relatively slow frequencies. The best-known example is the alpha rhythm which occurs in various areas of the cerebral cortex, neuronal discharges tending to occur in phase with the rhythmic waves at 8–12/sec[1,2]. In another example a single afferent input into the thalamus evokes a series of after-discharges of the thalamic neurones in which brief bursts of impulses tend to recur at intervals of about 100 msec[3-6]. Comparable rhythmic discharges may occur spontaneously in the lateral geniculate body and may be triggered by single orthodromic or antidromic volleys[7].

In general, the explanations of these rhythms fall into two classes[8]: one is that individual nerve cells have an intrinsic rhythmic property[9-11]; the other is that the cycle of the rhythm is set by the time of propagation along closed self-re-exciting chains of neurones[5,12-14]. There is an additional explanation that is essentially a compromise, for it postulates that the rhythm is determined by the resonance frequency which favours those neuronal chains that are most appropriately related to the recovery times of the individual neurones after their previous discharge[15,16].

The experimental evidence relating to the slow neuronal rhythms is very unsatisfactory, and does not allow any definitive statements with respect to any one of the hypotheses. For example, Chang[5] claimed that the rhythmic thalamic discharge was due to a reverberatory thalamocortical circuit because it was abolished by removal of the cortex or by interruption of the cortico-thalamic connexions. However, Adrian[3,17], and Bremer and Bonnet[6], found that cortical ablation did not affect the rhythmic thalamic discharge, and this has recently been confirmed (Andersen, Brooks and Eccles, unpublished observations). It appears that this rhythm is generated within the thalamus, and this recent investigation of the field potentials and intracellular potentials in the thalamus has led to the formulation of a more precise hypothesis of this neuronal rhythm than has hitherto been attempted.

The hypothesis is based on the surprising observation that the primary phenomenon in the rhythmic response of thalamic neurones is a large and prolonged inhibitory postsynaptic potential. It is now well established that many inhibitory postsynaptic potentials of cortical neurones are much longer than with neurones in the spinal cord, durations of about 100 msec being repeatedly observed[18–22]. As shown in Fig. 1*G–J* the inhibitory postsynaptic potentials of thalamic neurones always have this long duration, which has already been reported by Purpura and Cohen[23]. Furthermore, during rhythmic burst responses of the thalamus, such as those illustrated in Fig. 1*A* with extracellular recording, there are large inhibitory postsynaptic potentials in phase with the positive waves (*P*-waves) of the rhythm (Fig. 1*G*, *I*). The termination of each rhythmic burst response evidently is due to the sudden onset of large inhibitory postsynaptic potentials in each of a large population of thalamic neurones, regardless of whether they were actively responding or not.

This basic observation led to the development of the hypothesis that is illustrated in Fig. 1*K*, and which gives the simplest explanation of the interrelationship between the rhythmic inhibitory postsynaptic potentials and the rhythmic burst responses. A series of thalamic neurones is shown with their axons projecting to the cortex and each receiving one excitatory synapse from the medial lemniscus fibres. Collateral branches from the axons of the thalamic neurones (cf. ref. 24, II, Fig. 260, ref. 25) end in excitatory synapses on interneurones which in turn have inhibitory synapses on the thalamic neurones over an extensive area. A comparable recurrent inhibitory pathway has been demonstrated for motoneurones in the spinal cord[26,27] and for pyramidal cells in the neo-cortex[20,28], and the hippocampus[22]. It was also tentatively suggested as a possible but unlikely explanation of the large *P*-waves generated by both orthodromic and antidromic activation of lateral geniculate neurones[29].

The operational characteristics of this neuronal system at the simplest level would be that a volley of impulses in medial lemniscus fibres evokes the discharge of impulses from some thalamic neurones, which would travel to the cortex and also along the collaterals so that discharges of the inhibitory neurones would be evoked, with the consequent generation of inhibitory postsynaptic potentials in thalamic neurones, both the active and the quiescent. The thalamic discharge is thus terminated in the large and prolonged *P*-wave that is regularly observed in extracellular recording.

It is next postulated that, late on the declining phase of this inhibitory postsynaptic potential, a thalamic neurone would be in the hyperexcitable phase of post-anodal exaltation such as has been demonstrated after anodal polarization of spinal ganglion cells[30] or motoneurones[31,32], or after inhibitory postsynaptic potentials of motoneurones[33] or pyramidal cells of the hippocampus (ref. 34, Fig. 2*B*). Those cells with the most intensive inhibitory postsynaptic potential would be the most likely to generate a discharge late on its declining phase. All thalamic neurones recovering from large inhibitory postsynaptic potentials would be in a hyperexcitable phase at this time. Thus the first rhythmic burst response (Fig. 1*A–D*) may be sufficiently explained by the discharges of such neurones during the declining phases of their inhibitory postsynaptic potentials. Probably it may be necessary to postulate that the generation of discharge requires in addition some excitatory synaptic action, which could, for example, be produced by interneurones that were inhibited during the *P*-wave. Although less likely,

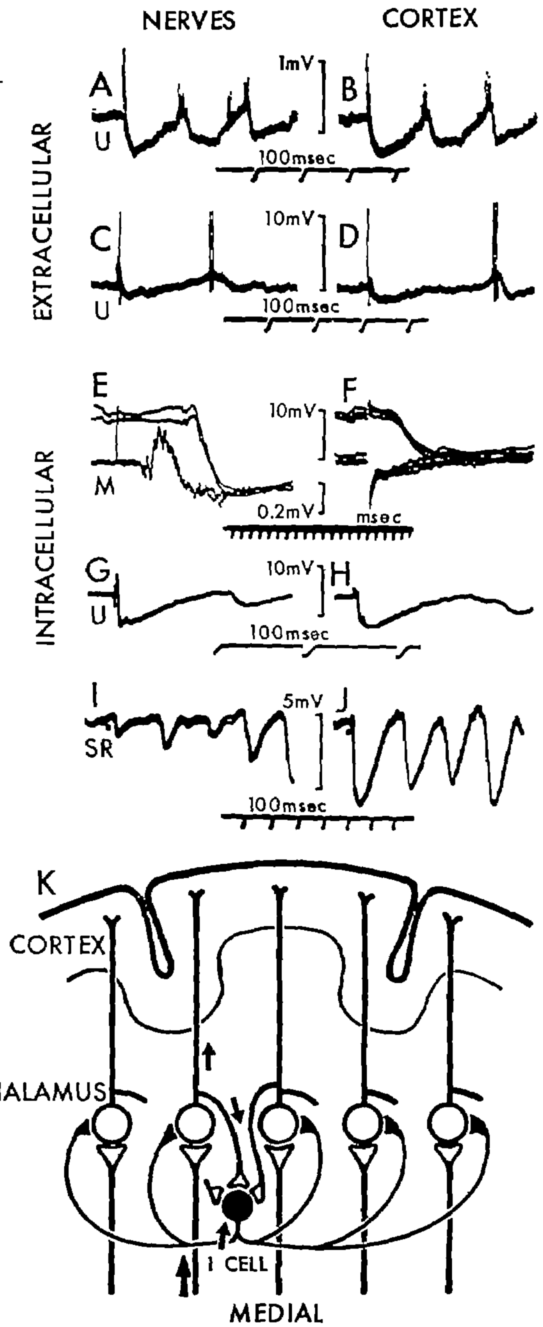

Fig. 1. Extra- and intra-cellular records from cells in the ventrobasal complex of the thalamus in response to stimulation of various contralateral foreleg nerves (left column), and of the foreleg area of the ipsilateral somatosensory cortex (right column). Each horizontal pair of records obtained from the same site (*A–D*) or cell (*E–J*). *A–D*, extracellular records. *A*, initial discharge followed by large positive wave (*P*-wave), then first and second burst response in response to single shock to ulnar nerve. *B*, as *A*, but stimulation of cortex, activating the thalamic neurones antidromically. *C* and *D* show the response of a single thalamic relay cell to the same stimuli as in *A* and *B*. The same cell discharges both in the initial response and in the first burst response. *E–J*, intracellular records from thalamic somæsthetic relay cells, recorded with potassium citrate filled electrodes. *E* and *F*, large inhibitory postsynaptic potential in response to stimulation of median nerve and cortex, respectively. Lower records are cuneate responses. *G* and *H* show large and rhythmic inhibitory postsynaptic potential induced by ulnar and cortical stimulation. *I* and *J*, slow sweeps illustrate the rhythmic nature of the inhibitory postsynaptic potential evoked both by a single superficial radial and by an antidromic volley

the ephaptic excitatory action of the impulses in adjacent neurones is a further factor that may help in generating the discharge of thalamic neurones once some have discharged, and so would contribute to the phasing of the successive burst responses.

The first burst response would again excite the inhibitory neurones via the axon collaterals, and so again terminate itself by the inhibitory postsynaptic potentials so generated, from which recovery would again recur in a phase of exaltation and generation of the second burst response and so on. Provided that the inhibitory phase generated by each burst response is distributed sufficiently widely to the thalamic neurones, there is no need to postulate any phasing device other than the rhythmically generated inhibitory postsynaptic potentials.

It would be predicted from Fig. 1*K* that the antidromic impulses evoked by stimulation in the sensory cortex of the axonal terminals of thalamic neurones would also activate the inhibitory neurones via the axon collaterals; hence this stimulation should be just as effective as orthodromic activation in evoking the rhythmic inhibitory postsynaptic potentials and the rhythmic burst responses from the thalamus. This is indeed observed with the somaesthetic cortex (Fig. 1, right column) and analogously Bishop and Davis[7] found that stimulation of the optic radiation evoked the rhythmic discharges of the lateral geniculate neurones.

It will be evident that the hypothesis here formulated belongs to the category in which the rhythm is generated intrinsically in the neurone; however, it is unique in that it has not previously been postulated that the essential phasing device is due to widely dispersed inhibitory postsynaptic potentials that are generated through a recurrent pathway from axon collaterals. It remains to suggest that the very large and prolonged recurrent inhibitory postsynaptic potentials of cortical pyramidal cells[30–32] may also be similarly concerned as the phasing device for the basic alpha rhythm, and themselves contribute very largely to the potential waves of this rhythm.

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REFLEX NATURE OF THE PHYSIOLOGICAL ANTICOAGULATING SYSTEM

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AN investigation of the physiological anticoagulating system[1–4] has led us to conclude that it is of a reflex-humoral nature[4]. When thrombin appears in the circulating blood of amphibians and mammals, the reflex arc becomes closed at the level of the medulla oblongata[3,4]. An effective act occurring in this case results in the secretion of heparin or heparin-like substances and of plasminogen activator into the blood. A sharp increase in blood fibrinolytic activity together with the action of natural anticoagulants as a background produces a blockage of blood clotting due to fibrinogenolysis and the formation of antithrombin VI (ref. 5) as well as a very rapid lysis of clots, if the latter has had time to form in some parts of the vascular bed.

In experiments carried out on rabbits new evidence was obtained confirming and extending the notion

Table 1. A CHANGE IN THE COAGULATION TIME OF BLOOD TAKEN FROM V. JUGULARIS OF RABBITS AFTER PERFUSING THE VESSELS OF THE KIDNEY ISOLATED FROM SYSTEMIC CIRCULATION WITH RINGER–LOCKE OR THROMBIN SOLUTIONS AND KEEPING THE INNERVATION OF THE ORGAN INTACT. (Thrombin dose 2–5 ml., clotting activity 4–5 sec)

No. of experiments	Before perfusion with Ringer–Locke solution	15 min after the beginning of the perfusion with Ringer–Locke solution	4 min after the beginning of the perfusion with thrombin solution	30 min after the cessation of the *thrombin* perfusion and the renewal of the Ringer–Locke solution flow
Experiment				
11	142	149	505	310
Control 1 (thrombin inactivated by heating)				
3	127	127	124	—
Control 2 (denervation of the kidney, active thrombin)				
3	106	117	120	—

Table 2. INFLUENCE OF THE SEVERANCE OF CONDUCTIVE PATHS IN THE VEGETATIVE NERVOUS SYSTEM ON THE FUNCTIONAL STATE OF THE PHYSIOLOGICAL ANTICOAGULATING SYSTEM IN RABBITS (AVERAGE RESULTS) (Thrombin dose 1·2–1·4 ml., activity 5–6 sec)

Experimental conditions	Time of blood coagulation (sec) Before severance	30 min. after severance	4 min after thrombin injection	No. of animals Total in the experiment	Survived	Died of thrombosis
Exp. 1. Severance of the vagus, unilateral at the level of the neck	124	127	over 600	5	5	0
Exp. 2. Severance of vagi, bilateral at the level of the neck	193	187	thrombosis	17	0	17
Exp. 3. Severance of the sympathetic chain at the level of the neck, bilateral	154	150	over 600	15	15	0
Exp. 4. Severance of the depressor nerve (a) unilateral	188	139	thrombosis of the heart and of pulmonary vessels	8	0	8
(b) bilateral	105	109		9	0	9
Control 1. Intact animals	105	109	from 360 to 900	22	22	0
Control 2. A vertical cut of the skin on the neck without trauma of adjoining tissues	98	103	from 360 to 900	20	20	0

Table 3. INFLUENCE OF THE SEVERANCE OF CONDUCTIVE PATHS IN THE VEGETATIVE NERVOUS SYSTEM ON THE FUNCTIONAL STATE OF THE PHYSIOLOGICAL ANTICOAGULATING SYSTEM IN RATS (AVERAGE RESULTS). (Thrombin dose 1·0–1·2 ml.; activity 5 sec)

Experimental conditions	Time of blood coagulation (sec) Before severance	30 min after severance	4 min after thrombin injection	No of animals Total in the experiment	Survived	Died of thrombosis
Exp. 1. Severance of the vagus, unilateral at the level of the neck	104	107	over 1,800	11	11	0
Exp. 2. Severance of vagal nerves, bilateral at the level of the neck	104	112	thrombosis	38	1	37
Exp. 3. Severance of the sympathetic chain, bilateral at the level of the neck	98	85	over 1,800	4	4	0
Control 1. Severance of vagal nerves, bilateral. Injection of thrombin inactivated by heating	90	98	98	5	5	0
Control 2. Cutaneous cut on the neck	134	131	over 1,800	21	21	0

of the anticoagulating system as a reflex mechanism serving to keep the circulating blood in a liquid state.

In rabbits weighing 2·5–3 kg the left kidney was isolated from the systemic circulation by ligating and severing the renal artery and vein, but maintaining the innervation of the organ fully intact. The operation was performed without narcosis or local anæsthesia to preserve the functional condition of the anticoagulating system. In the lumens of the kidney vessels cannulae connected with rubber tubes were inserted. Through the tube of the cannula entering the artery Ringer–Locke solution, heated to 37°, was passed. The back-flow of the nutritive solution from the kidney occurred through a cannula with the tube connected with a venous vessel. In test animals the time of the clotting of blood taken from the v. jugularis was determined before perfusion, 15 min after its beginning and 4 min after 2–5 ml. of thrombin solution coagulating at 4–5 sec an equal volume of oxalate blood at 37° C had been introduced into the tube connected with the artery. A perfusion with Ringer–Locke solution of the kidney isolated from the vascular bed, but with a normal innervation of the organ left unaltered, had no influence on the time of the coagulation of blood in the systemic circulation. Yet, 4 min after passing through the vessels of the isolated kidney a solution of active thrombin, the blood taken from the rabbit's v. jugularis was found to have lost its clotting capacity to a considerable degree (Table 1). Such a phenomenon was due to the appearance of anticoagulants in the blood, to a highly increased fibrinolytic activity of the plasma (experiment, 75 per cent; control, 25 per cent) and to a reduction of the fibrinogen concentration (experiment, 367 mg/per cent; control, 262 mg/per cent). As a control, thrombin inactivated by heating was used for the perfusion of the kidney. The washing of its vessels with an inactive thrombin solution produced no change in the clotting time of blood taken from the systemic bed (Table 1). The same negative result was obtained in perfusing renal vessels with active thrombin, but after the denervation of the organ (Table 1).

Therefore, to obtain a specific anticoagulating effect *in vivo*, an active irritant—thrombin acting on the chemoreceptive apparatus of blood vessels and on the conductive paths of the reflex arc—is necessary.

To study the latter paths, experiments involving a severance of nerve-trunks were made on rats and rabbits (Tables 2 and 3).

Following a bilateral severance of vagi as distinct from a unilateral disturbance of innervation, animals died of thrombosis after intravenous injections of adequate thrombin doses. However, with the preservation of the vagal nerve integrity, but with a bilateral severance of the sympathetic chain, injections of the same thrombin doses did not cause thrombosis. After a bilateral severance of vagi at the level of the neck in rabbits (Table 2), thrombosis occurred in the latter, mostly in the vessels of their abdominal cavity organs, after intravenous injections of moderate thrombin doses. In the case of a unilateral, and particularly in that of a bilateral, disturbance of the depressory nerve integrity, intravenous injections of moderate thrombin doses into rabbits produced a thrombosis of the heart coronary vessels or of pulmonary vessels as well as that of cardiac cavities.

An analysis of experimental results given in Tables 2 and 3 leads us to the conclusion that the reflex reaction determining the protective function of the physiological anticoagulating system is chiefly brought about with the participation of the parasympathetic section of the vegetative nervous system.

A temporary paralysis of the aforementioned section may result in the development of a general pre-thrombotic state of the organism, passing into thrombosis in the event of a sufficient thrombin concentration appearing in the blood. That assertion was confirmed in experiments on rats injected with atropine (0·08 ml. of 0·1 per cent solution) as well as with aminasine (0·04–0·06 ml of 2·5 per cent solution), which excludes the reticulary formation of the brain stem.

A disturbance of the parasympathetic innervation in separate portions of the body results in a local discontinuance of the function fulfilled by the anti-coagulating system. This conclusion may well be of importance for an understanding of the causes of post-operative thrombosis.

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SIALORESPONSIN: NEURAMINIC ACID-CONTAINING SUBSTANCE(S) WHICH ACCUMULATES IN CHORIOALLANTOIC FLUID DURING THE FIRST MINUTES OF VIRUS INFECTION

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THE nature of the physicochemical events which occur during the first minutes of virus infection is of interest with reference to our understanding of the mechanisms by which the virus actually moves from extracellular fluid into the host cells, our knowledge of the earliest defences of the host, and to the design of therapeutic agents for this phase of infection. Following the earlier demonstration in this laboratory that a neuraminic acid containing brain ganglioside preparation serves both as an inhibitor of the hæmagglutination reaction *in vitro*[1] and of the neurotoxicity of influenza *PR*8 and *NWS* viruses *in vivo* in mouse brain[2], our recent investigations have been directed to the occurrence of other natural neuraminic acid-containing substances in host systems and the part which they might play in virus infection. This article reports the chemical detection in chorioallantoic fluid of the intact host egg of the accumulation within the first few minutes of bound neuraminic acid in response to influenza viral infection. This substance(s) is distinguished from free neuraminic acid, as liberated by neuraminidase (sialidase)[3], since it is bound (presumably through its aldehydic group in a glycosidic linkage which is hydrolysed by 0·1 N sulphuric acid at 80° C for 1 h), and is distinguished from interferon[4] by its content of neuraminic acid and by the time of its appearance (see note added in proof). It has been named sialoresponsin since it appears as an immediate response to the introduction of virus.

The chorioallantoic cavities of intact 11-day-old embryos were injected with 0·2 c.c. of a *PR*8 strain of influenza virus which has been maintained in this laboratory by passage in the chorioallantois. The virus preparation had been dialysed against buffered saline, concentrated ten times by lyophilization, and contained on injection 2,048–3,280 hæmagglutinin units per 0·2 c.c. The eggs were inverted three to five times to mix the fluid and sampled by removing 1·5–2·5 c.c. of allantoic fluid with 2-ml. syringes and No. 23 needles for analysis of hæmagglutinins, infectivity, total neuraminic acid, free neuraminic acid and deoxyribose. A group of approximately 50 eggs were used for each experiment. Four to six eggs were used for each *T*-2 value (2 min before injection), and one egg was sampled every 2 min during the first hour giving values from 5 eggs every 10 min. Two to four eggs each were sampled at 80, 100 and 120 min after injection. Chorioallantoic fluid samples were centrifuged at 2,000 r.p.m. for 5 min and examined for sediment and red blood cells, but it was observed over 14 full experiments that the presence or absence of these did not significantly affect the values here determined. The entire injection and sampling procedure was done in a constant temperature warm room (37° ± 0·2° C), and the chemical and virological analysis at room temperature (25° ± 4° C).

Separate control experiments also carried out included the following: (1) sampling of uninjected eggs at the same time-intervals as for virus-injected eggs to obtain baseline values for total and bound neuraminic acid and deoxyribose; these included separate experiments with 'light' (under 53·2 g) and 'heavy' (over 53·2 g) eggs, which succeeded in excluding from relevance the size of the egg; (2) sampling at the same time-intervals eggs injected with normal saline; (3) injected with normal uninfected chorioallantoic fluid, concentrated and dialysed in the same way as in the case of virus-containing test fluids. The last two controls demonstrated the normal range of variations of values for eggs exposed to the 'trauma' of injection, and in addition, receiving either saline or normal chorioallantoic fluid constituents by injection (Fig. 1, shaded areas). The amounts of measured constituents transferred to each egg by injection of virus plus chorioallantoic fluid were 8·0 μg of total neuraminic acid, and 1·5 μg of deoxyribose, in 0·2 c.c. of innoculum. Each constituent then became diluted by diffusion approximately 30–40-fold in the chorioallantoic fluid so that its presence could not be detected beyond the range of variation per c.c. for uninjected normal eggs.

Free neuraminic acid and free deoxyribose were quantitatively measured by the method of Waravdekar and Saslaw[5] as modified by Warren[6]. The foregoing method for neuraminic acid measures deoxyribose (absorption peak 532 mμ) some two and a half times more sensitively, microgram for microgram, than it does neuraminic acid (absorption peak 549 mμ). For this reason, in the presence of a mixture of deoxyribose and neuraminic acid such as was encountered in chorioallantoic fluid in these experiments, it became essential to differentiate these two substances with greater precision than would be afforded by the use of correction factor formulæ based on the overlap of the curves[6]. Waravdekar and Saslaw had noted earlier[5] that the colour produced by neuraminic acid in the thiobarbituric acid test faded over a period of time in the presence of sodium hydroxide, whereas that due to deoxyribose did not fade. In seeking a more rapid modification of their method of differentiating these two chromophores, it was noted that if immediately following the

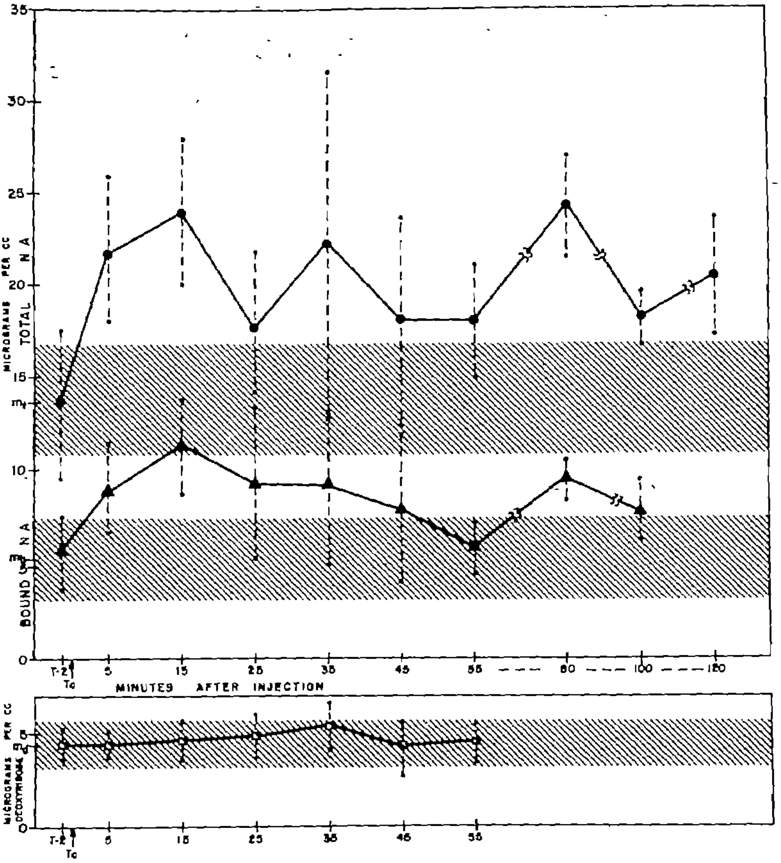

Fig. 1. Concentrations of total neuraminic acid (●), bound neuraminic acid (▲), and deoxyribose (□), as the means in μgm/c.c. of chorioallantoic fluid, 2 min before (*T*-2) and numbers of minutes after injection of influenza *PR8* virus. Vertical interrupted lines give standard deviation (± *S.D.*) for each mean value. Shaded areas indicate ± *S.D.* for saline-injected controls tested at same intervals as virus-injected eggs. Means for controls indicated as m_t (mean control total neuraminic acid), m_b (mean control bound neuraminic acid), and m_d (mean control deoxyribose). The lines joining the points between 50, 80, 100 and 120 min are broken in order to indicate the difference between these abscissa units and those charted between *T*-2 and 55 min. The arrows on the abscissæ indicate the point of injection of either saline or virus

addition of 1 c.c. 5 per cent sodium hydroxide the chromophore solution was extracted with *cyclo*-hexanone, the chromophore due to deoxyribose was quantitatively extracted, but that produced by neuraminic acid remained quantitatively unextracted in the lower phase. This modification (Evans, P., to be published) is highly accurate over a wide range of concentrations of both neuraminic acid and deoxyribose in quantitatively measuring and distinguishing these two substances. To the pipetted chorioallantoic fluid samples, aliquots of sodium periodate were added immediately (completed within 2 min after withdrawal of fluid from egg). Total neuraminic acid (after hydrolysis with 0·1 N sulphuric acid at 80° C for 1 h), free neuraminic acid (no prior hydrolysis), and deoxyribose were separately determined on 0·15, 0·2 and 0·2 c.c. aliquots respectively from each chorioallantoic fluid sample as above, and bound neuraminic acid was calculated as (µg/c.c. of total neuraminic acid) minus (µg/c.c. of free neuraminic acid).

Fig. 1 shows in its shaded portions the means plus the area covered by ± *S.D.* of total neuraminic acid, bound neuraminic acid and deoxyribose for saline-injected eggs. The values for deoxyribose in virus-injected eggs is seen not to vary significantly from those in the controls. Since the method used is even more sensitive to changes in deoxyribose than in neuraminic acid, the relative constancy of deoxyribose in both controls and virus-injected eggs is of interest not only of itself but also in that it serves as an indication of the reliability of the entire sampling procedure and of the chemical determinations used. The constancy of deoxyribose thus enhances the significance of changes observed in the values of neuraminic acid.

Virus-injected eggs are seen to show initial pre-injection (*T*-2) concentration of both total and bound neuraminic acid just at the levels observed for the uninjected and saline controls (Fig. 1). The concentrations of total neuraminic acid at 5 min and at 15 min after the injection of virus are seen to be significantly elevated above controls ($P < 0.05$ and < 0.001 respectively). In fact, the earliest sampled eggs, at just 2 min post-injection, demonstrate a significant elevation. This increase is shown in Fig. 1 to be due to not just the presence of more free neuraminic acid, expected due to virus sialidase action, but to the presence of greater amounts of bound neuraminic acid ($P < 0.25$ at 5 min, $P < 0.01$ at 15 min). Since this response does not occur for either saline-injected or normal chorioallantoic fluid-injected eggs, it must be related to the introduction of the virus itself. The response reaches a maximal mean value at 15 min, then decreases at 25 min. There is a second increase in the concentration of bound neuraminic acid at 80 min, suggesting a further release of a neuraminic acid-containing substance(s) here called sialoresponsin, and another decrease at 100 min. There is some evidence that additional responses occur beyond 100 min.

The results suggest that there is a constantly, although not uniformly, active viral sialidase which cleaves free neuraminic acid from sialoresponsin as the latter appears in the fluid, with a resultant increase in the level of free neuraminic acid as well as of bound neuraminic acid throughout the period measured. However, the free sialic acid liberated does not accumulate. Free sialic acid must therefore either be hydrolysed by an as yet unidentified neuraminic acid hydrolase in this system or it must be transported from the fluid into the chorioallantoic membrane.

It has been possible to demonstrate a drop in hæmagglutinins in chorioallantoic fluid as early as 30 min post-injection in these experiments, but the sensitivity of the hæmagglutination determination may not permit detection of the earliest evidence of virus disposition. Since the first few hours post-injection are those during which virus is known to be 'disappearing' from the fluid, and presumably entering the membrane ('eclipse phase'[7]), it is of interest that free neuraminic acid is observed here to 'disappear' during the same period. The possibility therefore occurs that virus and free neuraminic acid are involved in the same disposition system, perhaps the same transport system. The infecting virus may once again be 'utilizing' what may be a normal mechanism of the host tissue, in this case a transport mechanism. That is, by some physicochemical reaction, either directly as in some combination between virus and free neuraminic acid or indirectly via some as yet unelucidated steps linked to the removal of free neuraminic acid, the transport of virus from extracellular fluid into cell membrane may be accomplished. In this case, the utility of a viral neuraminidase which keeps producing more free neuraminic acid would be apparent.

Equally important is the possible relevance of these findings to certain membrane transport mechanisms in general where the 'bound-free' neuraminic acid system is found. Thus, earlier work in this laboratory had led us to the hypothesis[9,10] that neuraminic acid is involved in membrane, receptor and transport functions in the nervous system and elsewhere. The structure of brain ganglioside suggested its suitability for membrane interphase functions[8]. Brain ganglioside was shown to interact with influenza viruses in a manner suggesting membrane receptor properties[1,2] and brain ganglioside was found to excite the heart of *Venus mercanaria*[11] and to stimulate guinea pig intestine[12] in low concentrations. Additional evidence in support of this hypothesis has recently been provided by the demonstration that brain ganglioside has the properties of a receptor for tetanus toxin[13], that it stimulates isolated rabbit intestine[14], and that it restores the ability of brain slices to respond to electrical impulses[15]. The present findings may be viewed as adding further evidence of possible relevance to this hypothesis and, in addition, providing one experimental model for the detailed elucidation of its potential mechanism. To this end, the use of radioactive and fluorescent labelling techniques with both virus and neuraminic acid should permit the demonstration of their step by step course in the transport processes of this extracellular fluid membrane system. Work is also under way to characterize sialoresponsin by chemical and physical means.

The almost instantaneous response of the organism to virus introduced into the extracellular fluid, that is, the release of sialoresponsin, may represent one of the earliest defence mechanisms of the host. This release into the extracellular fluid of a neuraminic acid-containing substance(s), whatever its function, may be viewed as resulting in the provision of a natural receptor 'decoy' akin to that exogenously added in the form of brain ganglioside in earlier work in this laboratory[2]. In the balance achieved between host sialoresponsin and viral sialidase may rest one of the determinants of whether certain invading viruses are inactivated extra-cellularly or gain entry into the host cells.

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Note added in proof. Since the submission of this article, subsequent experiments have provided the following additional characterization of sialoresponsin: (1) it is freely dialysable, thus clearly distinguishable from the interferons and other large molecular weight viral inhibitors; (2) it can be preserved and obtained in yields double that described by shaking immediately with chloroform the freshly withdrawn chorioallantoic fluid; (3) it has been separated from free neuraminic acid and other dialysable constituents on Dowex-1-formate; (4) it has been produced by influenza PR8 virus which has been purified by red cell adsorption and by ultracentrifugal concentration; and (5) its production and hydrolysis have now been demonstrated in chorioallantoic fluid *in vitro* as well as *in vivo*.

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INITIATION OF IMMUNE RESPONSES BY SMALL LYMPHOCYTES

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Animals may develop a fatal wasting disease if they are injected with adult lymphoid cells which lack antigens present in the tissues of the host. The ability of a particular cell-type to induce such a disease is an index of its 'immunological competence', in the sense that it must be able to react with antigens in the tissues of the host and initiate an immune response against them[1,2]. Work on the graft-versus-host reactions which occur when thoracic duct cells from genetically appropriate donor rats are injected into new-born rats[3], adult F_1 hybrid rats[4,5] and lethally X-irradiated mice[5] has shown that the small lymphocyte is an immunologically competent cell. These investigations have also shown unequivocally that small lymphocytes can change rapidly into dividing cells with new morphological characteristics[4,6]. The change occurs in a proportion of the donor's small lymphocytes during their first 24-h residence in the lymphoid tissue of the host: the small lymphocytes enlarge, develop prominent nucleoli and a cytoplasm which stains strongly with pyronin; they then begin to divide. Such 'large pyroninophilic cells' do not appear when syngeneic (isologous) small lymphocytes are injected into normal hosts.

The large pyroninophilic cells which arise from small lymphocytes in graft-versus-host reactions resemble morphologically the cells which appear in regional lymphoid tissue during the reaction against homografts of skin[6] and during antibody formation[7,8,11]. This resemblance, together with the demonstration that the small lymphocyte is immunologically competent, suggest that primary immune responses in general may be initiated by the interaction of antigens with small lymphocytes. The experiments recorded here provide some support for this view: they show that, in the rat, small lymphocytes probably initiate the reaction against first-set homografts of skin and the primary antibody response to a particulate and a soluble antigen.

Two groups of experiments were performed. First, the identity of the cell-type formed by the dividing large pyroninophilic cells was determined by studying the fate of rat small lymphocytes in the spleen of lethally irradiated mice. The significance of these experiments for the homograft reaction was then examined. Secondly, rats were rendered immunologically unresponsive to skin homografts, sheep erythrocytes and tetanus toxoid. Suspensions of small lymphocytes from other rats were then injected intravenously to test their ability to abolish the unresponsive states.

Small lymphocytes. Almost pure suspensions of small lymphocytes were prepared by incubating rat thoracic duct cells *in vitro* for 24 h at 37° C in a conventional tissue culture medium[5]. This procedure kills most of the large lymphocytes in the culture.

In each experiment the proportion of small lymphocytes in the inoculum was estimated in smears from a count on 2,000 cells; the proportion ranged from 99·4 to more than 99·95 per cent.

Three methods were used for inducing immunological unresponsiveness in rats belonging to highly inbred strains.

(1) Immunological tolerance to skin homografts was induced in new-born albino rats by an intravenous injection of 10–40 million spleen cells from adult F_1 (albino × hooded) hybrid rats. A full thickness graft of F_1 skin was applied to the lateral thoracic wall when the injected rats were 6–8 weeks of age and they were regarded as highly tolerant if the grafts survived in good condition for more than 50 days. Four out of 40 grafted rats were highly tolerant, and these were used in the experiments described here.

Immunological tolerance to sheep erythrocytes was induced by an injection of $2{\cdot}5 \times 10^9$ erythrocytes on the day of birth; half the dose was given intravenously and half intraperitoneally. Twice-weekly intraperitoneal injections of $2{\cdot}5 \times 10^8$ erythrocytes were then given for 12 weeks, and the continued absence of hæmolysin from the serum was taken as evidence of tolerance. Cells were collected from the thoracic duct of these rats 4 days after the last injection of erythrocytes.

(2) Adult rats were made unresponsive to sheep erythrocytes and to tetanus toxoid by chronic drainage of cells from a thoracic duct fistula[9]. Lymph and cells were allowed to drain away for 5 days from the thoracic duct of restrained, unanæsthetized rats. They were then released from restraint and their fistulæ closed. 'Closure of the fistula' was performed by gently pulling the cannula out through the skin; a transient chylous ascites developed, but within a week normal lymph drainage from the intestine had been re-established. In all experiments antigen was given immediately after the fistula was closed.

(3) Exposure to 500 rads of whole-body X-irradiation rendered adult rats unresponsive to sheep erythrocytes.

Production of New Cells from Small Lymphocytes

Chromosome analyses have shown that when rat small lymphocytes are injected into a lethally irradiated mouse some of them colonize the white pulp of the spleen, develop into large pyroninophilic cells and start to divide[9]. The fate of these dividing cells was determined autoradiographically by labelling them in the mouse with a single pulse of tritiated thymidine. Individual mice were then killed at intervals after the single injection of thymidine and each spleen was divided into two parts: one part was sectioned and examined by stripping film autoradiography to identify the progeny of the originally labelled cells; the other part was used for a chromosome analysis to ensure that the labelled cells were 'rat' in origin.

Male *CBA* mice weighing 20–30 g were given an intravenous injection of 85 million rat small lymphocytes 24 h after 1,000 rads of whole-body X-irradiation. The inocula were contaminated with less than 0·05 per cent of any other cell-type. A single injection of tritiated thymidine (1 µc./g body-weight; specific activity, 4·4 c./m.mole) was given 48 h after the injection of cells. In the spleen, which was removed 12 h after the injection of thymidine, 28 mitotic cells were scored; all were 'rat'. Autoradiographs of this spleen showed heavy labelling over mitotic figures and over the nuclei of many large pyroninophilic cells in the white pulp. Other labelled cells in the white pulp possessed a less intensely pyroninophilic cytoplasm, no obvious nucleolus and a more pachychromatic nucleus; they were identified as 'large lymphocytes'. Labelled small lymphocytes were very rare at this time.

Cell division in the mouse spleens which were removed 37 and 61 h after the injection of thymidine was again confined almost exclusively to rat cells: 70 out of 71 and 72 out of 74 mitotic cells, respectively, were scored as 'rat'. At 61 h the white pulp of the spleen contained many labelled cells of which the great majority were lightly labelled small lymphocytes. No labelled mature plasma cells were found. It was concluded that small lymphocytes can form more small lymphocytes through the intermediary of the large pyroninophilic cell. The grain counts over the 'large lymphocytes' at 37 and 61 h suggest that they are intermediates between the large pyroninophilic cell and the small lymphocyte.

It could be objected that the labelled small lymphocytes were not derived from the originally injected rat cells but from mouse cells which had divided in the lymph nodes and had migrated by way of the blood into the spleen. This is very unlikely since, in another experiment, no dividing mouse cells were found in the mesenteric and brachial lymph nodes of *CBA* mice on the third, fifth and sixth days after 1,000 rads of X-irradiation. Rat small lymphocytes had again been injected 24 h after the irradiation.

Skin Homograft Reaction

During the response to a first-set homograft of skin in the rat the regional (axillary) lymph node becomes populated by large pyroninophilic cells which are identical in appearance to those described by Scothorne[6] in the regional node draining a homograft in the rabbit and to those which have been shown to develop from rat small lymphocytes[5]. The demonstration that the large pyroninophilic cell can itself generate more small lymphocytes suggests a possible origin for the small lymphocytes which heavily infiltrate first-set homografts in the rat: some small lymphocytes in the regional node react to antigens from the graft by differentiating into large pyroninophilic cells; these produce a specific crop of new small lymphocytes which leave the node, circulate in the blood and invade the graft. Two further experiments support this possibility. First, it has been shown that virtually all the small lymphocytes which infiltrate a first-set homograft are formed after the application of the graft. Secondly, injections of thoracic duct lymphocytes from normal rats will cause the breakdown of a long-standing homograft of skin in an immunologically tolerant rat. The small lymphocytes which invade such grafts are not those which were injected; they are also newly formed cells.

Full thickness grafts of skin were exchanged between rats belonging to a randomly bred albino colony The first signs of necrosis in such grafts occur at about 14 days. At the time of grafting and every 12 h thereafter tritiated thymidine was injected intraperitoneally (0·5 μc./g body-weight; specific activity, 4 4 c./m mole). Biopsies of the graft were taken at 7 and 10 days and examined by stripping film autoradiography. At both times the dermis was heavily infiltrated with small lymphocytes and in autoradiographs exposed for 30 days more than 90 per cent of them were labelled. At 7 days about 7 per cent of the small lymphocytes in the blood were labelled, while at 10 days the medullary sinuses of the regional (axillary) lymph node contained about 16 per cent of labelled small lymphocytes. Thus, the small lymphocytes which invade a homograft are not a random sample from the total lymphocyte pool. It is likely that they are a specific group of new cells formed in response to the graft.

Destruction of Grafts in Immunologically Tolerant Rats

The immunological inadequacy of a tolerant animal can be corrected by an injection of lymph node cells from a normal (non-sensitized) animal[10]. It can also be corrected by an injection of cells from the thoracic duct[4,11]. Thus, the injection of three fully tolerant albino rats with 700–1,000 million thoracic duct cells (99·6 per cent small lymphocytes) from normal rats of the same inbred strain destroyed healthy F_1 skin homografts in 8–10 days; the tempo of destruction was as rapid as that of first-set grafts on normal recipients. In a fourth tolerant rat twice-daily injections of tritiated thymidine were started immediately after the injection of lymphocytes and were continued for 7 days. Again, autoradiographs showed that more than 90 per cent of the small lymphocytes which were present in the graft at 7 days were labelled.

The small lymphocytes which were injected in these experiments did not pass into the graft and generate new cells within it. By labelling the small lymphocytes *in vitro* with tritiated adenosine[6] before their injection it was shown autoradiographically that they 'homed' rapidly into all the lymph nodes of the animal. The basis of this homing is the special affinity of small lymphocytes for the endothelium of the post-capillary venules in the cortex of the nodes[12]. An electron microscope study has shown that small lymphocytes pass from the blood into the nodes by actually entering the endothelial cells and traversing their cytoplasm[13].

The ability of albino-strain small lymphocytes to initiate a graft-versus-host reaction in F_1 (albino × hooded) hybrid rats[6] makes it very probable that they also initiate the destruction of F_1 skin grafts in tolerant rats. It seems likely that small lymphocytes interact with antigen derived from the F_1 graft and generate within lymphoid tissue a specific group of immunologically activated small lymphocytes through the intermediary of the large pyroninophilic cell. Soon after their formation the new cells are released into the blood. Consistent with this view is the demonstration that immunologically activated cells can be detected in the blood of homografted rats at approximately the same time as they appear in the regional lymph nodes[11].

Antibody Formation

Agents such as X-irradiation and cortisone which damage lymphoid tissue *in vivo*, and neonatal thymectomy which prevents its normal development[14], have provided circumstantial evidence that small lymphocytes are involved in primary immune responses. Animals subjected to these measures become severely depleted of small lymphocytes and this can be associated with a failure to produce circulating antibody[15]. However, the idea that the immunological deficiency is due solely to a lack of small lymphocytes would be strengthened if the unresponsiveness could be corrected by injections of small lymphocytes from normal animals. In the experiments reported here, adult rats have been depleted of lymphocytes by chronic drainage of cells from a thoracic duct fistula[9]. The primary antibody response of such animals is severely depressed but it can be restored by injections of small lymphocytes from other rats of the same highly inbred strain.

The loss of about 2 × 10^9 small lymphocytes over a 5-day period of drainage from a thoracic duct fistula is reflected in a reduction in the weight of all the lymph nodes, a gross depletion of small lymphocytes in the cortex of the nodes and in the white pulp of the spleen, and in a lymphopænia. In the rat, small lymphocytes normally recirculate from blood to lymph through all the lymph nodes[12,16]; thus, drainage from the thoracic duct withdraws lymphocytes from all the nodes and not merely from those in the splanchnic bed.

Fig. 1 shows that lymphocyte-depleted rats cannot be sensitized by a primary injection of tetanus toxoid. In a normal rat the second of two doses of toxoid given three weeks apart results in a brisk antibody response; but if the first dose is given immediately after lymph drainage then a second dose three weeks later elicits no response. On the other hand, the secondary response is not affected by lymphocyte-depletion: when the second dose is given after drainage from the thoracic duct a normal response is obtained. Thus, the cells which mediate the secondary response are 'fixed' within lymphoid tissue and cannot be withdrawn by drainage from the thoracic duct.

Fig. 2 shows that the primary immune response to sheep erythrocytes becomes progressively less as the period of lymph drainage is increased. The impairment of response is not due to stress or operative trauma[9] but to the progressive loss of small lymphocytes from the fistula: the unresponsiveness can be corrected by an injection of small lymphocytes from normal (non-immunized) rats of the same inbred

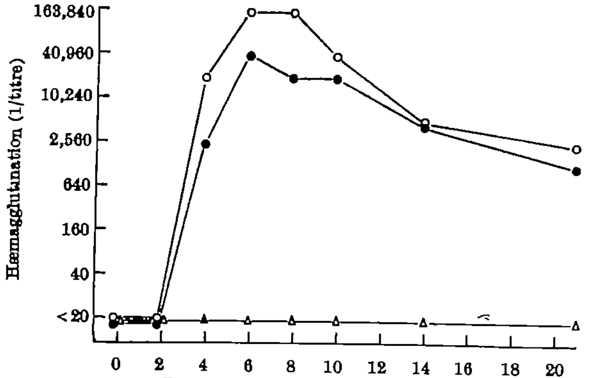

Fig. 1. Response to tetanus toxoid in a normal rat and rats depleted of lymphocytes by 5 days' drainage of cells from the thoracic duct. Each animal received two injections of 10 Lf of soluble toxoid 3 weeks apart, the first intraperitoneally, the second intravenously

●, Response of a normal rat, △, response of a rat given the first dose of toxoid immediately after the closure of a thoracic duct fistula; ○, response of a rat given the second dose of toxoid immediately after the fistula was closed. Antibody assayed by tanned red cell hæmagglutination (ref. 22)

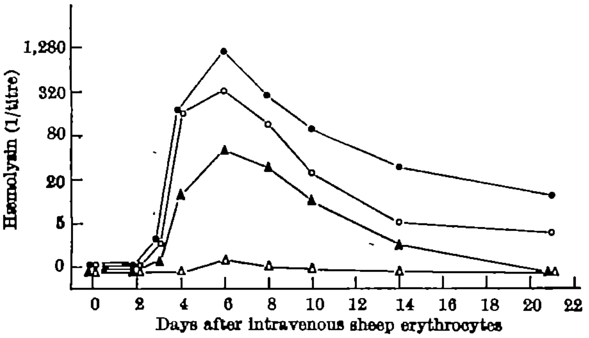

Fig. 2. Hæmolysin response to a single intravenous injection of 10^8 sheep erythrocytes in normal rats and rats depleted of lymphocytes by drainage of cells from a thoracic duct fistula

●, Mean response of 14 normal rats; ○, mean response of 4 rats after 2 days' drainage; ▲, mean response of 3 rats after 3 days' drainage; △, mean response of 10 rats after 5 days' drainage

strain. This restorative effect is related to the number of small lymphocytes injected. Thus, the injection of 10^9 thoracic duct cells (99·75 per cent small lymphocytes) restored the response of 5-day drained rats to approximately the level which was observed after 2 days drainage (Fig. 3). About 10^9 small lymphocytes emerge from a thoracic duct fistula between the second and fifth days after cannulation.

'Immunologically Tolerant' Small Lymphocytes

The hæmolysin response of rats to a single dose of 10^8 sheep erythrocytes is abolished by prior administration of 500 rads of whole-body X-irradiation. This unresponsiveness could be corrected with smaller doses of lymphocytes than were necessary in rats which had been depleted by drainage from the thoracic duct. Fig. 4 shows that an intravenous injection of 2·5 × 10^8 thoracic duct cells from a normal rat (99·4 per cent small lymphocytes) restored the hæmolysin response of an X-irradiated rat but that an equal number of fresh thoracic duct cells (not incubated *in vitro*; 95 per cent small lymphocytes) from a rat immunologically tolerant to sheep erythrocytes was without effect. This finding is important for two reasons. First, it shows that the restorative power of small lymphocytes is not merely a non-specific effect due to the injection of any cellular material. Secondly, it illustrates that immunological tolerance is a property of a population of small lymphocytes. Tolerant populations of cells have been described by others[17,18]. The present experiments identify a tolerant cell-type; they do not, of course, distinguish a population which is tolerant because reactive cells have been eliminated from one in which tolerant cells have been created.

Discussion

There is strong evidence that small lymphocytes can initiate graft-versus-host reactions[5]. Our experiments show that they may also initiate the response to homografts of skin and, by first differentiating into large pyroninophilic cells, give rise to the immunologically activated small lymphocytes which invade the graft. The sequence, small lymphocyte → large pyroninophilic cell → small lymphocyte has been demonstrated in a graft-versus-host reaction. This scheme may imply that there are two classes of small lymphocytes: those which are immunologically 'committed', in that they, or their precursors, have already reacted to antigen; and those which are 'uncommitted'. The latter class can be proposed without prejudice to the idea that the range of response in individual cells may be restricted in the sense suggested by Burnet[19]. The recirculation of small lymphocytes[13,16] could enable specifically reactive cells of either class to be selected from the total recirculating pool into any regionally stimulated node.

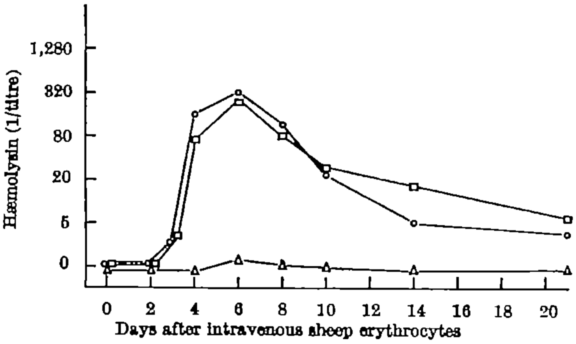

Fig. 3. Hæmolysin response to a single intravenous injection of 10^8 sheep erythrocytes in lymphocyte-depleted rats

○, Mean response of 4 rats after 2 days' drainage from a thoracic duct fistula; △, mean response of 10 rats after 5 days' drainage, □, mean response of 4 rats after 5 days' drainage; 10^9 small lymphocytes were injected intravenously when the fistula was closed and sheep erythrocytes were injected 48 h later

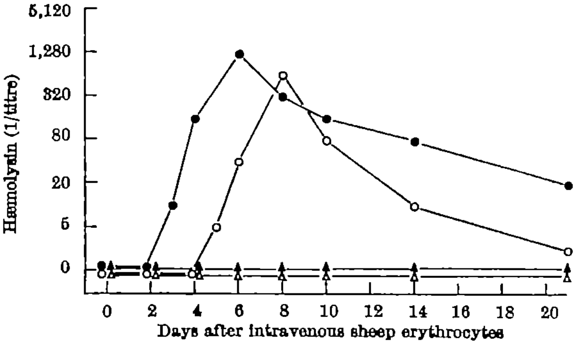

Fig. 4. Hæmolysin response to a single intravenous injection of 10^8 sheep erythrocytes in a normal rat and rats exposed to 500 rads of total body X-irradiation; sheep erythrocytes were injected 24 h after irradiation

●, Response of a normal rat; △, response of an irradiated rat; ○, response of an irradiated rat injected intravenously 2 h after irradiation with 2·5 × 10^8 small lymphocytes from a normal rat, ▲, response of an irradiated rat injected intravenously 2 h after irradiation with 2·5 × 10^8 lymphocytes from a rat immunologically tolerant to sheep erythrocytes

The demonstration that a population of small lymphocytes can carry the property of immunological tolerance is strong evidence that they are involved in the inductive phase of antibody formation[18]. The simplest view would be that small lymphocytes interact with antigen (or with antigen which has been 'processed' by reticuloendothelial phagocytes), become fixed in lymphoid tissue and give rise to a dividing cell-line of the kind identified by Nossal and Mäkelä[20]. This cell-line generates the cells which eventually synthesize antibody. Although there is strong morphological evidence that small lymphocytes are the ultimate precursors of antibody-forming cells[5,21], it must be emphasized that this has not yet been unequivocally demonstrated.

We are very grateful to Dr. A. J. Fulthorpe for help with the estimation of tetanus antitoxin. One of us (D. D. McG.) was supported by the Medical Research Council of Canada.

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USE OF *BHK 21* CELLS IN THE PREPARATION OF MOUSE ATTENUATED LIVE FOOT-AND-MOUTH DISEASE VACCINES FOR THE IMMUNIZATION OF CATTLE

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IN work reported previously[1] it was shown that the virus of foot-and-mouth disease could be grown in a cell line *BHK 21* (ref. 2) derived from hamster kidney. Both a strain of virus from the field, afterwards passaged only in cattle, and a vaccine strain modified in pathogenicity for cattle by passage in mice could be grown to high titre in these cells; and it was suggested that virus grown in this way could be of value in the preparation of vaccines.

In the experiments reported here a comparison has been made of the innocuity and immunogenicity for cattle of live vaccines prepared from modified strains (*a*) passaged once or several times in *BHK 21* cells and prepared from these cultures, and (*b*) passaged only in mice and prepared as mouse tissue suspensions[3].

The first experiment was made with a type *SAT 2* modified strain designated *RHO 1 AX/26 m 28 mo 118 m 1*, which was almost completely innocuous for cattle when inoculated by the intramuscular route. The two vaccines compared were: (*a*) One prepared after one passage in *BHK 21* cells as follows.

A 4-day-old monolayer culture of *BHK 21* cells (Clone 13) in a Roux bottle, that is, approximately 10^8 cells, was inoculated with 1·0 ml. of virus suspension having a titre of $10^{7\cdot0}$ mouse ID_{50}/ml. Following adsorption of the virus for 1 h at 37° C the culture was washed 5 times with phosphate-buffered saline ; 80 ml. of modified Eagle's medium[2] plus 10 per cent tryptose phosphate broth was then added. The tissue culture fluid was collected after 22 h incubation, the cell debris removed by centrifugation and to the supernatant an equal volume of glycerol was added. This preparation was stored at $-20°$ C until required. (*b*) Vaccine prepared from infected mouse tissue also contained 50 per cent glycerol similarly stored.

The two vaccines were tested for innocuity by inoculation at multiple sites intradermally on the tongues[4] of two groups of eight cattle. The dose of virus inoculated was $10^{5\cdot7}$ ID_{50}(mouse) for the mouse vaccine and $10^{6\cdot3}$ ID_{50}(mouse) for the *BHK 21* vaccine. One animal of the eight inoculated with the mouse vaccine and none of the eight inoculated with the *BHK 21* vaccine afterwards developed foot-and-mouth disease, indicating no significant difference in pathogenicity of the two vaccines.

In the second part of this experiment, the immunizing properties of the vaccines were compared in a protection test. Vaccinated cattle were challenged with a homologous cattle strain of virus. This type of test has been described elsewhere[5]. Each vaccine was tested in groups of 10 cattle at two dose-levels, and each animal received the appropriate dose of modified virus in a 6·0 ml. volume by the intramuscular route. It was intended that the vaccines be compared at similar dose-levels. To compensate for an apparent fluctuation in titre of the tissue culture vaccine during storage, the cattle receiving this vaccine were given doses which were adjusted to be similar to those of the mouse tissue vaccine. Subsequent re-titrations of the vaccines did not confirm this apparent drop in titre and in consequence the tissue culture vaccine was given at a higher dose-level than intended. Only 3 cattle of the 40 inoculated developed mild atypical foot lesions following vaccination. Twenty-one days after vaccination all the cattle were challenged by intradermal tongue inoculation with the homologous cattle strain of virus.

The result following the challenge shown in Table 1 indicates that the degree of protection given by the two vaccines is very similar.

Table 1. RESULTS OF A PROTECTION TEST COMPARING THE IMMUNITY PRODUCED IN CATTLE BY TYPE *SAT 2* LIVE MODIFIED FOOT-AND-MOUTH DISEASE VACCINES PREPARED FROM INFECTED MOUSE TISSUE, OR BY GROWTH IN *BHK 21* CELLS

Vaccine *RHO 1 AX/26 m28 mo118 m1* Method of preparation	Dose by intramuscular route $\log_{10}$ mouse ID_{50}	No. of cattle vaccinated	No. showing vesicular lesions after vaccination	No. protected against challenge	No. not protected against challenge
Mouse tissue	4·0	10	2	8	2
BHK 21 tissue culture	4·9	10	0	8	2
Mouse tissue	6 4	10	0	10	0
BHK 21 tissue culture	7 3	10	1	9	1

In a second experiment three vaccines prepared from the type *SAT 1* modified strain, designated *R V 11 mo 34 m 3*, were compared for their innocuity and immunogenicity in groups of 20 cattle. These vaccines were: (1) a mouse tissue vaccine; (2) a concentrated mouse tissue vaccine; (3) a *BHK 21* tissue culture vaccine. The mouse tissue vaccine was prepared in the usual way. The concentrated vaccine was prepared by Albertsson's phase separation method, using a polyethylene glycol, sodium dextran sulphate polymer system[6]. The concentrated vaccine was stored frozen at − 20° C for three weeks and then diluted with 50 per cent glycerol in M/25 phosphate buffer *p*H 7·6 immediately before use.

The tissue culture vaccine was prepared from the *R V 11 mo 34 m 3* strain of virus passaged three times in *BHK 21* cells by methods similar to those described for the first experiment. In this case, however, the collected virus was stored frozen at − 20° C for two days and then diluted with an equal volume of glycerol immediately before use.

All three vaccines were inoculated by the intramuscular route as a 3-ml. dose, and in each case the total amount of infective virus inoculated was $10^{7\cdot 5}$ mouse ID_{50}.

Table 2 shows the results obtained in this experiment. The reactions observed following vaccination in the 2 animals of the 60 inoculated were small lesions of a mild atypical character, involving one foot only of each of the animals affected. All cattle were challenged by intradermal tongue inoculation with homologous cattle strain virus at 21 days after vaccination. No significant difference was observed between the behaviour of the three vaccines in regard to their innocuity or immunogenicity when inoculated at this dose-level.

Table 2. RESULTS OF A PROTECTION TEST COMPARING THE IMMUNITY PRODUCED IN CATTLE BY TYPE *SAT 1* LIVE MODIFIED FOOT-AND-MOUTH DISEASE VACCINES, PREPARED FROM INFECTED MOUSE TISSUE OR BY GROWTH IN *BHK 21* CELLS

Vaccine *RV 11 mo 34 m 3* Method of preparation	Dose by intramuscular route $\log_{10}$ mouse ID_{50}	No. of cattle vaccinated	No. showing vesicular lesions after vaccination	No. protected against challenge	No. not protected against challenge
Mouse tissue	7·5	20	1	18	2
Concentrated mouse tissue	7 5	20	1	18	2
BHK 21 tissue culture	7 5	20	0	18	2

A third experiment has been made to assess the innocuity of the *R V 11 mo 34 m 3* strain after six serial passages in *BHK 21* cells. Following the injection of $10^{6\cdot 3}$ mouse ID_{50} of virus by the intramuscular route, no lesions were observed in a group of 8 cattle. This result and that of a similar test in the second experiment provided no evidence that there was any alteration in the degree of innocuity for cattle of this modified strain (*R V 11*) as a result of three or even six passages in *BHK 21* cells.

This work shows that effective live modified foot-and-mouth disease vaccines can be produced by growth of the virus in *BHK 21* cells. With two vaccines of different immunological types, growth in these cells did not cause alterations of the known characteristics of these strains in cattle, and the immunity stimulated by them was at least of the same order as that produced by the conventional vaccines prepared from infected mouse tissues.

The use of a tissue culture method for the production of established live modified vaccines offers advantages in terms of simplicity of preparation and eliminates the need for a large supply of unweaned mice. In addition, the successful growth of *BHK 21* cells in suspended culture has recently been reported[7], and this technique, coupled to the growth of virus in suspension culture, should provide a very economical method for the production of live modified foot-and-mouth disease vaccines.

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EFFECT OF ANTI-TUMOUR AGENTS ON NON-DIVIDING CELLS

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THERE is evidence that the tumour-inhibitory alkylating agents are divisible into two classes on the basis of their biological effects—the mustards and ethylene imines on one hand, and 'Myleran' and its analogues on the other. The former agents, often termed 'radiomimetic', differ from radiation in several ways. They cross-link significant amounts of DNA in living cells[1,2], immediately arrest mitosis in tissue cultures of mouse lymphoblasts[3], cause early onset of sterility in the male rat (apparently due to damage to spermatids and spermatozoa, non-dividing cells)[2,4,5] and inhibit antibody production, if given after the antigen[6]. Radiation and the 'Myleran', however, do not cross-link significant amounts of DNA in living cells[1], arrest mitosis in cultured lymphoblasts only after a delay[3], cause late onset of sterility[4] and inhibit antibody production only if given before the antigen[6,7]. These differences in biological effect may be due to a change in the susceptibility of the cell to toxic agents as it differentiates, early stages being specially susceptible to radiation and 'Myleran' and later stages to the mustards and ethylene imines. This susceptibility might be independent of any occasioned by cell-division.

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Megakaryocytes undergo marked changes during differentiation, but do not divide. It was therefore decided to investigate the effect on these cells of agents of the two classes mentioned here.

Male *A2G* mice, weighing 15–21 g, were killed and the marrow of both femurs expelled into 5 per cent bovine plasma albumin in physiological saline[8]. Total megakaryocyte counts were performed on the suspensions, and differential counts on films stained with May Grünwald–Giemsa. Cells were classified as megakaryoblasts (non-granular), basophil megakaryocytes (with azurophil granules occupying up to a third of the cytoplasm), granular megakaryocytes (with granules occupying more than a third of the cytoplasm) or platelet-producing megakaryocytes (with peripherally attached platelets). Results for normal mice are given in Table 1 (total counts on 30 mice and differentials on 24).

Table 1. ABSOLUTE NUMBERS OF MEGAKARYOCYTES/FEMUR IN NORMAL MICE

Total	Blasts	Basophils	Granular	Platelet-producing	Doubtful
2,990 ±252	48 ±33	360 ±86	2,136 ±199	370 ±92	98 ±41

Mice were given single subcutaneous injections of methyl *bis* (β-chloroethyl) amine hydrochloride (HN2), 2·4 mg/kg, or triethylene melamine (TEM), 2 mg/kg, in saline, 'Myleran', 125 mg/kg in arachis oil or a single irradiation of 400-r. γ-rays in a cobalt-60 unit at the rate of 10 r./min. Megakaryocytes were counted in groups of 5–10 mice at daily intervals after treatment, and the absolute number per femur of each cell type calculated. The numbers of blast cells and basophils tended to change in parallel fashion and so, for simplicity of presentation, the results for these two types were combined ('early cells'). For the same reason the results for granular and platelet-producing cells were also combined ('late cells'). The results, expressed as percentages of the absolute counts in normal mice, are given in Figs. 1 and 2.

There was a striking difference between the changes caused by radiation and 'Myleran', on one hand, and HN2 and TEM, on the other. The former agents caused a rapid fall in the number of early cells, whereas the fall in late cells was delayed until the third or fourth day. In contrast, with the latter agents it was the late cells which fell soonest, the absolute number of early cells rising markedly at first, to fall later. It is clear that the differences in biological effect between the two classes of agent noted in other systems are also shown in their action on megakaryocytes.

Elson[9] also found that the fall in number of peripheral blood platelets was more rapid after treatment with the mustards than after radiation or 'Myleran'. The number of megakaryocytes fell slowly after treatment with 'Myleran' and rapidly after chlorambucil, a mustard, which also caused a transient rise in early forms[10]. Simpson[11] showed that radiation caused cells of the early types in the rat to fall before the more mature ones did.

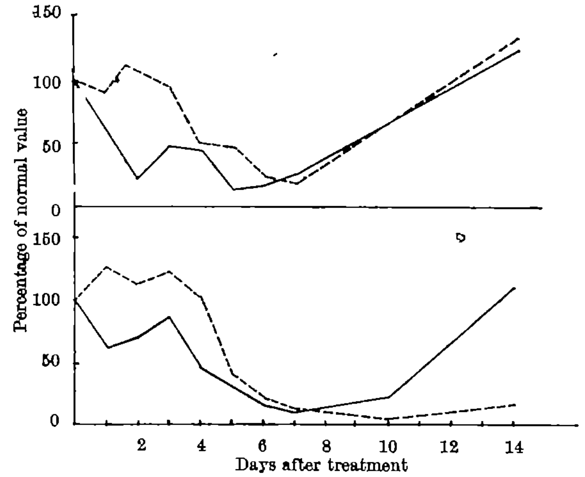

Fig. 1. Changes in megakaryocytes after radiation (top) and treatment with 'Myleran' (bottom) expressed as percentages of normal absolute values (arithmetic means of counts on 5–10 mice per group). The *S.D.* for early cells was usually 25–50 per cent of the mean, that for late cells 15–40 per cent. Early cells ——, late cells – – – –

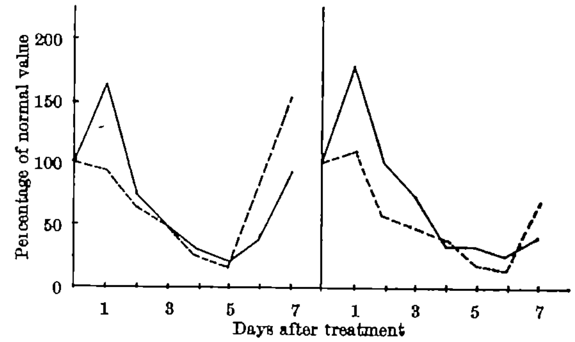

Fig. 2. Changes in megakaryocytes after treatment with HN2 (left) and TEM (right) expressed as percentages of normal absolute values (arithmetic means of counts on 5–10 mice per group). The *S.D.* for early cells was usually 25–50 per cent of the mean; that for late cells 15–40 per cent. Early cells ——, late cells – – – –

The detailed interpretation of these effects is difficult. The slow recovery after radiation and 'Myleran', compared with the rapid recovery after HN2 and TEM, suggests that the former damage cells at a considerably earlier stage of development than the latter. Possible mechanisms include destruction of megakaryocytes and destruction, delayed differentiation and arrested division of precursors, all of which would cause a fall in cell-counts. Each of these mechanisms may operate to a varying extent with all the agents used. An increase in a particular cell type immediately after administering an agent is most likely to be due to a delay in differentiation at that cell stage with unchecked differentiation of the immediate precursor. This effect is seen in early megakaryocytes after HN2 and TEM, so that it is likely that, whatever their action on the dividing precursors of the megakaryocyte, these substances act directly also on the non-dividing megakaryocyte.

It appears to be established, therefore, both for megakaryocytes and for spermatids and spermatozoa, that the mustards and ethylene imines can damage cells which are differentiating but not dividing, a consideration relevant for cancer chemotherapy. It is often assumed that tumour-inhibitory agents can differentially damage tumours because they are particularly noxious to rapidly proliferating cells. This does not explain their therapeutic effect in acute leukæmias, in which the neoplastic cells may divide less frequently than normal cells[12]. It is possible that part of the susceptibility of neoplasms to agents used in cancer chemotherapy is independent of cell proliferation *per se*. It may be due to special sensitivities arising in cells at particular stages of differentiation. Such stages may be prolonged in some neoplasms.

We thank Dr. J. F. A. P. Miller of the Chester Beatty Research Institute for irradiating the mice and Prof. F. Bergel for a gift of 'Myleran'.

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MECHANISM OF ACTION OF YEAST ALCOHOL DEHYDROGENASE

By DR B. R. RABIN and E. P. WHITEHEAD

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YEAST alcohol dehydrogenase, as prepared by Racker's method[1], is a 'thiol' enzyme[2] which contains tightly bound zinc[3]. It has a molecular weight of 150,000 and binds four molecules of coenzyme[4], the oxidized (NAD^+) and reduced (NADH) forms of which compete with each other for the binding site. Thus the enzyme probably possesses four active sites and there is evidence that each of these is associated with a zinc atom[5]. Estimates of the number of 'thiol' groups per mole vary from 18 (ref. 6) to 24 (ref. 7). The presence of ethanol[2], NAD[2], acetaldehyde[6] or NADH[6] is stated to reduce the number of 'thiols' reacting with silver but contradictory results have been obtained by other workers[7]. It has been claimed[2] that NAD and ethanol protect the enzyme from inhibition by iodoacetamide. We have confirmed the protection by NAD but have been unable to observe any protection by ethanol. It is essential to use only enzyme which is maximally active in the absence of cysteine for protection experiments and such a preparation has been used throughout in the work described here.

We have found that the total number of 'thiol' groups determined by reaction with para-chloromercuribenzoate[8] (PCMB) is variable and depends on the previous history of the preparation. However, the number which react with iodoacetamide, as determined differentially by the PCMB method before and after treatment of the enzyme with iodoacetamide (1·5 10^{-3} M, *p*H 9·0, 40 min), is constant and appears to be approximately equal to the number of molecules of coenzyme bound. This is consistent with the presence, per active site, of one 'thiol' group as a 'contact' residue[9] reactive towards iodoacetamide. These groups will be referred to as reactive thiols.

Progress curves for the reaction of the enzyme with iodoacetamide and the effects of substrates and coenzymes on this reaction are shown in Fig. 1. The reactions are accurately first order in both iodoacetamide and enzyme. Both NAD^+ and NADH protect the enzyme against reaction with iodoacetamide suggesting that the reactive thiol interacts with the coenzyme. The considerable quantitative difference for the reduced and oxidized form of the coenzyme would seem to implicate the nicotinamide ring as the site of interaction with the reactive thiol as suggested for the liver enzyme[10]. We believe a covalent adduct is involved rather than the hydrogen bonded structure of Kaplan and Ciotti[11].

To gain further insight into the nature of the reactive thiol the effect of *p*H on the rate of the reaction with iodoacetamide was investigated and the results are shown in Fig. 2. The outstanding feature observed is that the reaction is independent of *p*H over the range 4·5–10·0. A similar phenomenon has been previously reported for creatine phosphokinase[12,13]. As already discussed the most plausible explanation is that the reactive thiol is hydrogen

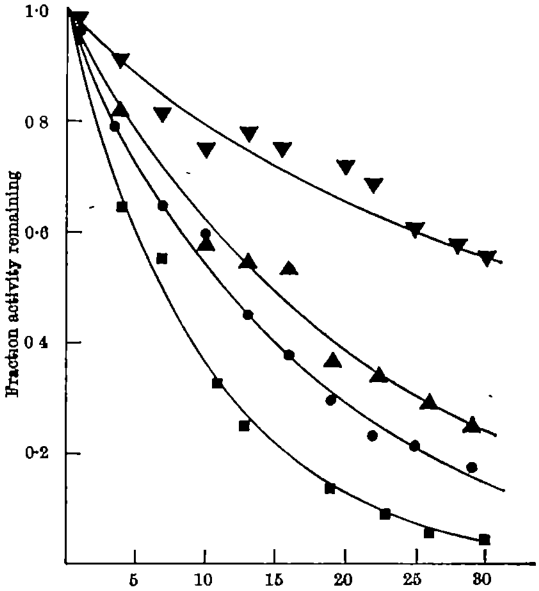


Fig. 1. Progress curves of inactivation of yeast alcohol dehydrogenase by iodoacetamide (1·5 mM) at *p*H 9·0, I 0·1 and 25° C. ●, no additions; ▲, in the presence of NAD^+, 1·35 mM; ▼, in the presence of NADH, 1·35 mM; ■, in the presence of acetaldehyde, 83·5 mM

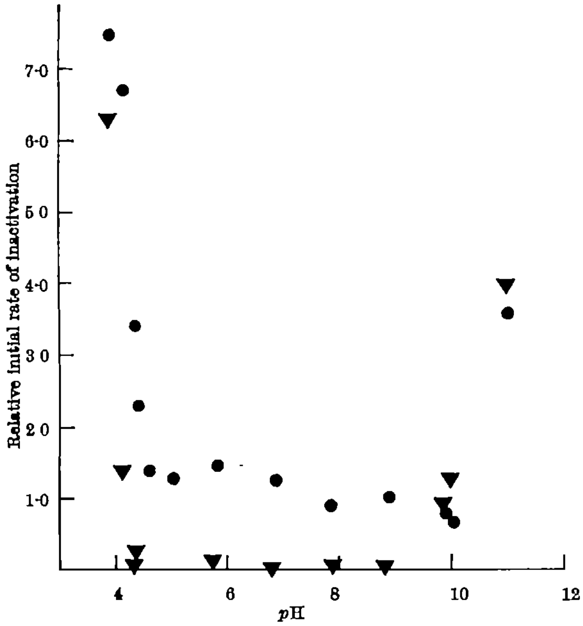


Fig. 2. Variation of rate of inactivation of yeast alcohol dehydrogenase with pH at 25° C. ▼, spontaneous, no additions; ●, in presence of 0·85 mM iodoacetamide (corrected for spontaneous rate). The first-order rate constants are relative to that of pH 9·0 in the presence of iodoacetamide, which is taken as unity

bonded to a base species on the enzyme. At the extremes of the pH range investigated the reaction-rate increases rapidly. The rate of spontaneous inactivation parallels these increases which are probably associated with structural changes in the protein making available additional reactive groups. The dominating effect here could be the addition or removal of a proton from the hydrogen bonded pair involving the reactive thiol. The results suggest that the base species is an imidazole giving the structure shown in Fig. 3.

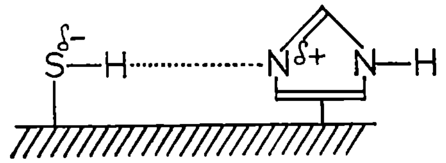


Fig. 3. Structure of the reactive thiol group of alcohol dehydrogenase

The predicted effects of hydrogen bonding on the pK's of the groups are to raise that of the thiol and lower that of the imidazole: this is consistent with the pH effects observed.

The sulphur atom of the thiol–imidazole pair should be quite a powerful nucleophilic reagent over a wide pH range and we believe that it functions catalytically by forming a covalent adduct with the nicotinamide ring probably at carbon atom 2. For NADH this reaction would be the familiar nucleophilic addition of a thiol to an activated double bond similar to the well-known reaction with N-ethyl maleimide[14]. The activation is provided by the amide oxygen on carbon (3). Similar activation can be provided by the coenzyme analogues[15] which function with dehydrogenases. The adduct formation would be catalysed by the co-ordination of the amide oxygen to a Lewis acid, perhaps zinc. Interaction of this sort has been suggested for both yeast and liver[16] alcohol dehydrogenases, but alternative roles for zinc have also been proposed[17-19]. Amide complexes of metals, including zinc, are well authenticated and the site of interaction is with the amide oxygen atom[20]. The reaction involved in the formation of the adduct with NADH is shown in Fig. 4.

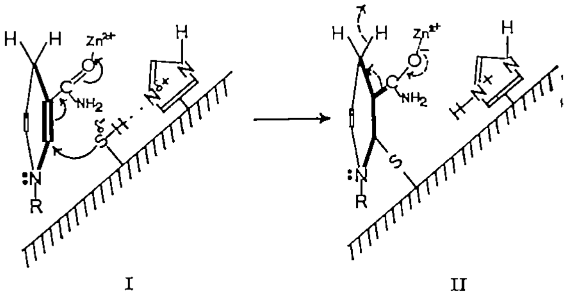


Fig. 4. Formation of NADH-reactive thiol adduct

The attack of the sulphur would doubtless be specific for one face of the ring. The adduct would have a greater tendency than NADH to transfer a hydride ion to acetaldehyde because of the electronic tendencies shown by the dashed curved arrows which are not present in NADH. If the transfer of the hydride occurs from the same face of the nicotinamide ring as the sulphur addition, then it can easily be shown with models that the acetaldehyde oxygen can approach very near to the imidazole hydrogen. Thus acetaldehyde could affect the reactivity of the thiol, in the absence of the coenzyme, as is in fact observed in Fig. 1. The predicted shift would be towards increased nucleophilic reactivity, in accord with the experimental data. No such effect is expected for ethanol, nor is it observed. On formation of the NADH-thiol adduct the imidazolium ion is released and the hydride transfer to acetaldehyde could be further catalysed by interaction of the aldehyde oxygen with this ion as illustrated in Fig. 5. The reduction of acetaldehyde requires the imidazole to be in the cationic form.

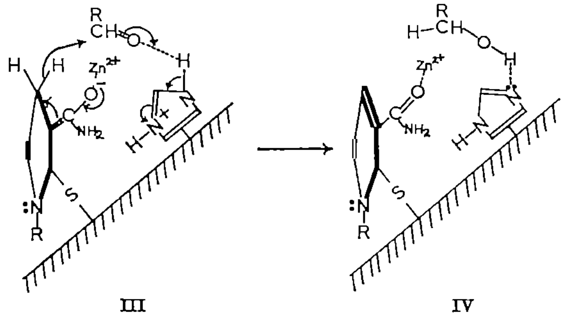
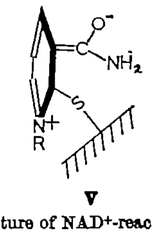


Fig. 5. Mechanism of hydride transfer from NADH to acetaldehyde

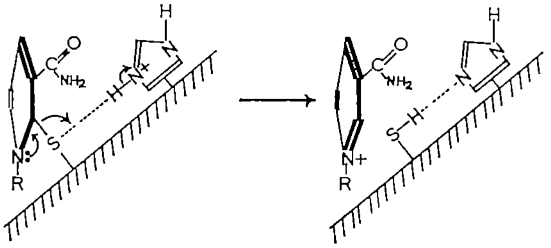
The product of the reaction is one canonical form of a thiol-NAD+ adduct. The other canonical form is shown in Fig. 6.



V

Fig. 6. Structure of NAD+-reactive thiol adduct

Dissociation of the alcohol from the enzyme surface would make available the imidazole which could acquire a proton and catalyse the decomposition of the thiol-NAD+ adduct as shown in Fig. 7.



VI VII

Fig. 7. Decomposition of NAD+-reactive thiol adduct

As required by the principle of microscopic reversibility the reverse reaction would take place by complete reversal of the pathway of the forward reaction by the sequence VII→VI→IV→III→II→I. This mechanism provides a simple explanation of how the coenzymes tend to select their appropriate reaction partners. The thiol-NAD+ adduct (IV and V) has one more unit of positive charge than the thiol-NADH adduct (II). Thus the thiol-NAD+ adduct favours the base form of the imidazole and hence the binding of alcohol whereas the thiol-NADH adduct favours the acid form of the imidazole and the binding of acetaldehyde.

This mechanism is entirely different from previous proposals[10,16,18,21] and we believe it to be general for alcohol dehydrogenases. Adducts of mercaptans and NADH have been prepared[22] and the spectral[23] and optical rotatory[24] changes associated with NADH binding are consistent with our hypothesis. Proof must, however, await the isolation of a cysteine derivative appropriately substituted and we are at present attempting to achieve this.

We thank the following for grants in aid: the Wellcome Trust, for the purchase of a Cary recording spectrophotometer; the Central Research Fund, University of London, for the purchase of automatic titration equipment; the Medical Research Council for a personal grant to one of us (E. P. W.).

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INHIBITION OF THE EVOLUTION OF ETHYLENE AND THE RIPENING OF FRUIT BY ETHYLENE OXIDE

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AND
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INVESTIGATIONS of the biosynthesis of ethylene in plants have led to the recognition of a cell-free enzymatic system from fruits which produces ethane in the presence of thiomalic acid and linolenic acid. The details of this system and its possible relationship to ethylene biosynthesis is discussed elsewhere[1]. The pertinent point in the present discussion is the tentative identification of ethylene oxide (epoxy ethane) as an additional product of the ethane-producing reaction system. Following this discovery, ethylene oxide was also tentatively recognized in the volatiles emanating from tissue slices and tissue homogenates of fruit, and has also been found in very low concentrations, on a few occasions, in volatiles of intact fruit. The physiological significance of this finding was realized in the course of experiments which demonstrated that ethylene oxide inhibited ethylene production and ripening of intact fruit. We will discuss here some of these findings and suggest a role for ethylene oxide in fruit ripening.

Ethylene oxide was tentatively identified by co-chromatography with authentic compounds on two columns. The first column, previously described[2], contained silicone oil 550 as a stationary phase and gave a corrected retention time of 26 min at 25° C. Under identical conditions an 18-ft. column containing 30 per cent squalane as a stationary phase gave a corrected retention time of 13 min.

In seven experiments the ripening of green tomato fruits was retarded by holding them for 16–22 h in an atmosphere containing 0·75 per cent ethylene oxide. The treated fruit then ripened normally at 20° C after a delay of 5–21 days, depending on the ripeness of the fruit at the start of the experiment. Ripeness was judged by ethylene production, colour and softness of the fruit.

Retarded fruit could not be ripened after large doses of ethylene oxide (more than 2 per cent) or after extended contact (2 or 3 days) with 1 per cent of ethylene oxide in the atmosphere. In these cases

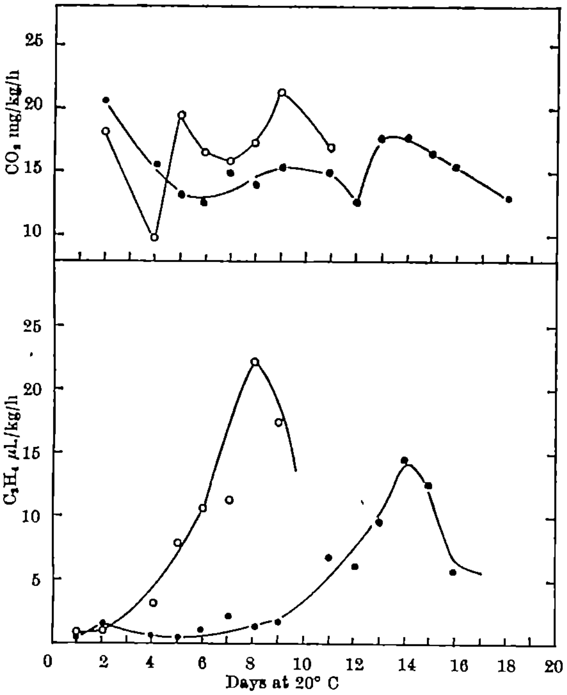

Fig. 1. Ethylene production (bottom) and respiration (top) of two tomatoes during ripening at 20° C. Both fruits were green at the start of the experiment and were emanating approximately equal quantities of ethylene. The control started to yellow after 2 days and was completely red after 7 days. The ethylene oxide-treated fruit was green for 7 days, started to yellow on the 8th day and did not turn completely red until the 18th day. Ethylene was determined by gas chromatography as previously described (ref 2). Respiration was determined by absorbing evolved carbon dioxide in alkali and titrating with standard acid. ○, Control fruit; ●, fruit treated with 0·65 per cent ethylene oxide for 20 h at 20°

the tissues were permanently damaged, as evidenced by a breakdown around the stem scar of the fruit.

Fruit already on the climacteric rise, producing fairly large amounts of ethylene and showing some red colour, was not appreciably retarded by treatment with ethylene oxide. Some fruit showed retardation in colouring but no, or only slight, inhibition of ethylene production. Treatment with ethylene oxide did not affect the subsequent flavour of any of these fruits after ripening.

Respiration was not significantly altered in the treated fruit in most of these experiments. However, in a few experiments there was an increase in respiration in the treated fruit. Fig. 1 shows results obtained with green fruit in a typical experiment.

Preliminary experiments with apples indicated that ethylene oxide was acting similarly in this tissue. Apples producing high levels of ethylene and therefore on the climacteric rise, were not retarded in ripening; however, the production of ethylene was very greatly depressed by 0·75 per cent ethylene oxide.

Acetaldehyde also retarded ripening. However, it was far less effective than ethylene oxide, for a given dosage. It seems possible that acetaldehyde may be converted to ethylene oxide; the compounds are isomeric and both appear to retard ripening in a similar manner.

The fact that ethylene oxide retards ripening and simultaneously inhibits the production of ethylene by the fruit may support the concept that ethylene is actually a ripening hormone and not simply a by-product of senescent metabolism, which only incidentally accelerates ripening of fruit[3]. Ethylene oxide may actually be an endogenous metabolic antagonist of ethylene and, as such, inhibit the ripening process. During growth and development there may be an interplay between ethylene and ethylene oxide that determines the degree of ripening which occurs at any stage. Indeed, in one experiment it was possible to antagonize the retarding effect of ethylene oxide (0·3 per cent) on the ripening of tomatoes by including ethylene (0·005 per cent) in addition to ethylene oxide in the atmosphere. It may be possible to control maturation and ageing to some extent in this way by the proper proportioning of ethylene and ethylene oxide to promote and regard reactions associated with ageing and ripening. The biochemistry of fruit ripening should consider not only the influence of ethylene but also that of ethylene oxide, acetaldehyde and related volatiles on tissue metabolism.

This work was carried out at the Low Temperature Research Station, Cambridge.

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AN APPARENT 20 MAP-UNIT POSITION EFFECT

By Dr. J. B. GIBSON and Prof. J. M. THODAY

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IN investigating a polymorphism affecting sterno-plural chaeta number in *Drosophila melanogaster*, we have demonstrated[1,2] the existence of two 'loci' with remarkable properties. We have since obtained new information about these loci which, together with a summary of the earlier knowledge, is presented here.

The two loci are close to positions 27 and 47 in the second linkage group and at each locus two 'alleles' are known, one (designated +) giving a higher chaeta number than the other (designated −). There are thus four possible kinds of chromosome with respect to these two pairs of factors, − −, + −, − +, and + +. We have already shown[2] that the − − chromosomes were responsible for the low chaeta number flies in the polymorphic population that we were investigating, and this chromosome was proved homozygous lethal. That both + − and − + chromosomes occur in the polymorphic population and also in the wild-stock (Dronfield) from which the polymorphic population had been established was demonstrated by recombination tests: + −/− + heterozygous females produced − − eggs with regularity. There was, however, no evidence that such heterozygotes produced the reciprocal recombinant class of progeny and the chromosome + + therefore seemed to be dominant lethal. Appropriate marker tests using *dp* + − *cn*/− + females mated to

$dp + - cn/dp + - cn$ males substantiated this for the relevant marker recombinant class ($dp\ cn^+$) was deficient in quantities equivalent to the frequency of $dp^+ - - cn$ recombinants.

We have now investigated the viability of the eggs produced by the mating $+-/-+$ ♀ × $+-/+-$ ♂, in order to test whether lethal eggs are actually laid. The results are given in Table 1. The proportion of eggs produced by this mating that fail to hatch significantly exceeds that produced by the reciprocal mating and by the $+-/+- \times +-/+-$ mating. This excess is numerically very close to that to be expected on the hypothesis that the $++/+-$ recombinant progeny are laid but fail to hatch.

To summarize, the investigation reported by us previously[2] involved extensive production of the following genotypes: $+-/+-$, $-+/-+$, $+-/-+$, $--/+-$ and $--/-+$ each of which is viable, and $--/--$ which is lethal. We also involved extensive attempts to produce the genotype $++/+-$, none of which was successful. We have since done appropriate experiments to test for the occurrence of $++/-+$ flies which also prove lethal.

In view of the impossibility of obtaining flies containing $++$ chromosomes, we at first thought that the two remaining genotypes, each of which involves $++$, could not be investigated. However, an experiment has since occurred to us that makes it possible to test the viability of the most interesting of them, namely, $++/--$.

The test was as follows. A $dp\ --\ cn$ chromosome was bred and dumpy non-cinnabar males were selected from the mating:

$$dp + - cn/- + ♀ \times Cy\ L/dp - - cn ♂ \rightarrow dp\ cn^+/dp - - cn ♂$$

The map distances[2] are dp 15 + 20 − 10 cn, so that if $++/--$ is viable, of the $dp\ cn^+$ chromosomes in these males 15/45 should be $dp - + cn^+$, 20/45 should be $dp + + cn^+$ and 10/45 should be $dp + - cn^+$, so that approximately 45 per cent of the males should contain $dp + + cn^+$ chromosomes.

To test whether this was so, 200 such males were mated singly to $dp + - cn$ homozygotes. Since we know that $++/+-$ flies do not occur, any of the tested males that were in fact $++/--$ should fail to produce non-cinnabar progeny, for the non-cinnabar class of zygotes they would produce is necessarily $dp + + cn^+/dp + - cn$. Of the 200 males, 185 produced progeny. All the progenies contained both cinnabar and non-cinnabar flies. None of the males can have been $++/--$.

The information we have concerning the viability interactions of these two loci is therefore as follows:

	Apparent position effect				
Viable	$\frac{+\,-}{-\,+}$	$\frac{+\,-}{+\,-}$	$\frac{-\,+}{-\,+}$	$\frac{-\,-}{+\,-}$	$\frac{-\,-}{-\,+}$
Lethal	$\frac{+\,+}{-\,-}$	$\frac{+\,+}{+\,-}$	$\frac{+\,+}{-\,+}$	$\frac{-\,-}{-\,-}$	
Presumed lethal		$\frac{+\,+}{+\,+}$			

The demonstration that the genotype $++/--$ is unobtainable eliminates certain explanations that, hitherto, might have been given for our results, and were, in fact, put forward before the test of $++/--$ was made. One explanation, suggested by Dr. J. A. Pateman, was that the loci are duplicate loci having the same essential biochemical function, that − genes do not function, but that more than two + genes as in $++/+-$ produce an excess of whatever metabolites are involved, such excess being lethal. This explanation would not explain the lethality of $++/--$.

Table 1. NUMBERS OF EGGS FAILING TO HATCH FROM 100 EGGS

Type of cross	♀		♂	Replicate experiments 1	2	3	4
(1)	$\frac{+\,-}{+\,-}$	×	$\frac{+\,-}{+\,-}$	5	8	8	10
(2)	$\frac{+\,-}{+\,-}$	×	$\frac{+\,-}{-\,+}$	6	10	9	11
(3)	$\frac{+\,-}{-\,+}$	×	$\frac{+\,-}{+\,-}$	18	20	16	15

Cross 3 v rest:	$\chi^2_{(1)}$	20 6076	P small
Heterogeneity:	$\chi^2_{(10)}$	4·7352	$P \simeq 0{\cdot}9$
Total:	$\chi^2_{(11)}$	25 3428	$P < 0{\cdot}01$

Another explanation suggested to us by Prof. K. Mather was that the two chromosomes $+-$ and $-+$ differ by a translocation such that $+-$ is the normal chromosome and $-+$ involves a translocation of the right material to the left-hand position. The $++$ recombinant would then involve a deficiency, and the $--$ a duplication. This hypothesis, too, fails to account for the inviability of $++/--$ zygotes.

We are therefore left with a surprising situation in which we not only have a reproducible located synthetic lethal, with the reciprocal recombinant a synthetic dominant lethal, but we also have an interaction over 20 map units that is formally a position effect of *cis–trans* type, $++/--$ being lethal, whereas $+-/-+$ is viable. It should be noted that although this position effect looks like a *trans–cis* position effect, it is, in fact, arbitrary whether $+-/-+$ is to be called the *trans* or the *cis* arrangement, for + and − merely designate the direction in which the genes affect bristle number. If we were to make any decision, we would say that, since $+-$ and $-+$ are wild-type chromosomes, that is have both been obtained from a wild-type population and both give wild-type flies when homozygous, $+-/-+$ is the *cis* arrangement. Apart from this, there are two striking differences distinguishing the present apparent position effect from hitherto known position effects. The first arises from the very large map distance between the loci. In this respect there is some similarity with the non-complementation of some methionine suppressors in *Coprinus*[3,4]. Here, however, the genes that fail to complement are in separate nuclei in a dikaryon, whereas in our case the genes are on different chromosomes in the same nucleus. The second difference involves the fact that both '*cis*' arrangements ($+-$ and $-+$) are non-mutant, that is, viable as homozygotes, whereas it is usual in *cis–trans* position effects for only one of the *cis* arrangements to be wild-type.

By what mechanism such an apparent position effect interaction as this could occur is obscure. Perhaps we shall be forced to the view that a form of non-allelic pairing, possibly mediated by heterochromatin, is sometimes involved in the control of gene function. Whatever the explanation may be, however, the evidence that we have here a new and striking kind of position effect seems strong.

We thank Prof. K. Mather, Dr. J. L. Jinks and Dr. J. A. Pateman for valuable comments.

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LETTERS TO THE EDITOR

PHYSICS

A Method for Unravelling Complex Spectra

ONE of the central features of any spectroscopic investigation is the problem of unscrambling the patterns of lines. In general one has some knowledge of individual line shapes and the physical principles through which the spectra are built up, but one does not know how the particular components or line patterns are intermixed and shifted with respect to one another. This communication describes a systematic correlation method for decomposing complex, high-resolution spectra made up of many interleaving and overlapping lines.

Examples of spectra for which the method is applicable include high-resolution electron spin resonance, spin–spin coupling in nuclear magnetic resonance, and X-ray crystallography. In the third case the Patterson map can be treated as a centrosymmetric three-dimensional spectrum and the methods we describe generalize the traditional image seeking and superposition techniques used in this field. Energy-level assignments in complicated atomic and molecular spectra or unravelling the order present in sets of nuclear energy-levels are other possible applications. The checking and unscrambling of messages may also be aided by these methods. For concreteness we will describe the methods in a manner suitable for the determination of the proton hyperfine interaction coupling constants in isotropic electron spin resonance spectra. In these spectra a given coupling constant gives rise to a set of (n_i+1) Lorentzian shaped lines binomially distributed in intensity, where n_i is the number of equivalent protons. Magnetic field is the independent variable, and the spacing between successive lines is the coupling constant measured in gauss. A second set of equivalent protons corresponding to a second coupling constant splits each of the first lines so that the intensity of any observed line is a product of the binomial coefficients and the number of lines in the spectrum is $(n_i+1)(n_j+1)$. The spectra have a centre of symmetry. A number of cases with a relatively few lines or widely separated coupling constants have been broken down by trial and error; but there exist many spectra with 300–3,000 interspersed and overlapping lines which have not been resolved. The limited resolution of spectrometers increases line overlap, and limited sensitivity causes lines to be lost in noise at the ends of spectra, both of which increase the difficulty of the problem.

Insight into the nature of the problem can also be gained by a brief consideration of classical methods of analysis. A least-squares fit accomplished by a steepest descent technique or another scheme is a refinement rather than a search procedure and will generally yield false solutions for complex spectra. This situation is well known in X-ray crystal structure analysis. Fourier analysis or expansions in orthogonal polynomials are not suited to the problem because one is interested in shifts and interweaving patterns rather than frequencies. Analogue computer simulation fails because there are too many interdependent variables.

We have developed a variety of correlation methods to handle the problem. The first two of these, autocorrelation and cross-correlation, are familiar from signal analysis and communication theory, but there they are generally applied to separate a coherent signal from random noise while our problem is to separate one pattern or signal from a highly ordered background of other patterns. The autocorrelation of complex spectra is the appropriate tool when one has no *a priori* knowledge of the structural pattern in the spectrum and it tends to bring out regularities and the separations between major components, but it is frequently as difficult to interpret as the spectrum itself. On the other hand, the cross-correlation coefficient:

$$C(H_i) = \int_{-\infty}^{\infty} V_{\text{exp}}(H)\, V_{\text{test}}(H_i;\, H)\, \mathrm{d}H$$

enables us to utilize our knowledge of the number, intensity, and shape of the lines. Fig. 1 is a typical test pattern for four equivalent protons characterized by a single coupling constant, H_i. The cross-correlation coefficient is simply the inner or dot product of the two vectors representing the experimental trace with the test pattern. The test vector is moved by varying H_i and a maximum projection is used as the criterion for selecting the desired H_i. Sequential search for successive H_j's uses the knowledge of the previous H_i's to enhance the discrimination of the test pattern, and this scheme has been successful in decomposing a number of electron spin resonance spectra of intermediate complexity.

Cross-correlation, however, can lead to false maxima in the correlogram ($C(H_i)$ versus H_i) because

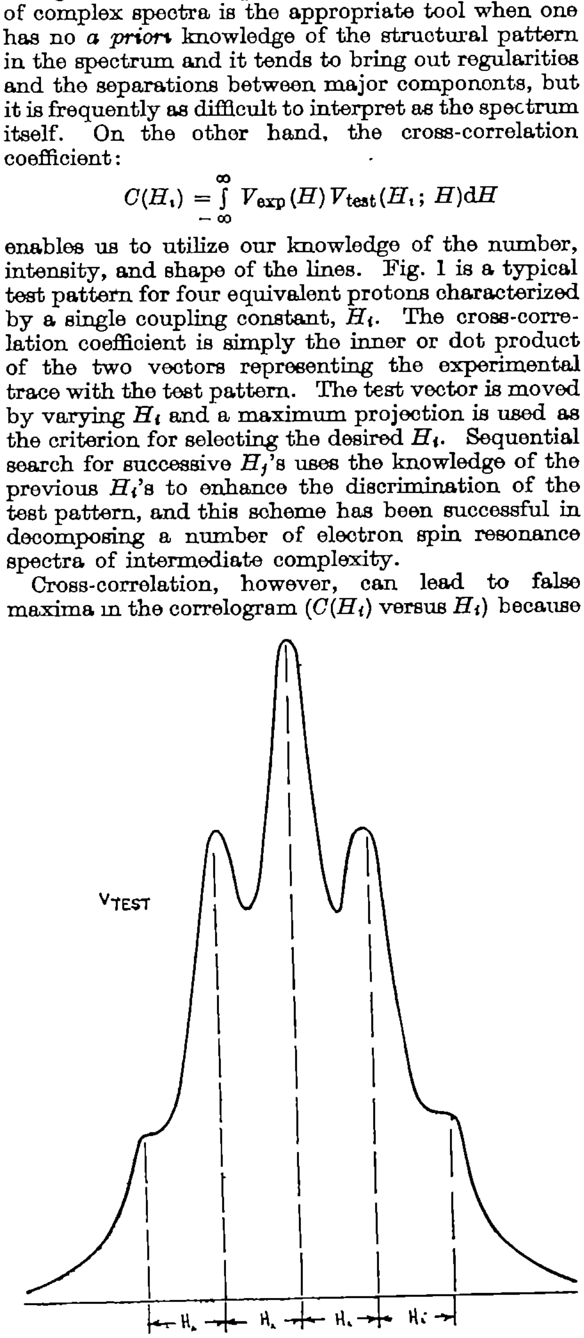

Fig 1. Test pattern for four equivalent protons

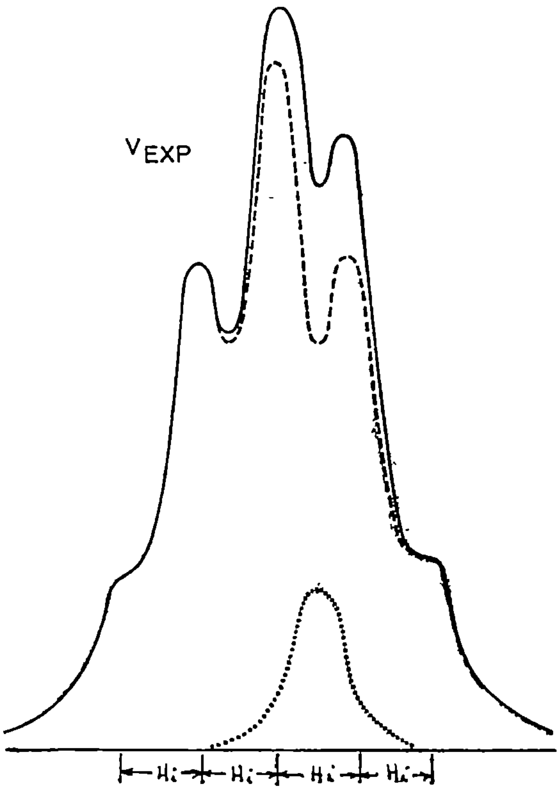

Fig. 2. Section of experimental trace containing both the pattern of lines for which one is searching and an interleaving line from another set

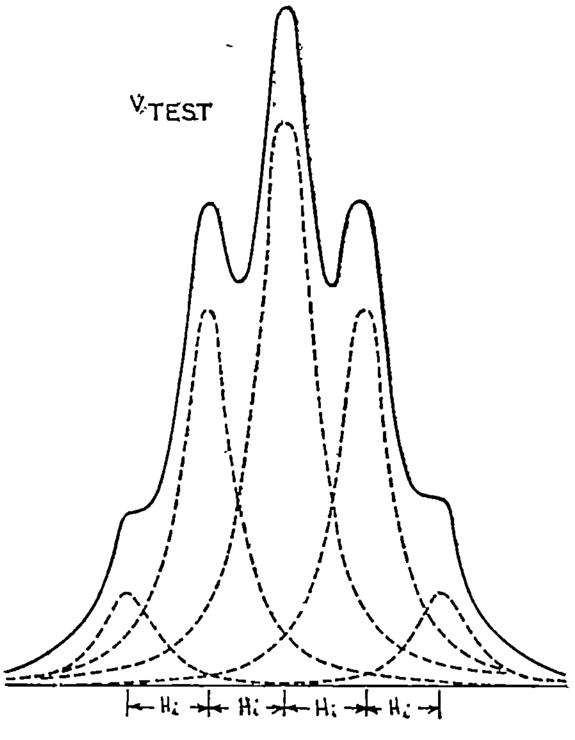

Fig. 3. Individual lines which comprise test pattern

of interlaced lines from other patterns. This always gives too large an amplitude in the correlogram (Fig. 2; the dotted curve represents an interloping line) and intrapattern correlation can be introduced to eliminate this. Intrapattern correlation makes use of redundancy in the test pattern as illustrated in Fig. 3. In this example the test pattern is built up from five individual lines, and to introduce an additional correlation we compute the cross-correlation coefficient for each of the five components of the test pattern separately and normalize them (taking proper account of the overlap of the components). As can be seen from Figs. 2 and 3, an interleaving line will distort the correlation coefficient for only one or two of the components and so we select only the minimum of the five individual correlation coefficients to represent the correlation for the whole test pattern. This largely eliminates false peaks in the correlogram.

In addition to these correlation methods there are a number of auxiliary schemes which can aid the decomposition. If all the lines have the same width and form one can, in principle, transform the spectrum into a series of delta functions located at the line centres (matched filter). Another technique which frequently helps in the identification of particular patterns is artificial broadening of both experimental and test spectra (low pass filter). Specific results for several types of complex spectra and details of a digital computer programme for the electron spin resonance case will be reported elsewhere.

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Photoelectrons and their Back Diffusion in Self-quenching Geiger–Müller Counters

THE Thomsons's[1] equation is the only satisfactory theoretical approach to the problem of back diffusion of electrons. Though most simple in form, the value of its term C relating to the energy of electrons is very uncertain. Theobald's[2] results on photoelectric current measurements at varying X/p are in agreement with the Thomsons's theory in molecular gases, taking C equal to the most probable energy of emission of photoelectrons. The lack of agreement in the case of argon is attributed to the uncertainty in the electron drift velocity near the cathode. Likewise, Bradbury's[3] values for mobility of electrons calculated using the Thomsons's equation are in agreement with those determined by direct methods. Few data are, however, available concerning the investigation of this subject in Geiger–Müller counters.

We believe that in a self-quenching counter back diffusion of photoelectrons takes place in a narrow zone near the surface of its cathode. Those which escape out of this zone move towards the counter anode and form Townsend avalanche. Whether the Thomsons's equation would be valid in this case is difficult to test. First, because the source of photoelectrons, the soft ultra-violet photons, produced in its pulse extends over a wide range of spectrum; secondly, the mobility of electrons in a mixture of argon–alcohol cannot be known with certainty in the zone of back diffusion; and thirdly, the cathode work function alters considerably under counter discharges.

It appears to us, however, that over a range of the counter plateau, 'C' may be assigned a constant value as in Theobald and Bradbury's experiments.

The soft ultra-violet photons are produced in a self-quenching counter due to recombination of argon ions with electrons to their excited states[4]. As there are more than one excited level of argon to which electrons, present in the vicinity of the anode and possessing some energy distribution, may recombine, the spectrum of the soft ultra-violet photons would stretch over a wide range. We have observed that this is so.

Furthermore, if photoelectrons are released from the counter cathode by these soft ultra-violet photons their flow into the body of the counter may be accompanied by back diffusion, the extent of which would depend on the electric field or overvoltage of the counter. Our experimental results show that this is probably happening.

The technique of our measurements is similar to that described earlier[4]. Two end-on counters about 5 cm apart and with their axes inclined to each other at an angle of 40° are enclosed in a steel chamber filled with 10 per cent argon–alcohol mixture at a total pressure of 10 cm. A collimated beam of photons from one of them, the source counter, giving 1,500–2,000 c.p.m., strikes against the cathode of the other, the target counter, counting about 400 c.p.m. The pulses of the two counters are fed into a coincidence unit of resolving time 1 μsec, and the rate of coincidences obtained by keeping the overvoltage on the source counter fixed but varying it on the target is recorded in a scaler. The spectrum of soft ultra-violet photons is examined using monochromatic interference filters, U–537, U–532, U–636, U–507 and U–510, peaking at 2155 Å, 2310 Å, 2537 Å, 2680 Å and 2940 Å, respectively, and having peak transmission of 16–25 per cent and a band-width of 160 Å. Coincidences are measured with and without interposing the filters between the two counters.

A set of typical curves obtained with different filters as indicated against each is given in Fig. 1. All curves gradually rise to a maximum and have flat peaks.

Experiments performed with target counters with cathodes of copper, nickel, aluminium and brass, all having good plateau characteristics, yield similar results.

The increase in the coincidence-rate in any one curve (Fig. 1) with overvoltage of the target counter is, we believe, due to the decrease of back diffusion of photoelectrons to the cathode as the electric field on the counter is raised.

The photo-sensitivity of a self-quenching counter rises rapidly with decrease in wave-length in the range 2000–3000 Å while the intensity of ultra-violet photons increases with wave-length in this region[5]. We are thus unable to determine from the curves (Fig. 1) the extent to which back diffusion is dependent on the energy of emission of photoelectrons. The fact that the maxima of the curves for different filters show a tendency to appear at varying overvoltages makes us believe, however, that it is probably dependent on their energy of emission.

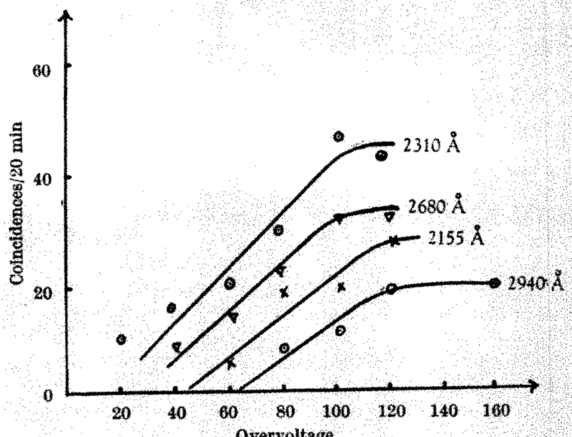

Fig. 1

These experiments establish that soft ultra-violet photons lying in the range 2000–3000 Å are produced in a self-quenching counter and that they all release photoelectrons from its cathode, a number of which back diffuse to it, depending upon the electric field close to its surface. It would also appear from the curves (Fig. 1) that in coincidence measurements with counters the operating voltages have to be carefully selected for accurate measurements.

In our second device, following Korff[6], a cylindrical grid of very fine mesh is placed inside a counter very close to its cathode to establish photoemission from it. It is observed that its counting-rate decreases by more than 25 per cent by changing the potential of the grid with respect to the cathode from +1·5 V to −1·5 V. This decrease is due to the influence of the negative retarding field on the photoelectrons released from the cathode which stops their flow into the body of the counter.

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GEOPHYSICS

Confirmation of the Absolute Age of the Granodiorite Outcrop in Elba Island with Potassium/Argon Measurements

Some of the islands of the Tuscan Archipelago are formed by outcrops of granitic intrusions which also outcrop along the coast. According to the general scheme of the Apennine orogenesis the age of these intrusions is believed to become progressively younger proceeding from south-west to north-east. This fact is in agreement with pleochroic halo measurements[1].

The granodiorite outcrop forming the Monte Capanne on Elba is one of the oldest in this intrusive series. For its age we must assume as lower limit the upper Eocene (nummulitic microbreccias disturbed and metamorphosed by the intrusion). The upper limit is the period of deposition of the pebbles of Elba magmatic rocks into the conglomerate at the base of the marine transgression following lacustrine lignites of southern Tuscany. These are normally attributed by Italian authors to the upper Miocene[2].

The Monte Capanne granodiorite contains pegmatitic veins with rubidium-rich minerals (lepidolite, pollucite). Rubidium/strontium ages of these have already been published[3].

Two samples already dated with rubidium/strontium have been analysed using the potassium/argon method. Argon analyses were carried out with isotopic dilution technique; potassium analyses with a Perkin–Elmer flame photometer using lithium as internal standard. In the case of the lepidolite measurement, the lithium content of this mineral has been previously determined. The results are given in Table 1. The argon ages are the average of several independent determinations.

Table 1. POTASSIUM/ARGON AND RUBIDIUM/STRONTIUM AGES

	Sample	Potassium/argon age (m.y.)	Rubidium/strontium age (m.y.)
1	Pollucite		5·4 ± 1·3
2	Pollucite		6·2 ± 0·7
3	Pink lepidolite II		7·1 ± 0·8
4	Pink lepidolite III	6·4 ± 0·6	6·3 ± 0·6
5	White lepidolite II		7·1 ± 0·6
6	White lepidolite III		6·1 ± 0·5
7	Biotite from granodiorite	7·6 ± 0·4	9·8 ± 3·6

The potassium/argon ages were calculated with the following constants: $\lambda = 5{\cdot}30 \times 10^{-10}\ y^{-1}$; $\lambda_K = 5{\cdot}85 \times 10^{-11}\ y^{-1}$; $^{40}K/K = 1{\cdot}19 \times 10^{-4}$. The rubidium/strontium ages were recalculated with respect to those published in 1961 using the rubidium-87 decay constant of $1{\cdot}47 \times 10^{-11}\ y^{-1}$ (ref. 4). The errors of the ages do not include the uncertainty in the decay constants.

The two ages of the pink lepidolite III agree very well. The rubidium/strontium age of sample 7 (biotite from granodiorite) is relatively higher than the other ones; but considering the large error, this value is still in accordance with the mean value of 6·5 million years of the other samples; the potassium/argon age is slightly higher than the age of the pink lepidolite and the average rubidium/strontium age of the pegmatitic material.

This age, more recent than it was supposed for the Elba granodiorite, suggests two different conclusions: (1) The absolute age of the Miocene–Pliocene limit, as established in Italy, might be more recent than the age assumed on the basis of data obtained in North America (12 m.y.)[5]. (2) It is possible that the effusive rocks along the coast of Tuscany are recent phenomena (upper Pliocene and Quaternary period) of a magmatic history in which the Elba intrusion is one of the oldest.

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RADIATION CHEMISTRY

γ-Ray-induced Formation of Gold Sol from Chloroauric Acid Solution

IN the course of investigations on the effect of γ-rays on inorganic colloids, we observed formation of gold sol from an aqueous solution of chloroauric acid. This communication presents our preliminary findings since very little is known about the formation of a heterogeneous system of this nature induced by irradiation[1,2].

When an aqueous solution of chloroauric acid was irradiated with γ-rays from a cobalt-60 source, colloidal gold particles formed under the following conditions: initial concentration of chloroauric acid, $4{\cdot}9 \times 10^{-4}$ M; bubbling with oxygen; radiation dose-rate of $9{\cdot}3 \times 10^{3}$ r./min for 70 min (total dose, $6{\cdot}51 \times 10^{5}$ r.). The particles formed had an average diameter of about 80 mμ (Fig. 1). However, as shown in Fig. 2, if a solution of the same concentration was exposed to a smaller total dose (that is, $0{\cdot}89 \times 10^{5}$ r., with a dose-rate of $9{\cdot}8 \times 10^{3}$ r./min for 10 min) in the same atmosphere, flat and thin 'hexagons' or 'trigons'[3,4] were observed.

The difference in shape and size of particles between Fig. 1 and Fig. 2 may be attributed to the difference in reaction-rates between hydrogen peroxide molecule and hydroperoxide radical (or atomic hydrogen) which are produced by radiolysis of water. The reduction with hydrogen peroxide may contribute to the growth of crystals rather than to the nucleation because of its slow reaction-rate. Actually, larger

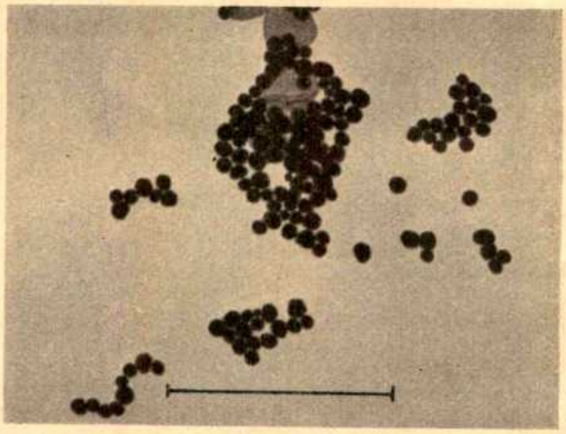

Fig. 1. Electron micrograph of gold particles formed from $4{\cdot}9 \times 10^{-4}$ M chloroauric acid solution by γ-irradiation ($9{\cdot}3 \times 10^{3}$ r./min × 70 min). Scale, 1μ

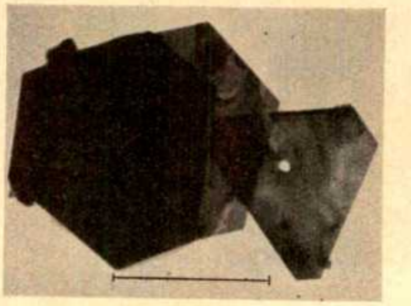

Fig. 2. Electron micrograph of gold 'hexagons' formed from $4{\cdot}9 \times 10^{-4}$ M chloroauric acid solution by γ-irradiation ($9{\cdot}8 \times 10^{3}$ r./min × 10 min). Scale, 1μ

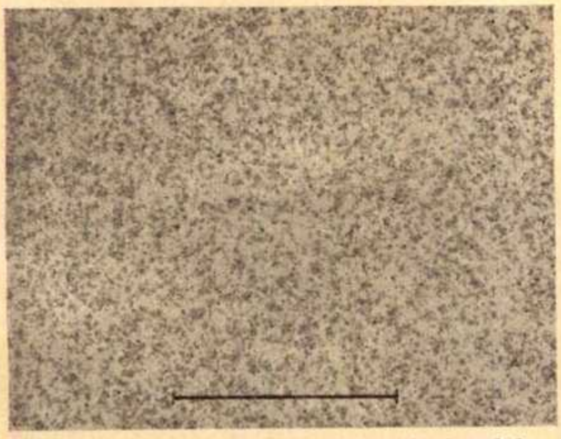

Fig. 3. Electron micrograph of gold particles formed from $4 \cdot 9 \times 10^{-4}$ M chloroauric acid solution containing 450 mg/l. gelatin by γ-irradiation ($7 \cdot 8 \times 10^{3}$ r./min × 70 min). Scale, 1μ

flat 'hexagons' were obtained when chloroauric acid ($2 \cdot 5 \times 10^{-4}$ M) was reduced with hydrogen peroxide ($2 \cdot 3 \times 10^{-3}$ N). Therefore, when a solution of chloroauric acid was irradiated with a sufficiently high dose to reduce chloroauric acid completely in a comparatively shorter time, most of the particles formed were the smaller ones as seen in Fig. 1, owing possibly to the formation of many nuclei. On the other hand, when the irradiation dose was insufficient for complete reduction of chloroauric acid, gold crystals grew larger as the remaining hydrogen peroxide in solution reacted with chloroauric acid slowly until a sample solution sprayed on a collodion film for electron microscopy was dried up (Fig. 2). The flat large 'hexagons' were also formed in cases of irradiation of chloroauric acid solution of the same initial concentration at the lower dose-rate, namely, 950 r./min or 105 r./min, and of a solution of higher concentration, namely, $6 \cdot 9 \times 10^{-4}$ M or $9 \cdot 9 \times 10^{-4}$ M, with a dose-rate of $9 \cdot 3 \times 10^{3}$ r./min. Hydrogen molecules may also reduce chloroauric acid, but no detectable formation of colloidal particles was observed by bubbling hydrogen gas for 70 min through a solution of chloroauric acid of $2 \cdot 5 \times 10^{-4}$ M. The dependence on temperature of the rate of this radiation-induced reaction was low. The apparent activation energy in the reaction was found to be approximately 1 kcal/mole as a rough estimate. Since this value is rather small, atomic hydrogen or radicals may be the primary inducers of this reduction reaction.

The gold particles produced in this manner had a negative charge and the sols were not stable. When irradiation was carried out in the presence of gelatin or polyvinyl alcohol of suitable concentration as protective colloid, very stable sols were obtained. Particles produced in the latter manner were strikingly smaller than those produced in absence of the protective colloid. As an example, Fig. 3 shows gold particles formed by irradiating an aqueous solution of $4 \cdot 9 \times 10^{-4}$ M chloroauric acid containing 450 mg/l. gelatin with the irradiation dose-rate of $7 \cdot 8 \times 10^{3}$ r./min for 70 min (total dose, $5 \cdot 5 \times 10^{5}$ r.).

Several physicochemical properties of the aqueous solution were altered as the reaction proceeded. Thus, the absorbance at 550 mμ, turbidity and specific conductivity increased, while the *p*H decreased with increasing γ-ray dose. These changes in relation to the irradiation dose were approximately linear. The G value was estimated to be $G(\text{—HAuCl}_4) = 0 \cdot 71$. It was not dependent on the initial concentration of chloroauric acid up to 10^{-3} M, and was not affected by the quantity of oxygen dissolved in aqueous solution.

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Formation by Oxidizing Agents of Free Radicals in Wool and Silk

INVESTIGATIONS into the polymerization of vinyl monomers in wool initiated by certain chemical catalysts have yielded some indirect evidence which suggests that the wool itself may acquire the property of a free radical in addition to the radicals formed from the catalyst[1-3]. Free radicals have been produced in wool by exposure to ionizing radiation, and afterwards used to initiate the polymerization of acrylonitrile within the fibre[4]. We have recently found that wool and silk may be modified by the action of certain oxidizing agents to produce free radicals, and in the case of wool these radicals are also able to initiate addition polymerization. These free radicals have been investigated using electron spin resonance techniques, and the spectra thus obtained have been found to be similar to those produced in wool and silk by γ-irradiation.

Wool samples (approximately 20 mg) were first pretreated for 10 min with dilute solutions of the following oxidizing agents: sodium perborate, sodium persulphate, peracetic acid, performic acid, hydrogen peroxide, and ferrous ion–hydrogen peroxide. The samples were then centrifuged, packed into small-bore glass tubes and then simultaneously evacuated and heated to 110° C for 10 min. The tube and contents were then sealed under vacuum and examined in an electron spin resonance spectrometer. Of the oxidizing agents investigated, only sodium persulphate was found to produce relatively large concentrations of radicals. Small concentrations of free radicals could also be produced in wool with water alone using the same experimental procedure, and the other oxidizing agents did not give significant increases above these levels. No free radicals could be detected in pure sodium persulphate, moistened and heated as outlined here.

In Fig. 1 are shown typical spectra of free radicals produced in wool by (*a*) sodium persulphate (0·1 per cent), (*b*) ferrous ammonium sulphate–hydrogen peroxide (0·2 and 0·3 per cent respectively), (*c*) water alone, and (*d*) γ-radiation. It is clear that (*a*) and (*d*) are similar, although the hyperfine structure is better resolved in the latter. Fig. 2 shows comparison spectra of free radicals produced in silk: (*a*) treated with sodium persulphate (0·1 per cent), as for wool, and (*b*) irradiated by γ-rays. These also show similarities.

The actual location of the free radicals produced by these techniques has not been definitely established,

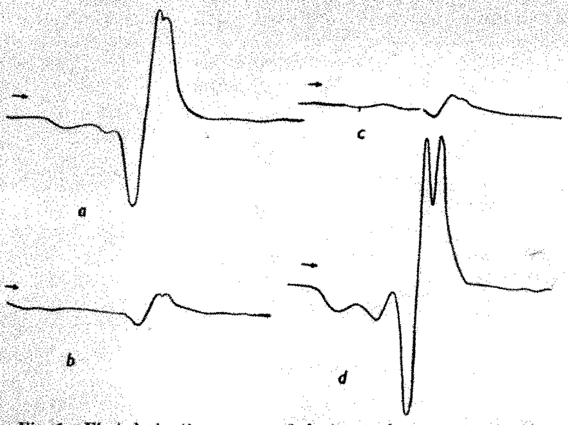

Fig. 1. First derivative curves of electron spin resonance spectra of wool, treated (*a*) with sodium persulphate, (*b*) with ferrous ammonium sulphate–hydrogen peroxide, (*c*) with water alone, and (*d*) by irradiation with γ-rays. The spectra, taken at room temperature and *in vacuo*, were observed at 9,000 Mc/s about $g = 2 \cdot 00$ for the magnetic field. The total span of the traces is 250 gauss with the magnetic field increasing in the direction of the arrow

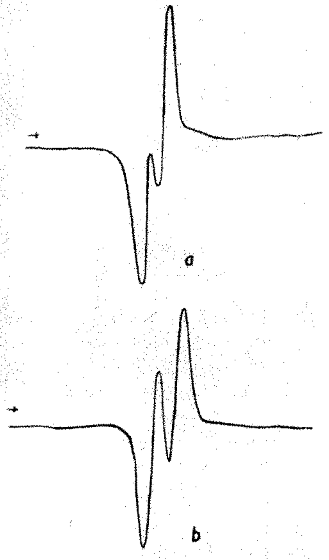

Fig. 2. First derivative curves of electron spin resonance spectra of silk, (*a*) treated with sodium persulphate, and (*b*) irradiated with γ-rays. The spectra were observed under the same conditions as for Fig. 1

although suggestions as to the sites of the unpaired electrons in irradiated wool and silk have been given by Gordy and Shields[5]. Work is now proceeding to determine the sites of these chemically induced free radicals and to investigate the graft polymerization of addition polymers to wool initiated by these radicals.

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CHEMISTRY

Secondary Processes in Chemiluminescent Solutions

Excitation energy transfer and quenching of excited states are known to be of importance in many phenomena, for example, in photochemistry[1], photoluminescence[2], radioluminescence[3], radiolysis[4]. Evidence is presented in this communication that processes of the kind may occur in chemiluminescent solutions as well.

Chemiluminescence has been shown recently to be a widely spread phenomenon accompanying many chemical reactions[5]. It was concluded that excitation in liquid phase radical reactions occurred by elementary radical recombination. The theoretically expected proportionality between the reaction-rate and the light intensity was found in many cases. However, this correlation was not observed for some of the systems studied. It was suggested that this feature might be connected with the formation or disappearance of certain components which were capable of interacting with primary excited recombination products, the components being quenchers or activators depending on their properties.

These considerations have been verified experimentally.

The quenching action of oxygen was observed in the oxidation of the organic compounds initiated by thermal decomposition of certain substances[5], as, for example, in methylethylketone oxidation (Fig. 1). With a gradual consumption of dissolved oxygen, quenching weakens and the light intensity increasingly rises, although the oxidation and recombination-rates remain constant (the sharp drop of intensity at the end of the reaction is due to total disappearance of oxygen in the reactant mixture[6]).

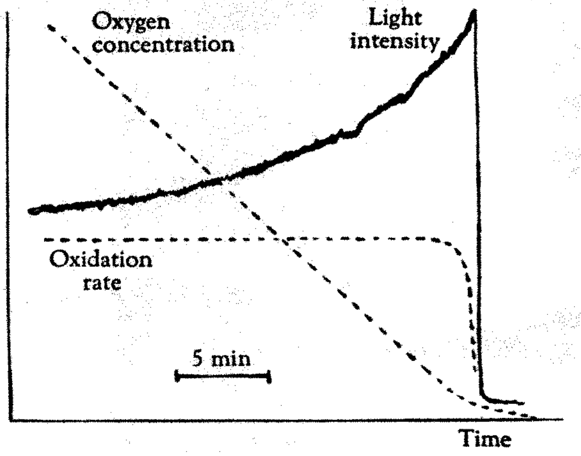

Fig. 1. Experimental kinetic curve of the chemiluminescence intensity in methylethylketone oxidation at 60° (solid line). The dashed lines represent the qualitative aspects of dissolved oxygen concentration and oxidation-rate

Activation of chemiluminescence was obtained by adding luminescent materials to the reactants[7]. Anthracene, its alkyl-, phenyl-, halogen-derivatives, oxazoles and pyrazolines have been used as activators. Chemiluminescence spectra were recorded and the intensity was studied as a function of activator concentrations in the oxidation of benzene solutions of *cyclo*hexane and ethylbenzene.

Activated chemiluminescence spectra were found to be identical to the fluorescence spectra of activators. (Similar results were obtained in strongly exothermic heterogeneous oxidation of silicons by H. Kautsky[9], who observed energy transfer from excited products to dyes adsorbed on the reactant.) This indicates that the singlet state of the activator is the excited one.

The enhancing action of activators becomes stronger with increasing concentration. Their efficiency builds up with halogen substitution in spite of the fact that substitution-induced changes lead to decreasing fluorescence yield (Table 1).

Table 1. ACTIVATION OF THE CHEMILUMINESCENCE ACCOMPANYING *Cyclo*HEXANE OXIDATION AT 50°; ACTIVATOR CONCENTRATION 10^{-4} MOL./L.

Activator used	9,10-Dibromo-anthracene	9-Bromo-10-phenyl-anthracene	9,10-Dichloro-anthracene	9,10-Diphenyl-anthracene
Fluorescence yield of the activator	0 095	0·16	0·48	0·74
Activation coefficient*	47	27	12	1·8

* Ratio of the intensity of activated chemiluminescence to that of chemiluminescence without an activator.

Formally these results would mean that the increase in the probability for energy transfer is greater than the decrease in that for radiation transition (fluorescence yield), but it is not clear yet what are the properties of the activator molecule that influence the transfer probability in the experiments reported. In any event neither the overlapping integral for absorption and fluorescence spectra (determining 'resonance transfer') nor the absorptivity and fluorescence yield (determining 're-absorption transfer') are of importance and do not explain the hundred-fold variations in the transfer probability. It might be noted that the introduction of halogens is known to raise the probability of 'forbidden' singlet–triplet transitions in the molecule. This correlation should possibly be taken into account in further theoretical treatment of the phenomenon as well as a probable role of the solvent (benzene) and formation of complexes.

Activation of chemiluminescence is of practical use since it extends the possible applications of kinetic chemiluminescent methods. For example, oxidation of tetraline is not accompanied with detectable chemiluminescence at 60°. 9,10-di-bromo-anthracene ($\sim 10^{-4}$ mol. $l.^{-1}$) 'develops' the luminescence and the ratio of the elementary constants can be measured by using the chemiluminescent technique[6].

This work will be published in more detail elsewhere[8].

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Thermophoresis of Aerosol Particles

THE velocity of thermophoresis v of small aerosol particles of radius R, much smaller than the free path λ of the surrounding gas molecules, has been calculated by us[1] in two ways: (1) as a limiting case of thermodiffusion; (2) by finding the momentum imparted to the particles by impact of molecules the velocity distribution of which is not disturbed by its presence, using the method of Chapman *et al.*[2]. The formula obtained for the case of diffusional molecular scattering:

$$v = \frac{6}{8+\pi}\,\frac{\eta}{\rho}\,\frac{\text{grad}\,T}{T} \qquad (1)$$

where T is the Kelvin temperature, η and ρ the viscosity and density of the gas, reveals that v is independent of the thermal conductivity of the particles, in contrast to the case where $R \gg \lambda$.

However, the commonly known Maxwell–Epstein formula covering the latter case is based on the assumption that the colliding molecules retain the velocity distribution which they have in the bulk, this assumption being valid only for $R \ll \lambda$.

We have developed a thermophoresis theory for the case where $R \gg \lambda$, applying the Onsager principle of symmetry of kinetic coefficients to flows of mass and heat across an imaginary plane-parallel partition consisting of spherical particles arranged disorderly but motionless and 'uniformly' in the gas. It is presumed that $4/3\ \pi R^3 N \ll 1$, where N is the number of spheres per unit volume. Under Knudsen conditions, as is known, we get a heat of transfer resulting in the Knudsen formula for a thermomolecular flow. In the opposite limit case $R \gg \lambda$ the heat of transfer per unit area can be shown to be generated mainly by the heat of the transfer Q^* (per unit area) in the bulk of the gas (beyond the limits of boundary layers of thickness λ), equal, according to the third approximation of the Chapman theory, with allowance for the Navier–Stokes equation of viscous flow, to:

$$Q^* = \frac{3}{2}\,\frac{\eta}{\rho}\,\text{grad}\,p \qquad (2)$$

where p is the pressure in the gas, and η and ρ are its viscosity and density respectively.

This heat of transfer satisfies the equation div $Q^* = 0$ and is evolved partially on the surfaces of all the spheres, covering their surface with heat sources and sinks of intensities governed by the law:

$$q = \frac{9}{2}\,\frac{\eta^2}{\rho}\,\frac{u\cos\theta}{R^2} \qquad (3)$$

where u = rate of gas flow at infinite distance from the particle, θ = polar angle, and only partially on the partition surfaces. Calculation of this residue results in the expression:

$$Q^*_{\text{Res}} = \frac{1}{2}\,\frac{\eta}{\rho}\,\text{grad}\,p \qquad (4)$$

When as usually $H_i/H_e \gg 1$, where H_i is the thermal conductivity of the particles, H_e that of the gas, it can hence be derived by conventional methods of steady process thermodynamics that if there is an average temperature gradient across the partition, there will appear a linear gas flow velocity equal to:

$$v = -\frac{1}{2}\,\frac{\eta}{\rho T}\,\text{grad}\,T \qquad (5)$$

The same formula gives the velocity v of thermophoresis of infinitely heat-conductive particles, equal, according to the Epstein formula, to zero, in sharp contradiction to experiment.

When H_i and H_e are comparable the 'thermal polarization' of the particles by an originally 'isothermic' gas flow results in the appearance of a drop in temperature ΔT on both sides of the partition thickness H:

$$\Delta T = \frac{18\pi RN\eta^2 H_e u}{\rho(2H_e + H_i)}$$

equivalent to the additional heat transfer equal to:

$$Q_a^* = \frac{18\pi RN\eta^2}{\rho} \frac{H_e}{(2H_e + H_i)} u$$

allowing for which we get for the velocity of thermophoresis:

$$v = -\frac{1}{2} \frac{8H_e + H_i}{2H_e + H_i} \frac{\eta}{\rho T} \operatorname{grad} T \qquad (6)$$

Thus, the velocity of thermophoresis may grow slightly in the case of particles of low heat conductivity. However, the limit case, $H_i/H_e \ll 1$, is evidently practically unrealizable.

It should be emphasized that, contrary to the Maxwell–Epstein theory, the velocity of thermophoresis according to equation (6) depends only on the bulk properties of the gas and the particles, but not on the nature of the collisions of the molecules with the particle surfaces (that is, it is independent of the percentage of diffusionally scattered molecules).

Similar treatment has made it possible to find an expression for the thermomolecular pressure drop in capillaries[3] with $R \gg \lambda$, compensating thermo-osmotic motion at a steady state corresponding to a zero total gas flow:

$$\Delta p = 12\eta^2 \frac{\Delta T}{R^2 \rho T}$$

This formula was found to be in good agreement with Knudsen's experiments[4], while the value obtained from Maxwell's formula for thermal sliding is twice as small.

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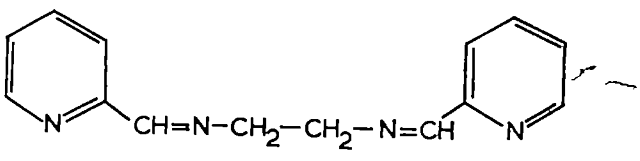
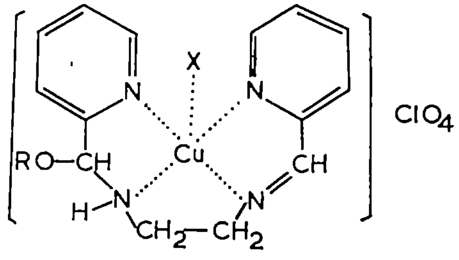
Addition of Alcohols to a Schiff Base co-ordinated to Copper(II)

WE have been interested in the Schiff base quadridentate I (1,2-*bis*-(2′-pyridylmethyleneamino)-ethane, BPE) in order to ascertain whether it forms five-co-ordinated copper(II) complexes $[Cu(BPE)X]^+$ (X = univalent anion) similar to those reported with 2,2′-bipyridyl and 1,10-phenanthroline[1]. These are of the type $[Cu(chelate)_2X]^+$, and the iodo-complex $[Cu(bipy)_2I]^+$ possesses an approximately trigonal bipyramidal arrangement about the copper atom[2].

Some complexes with this ligand (I) have been reported[3,4] and Busch and Bailar[3] presented infra-red evidence that water adds across both the C=N bonds when the ligand is co-ordinated to copper(II) chloride.


I


II

In attempting to prepare a series of five-co-ordinated copper(II) complexes of I, we observed an interesting reaction in which methanol or ethanol adds across only one of the double-bonds of the co-ordinated ligand. The green complexes $[Cu(BPE)I]ClO_4$ and $[Cu(BPE)Br]Br$, which are uni-univalent electrolytes in nitromethane, were prepared from acetonitrile or acetone solutions by reacting blue $Cu(BPE)(ClO_4)_2$ with the appropriate amount of halide. However, when this type of reaction was performed in boiling methanol or ethanol solution, green compounds of the general type $[Cu(BPE+ROH)X]ClO_4$ (X=Cl, Br, I or NCS and R=Me or Et) were obtained in which the alcohol has added across one double bond as in II.

Table 1. INFRA-RED SPECTRA IN THE REGION 4,000–1,500 CM⁻¹. (CH bands neglected, measurements on a Perkin–Elmer 'Infracord' 137 as paraffin oil mulls)

	Band assignments				
Compound	OH	NH	C=N	Pyridyl ring	
$Cu(BPE)(ClO_4)_2$	—	—	1,680(*s*)	1,620(*s*)	1,580(*w*)
$[Cu(BPE)Br]Br.H_2O$	(H_2O) 3,400 (v.b.)	—	1,680 1,665	1,610	1,570
$[Cu(BPE)I]ClO_4$	—	—	1,670 1,655	1,610	1,570
$Cu_2(BPE)X_4.H_2O$ (X=Cl,Br)	(H_2O) 3,400 (v.b.)	—	1,650	1,650	1,570
$Cu_2(BPE)(NCS)_4$	—	—	1,700 (*w*) 1,670(*s*)	1,620	1,580
$Cu(BPPr)(ClO_4)_2$	—	—	1,650	1,610	1,570
$[Cu(BPPr)I]ClO_4$	—	—	1,650	1,610	1,570
$[Cu(BPE+MeOH)X]ClO_4$ (X=Cl,Br,I,NCS)	—	3,250–70	1,670–80	1,610–20	1,570–80
$[Cu(BPE+EtOH)X]ClO_4$ (X=Br,I)	—	3,250	1,670	1,610–20	1,580

s, Strong; *w*, weak; *v.b.*, very broad.

Evidence for this is provided by: (*a*) analyses, including alkoxyl determinations; (*b*) the presence of a strong absorption band in the N—H 'stretch' region[5] of the infra-red spectra, along with a diminished, but still strong, absorption in the C=N region and the absence of a band in the OH 'stretch' region (Table 1); (*c*) two bands at ~1,350 cm⁻¹ in the spectrum of the methoxy compounds which probably can be assigned to the methoxy group, and which are markedly different from the position in methanol[6].

Compounds of the type $Cu_2(BPE)X_4$ (X=Cl, Br, NCS) were obtained by reacting the ligand with excess of the copper(II) salt in ethanol. Their infra-red spectra, together with those of the other copper(II) complexes of the ligand(I) (Table 1), provide a useful comparison.

The alcohol addition reaction may be rationalized in terms of a trigonal bipyramidal arrangement in these compounds. Steric interaction between the aromatic rings of I would seriously hinder the formation of a square–planar arrangement with normal nitrogen–metal bond angles and suggests that the ligand may prefer to occupy four positions of a distorted trigonal bipyramid as do the bipyridyl molecules in the $[Cu(bipy)_2I]^+$ ion. Some distortion of the ligand bond angles is necessary for this arrangement and this may render the double bonds more reactive. However, Catalin-type models indicate that the new ligand resulting from the addition of one molecule of alcohol can adopt this arrangement with little strain.

Preliminary results with 1,3-*bis*-(2′-pyridyl-methylene-amino)-propane (BPPr) support this explanation in that the longer carbon chain further hinders a square planar arrangement but allows the ligand to occupy a less strained arrangement in a trigonal-bipyramidal copper complex, for example, $[Cu(BPPr)I]ClO_4$. In this case no tendency was observed for the occurrence of an alcohol addition reaction.

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BIOCHEMISTRY

Labelling of Phospholipids with Phosphorus-32 in Rat Liver Homogenates

KENNEDY[1] showed that the phospholipids in a rat liver homogenate, suitably fortified and buffered, became labelled in the presence of inorganic phosphorus-32. After the addition of adenosine triphosphate but not cytidine triphosphate most of the phosphorus-32 was present in an acidic fraction which appeared to be mainly phosphatidic acid but which was not fully characterized. Later work[2] showed that phosphorus-32 exchange with lecithin, sphingomyelin, phosphatidyl ethanolamine and phosphatidyl inositol was dependent on cytidine triphosphate and the labelling with adenosine triphosphate alone was assumed to involve only phosphatidic acid. More recent work[3] has shown that labelling with adenosine triphosphate could occur also with some inositol phospholipids.

In the course of the examination of phospholipid derivatives, an investigation was made of the incorporation of phosphorus-32 into the phospholipids of liver homogenates under conditions that resulted in minimal exchange with the major phospholipids.

Rat liver (10 g) was homogenized in 10 volumes of isotonic sucrose (0·25 M), buffered at *p*H 7·4 (0·025 M *tris*(hydroxymethyl)aminomethane) and to this was added disodium hydrogen phosphate (10^{-4} M containing 0·2 mc. phosphorus-32). This mixture was shaken in air at room temperature for 2 h, and was then frozen and dried. The dried material was extracted, the lipids dinitrophenylated and methylated and fractionated by counter-current distribution as described by Collins and Shotlander[4]. The lecithin, phosphatidyl ethanolamine and phosphatidyl serine had little radioactivity but four radioactive peaks were observed (see Table 1). Two were well separated from other phospholipids: Fraction 1 corresponded to phosphatidic acid which was located between the methyl ester of N 2 : 4-dinitrophenyl phosphatidyl ethanolamine and cardiolipin as described by Collins and Shotlander[4]. Fraction 2 was an unknown material located in the extreme non-polar region of the distribution of the phospholipids which remained acetone insoluble after dinitrophenylation and methylation.

The remaining two fractions were located in the 'complex' portion[4] of the phospholipids and were only partially separated. The 'complex' fraction (0·001 M) was reacted with 0·002 M triethylamine hydrochloride and 0·02 M diazomethane in equal parts of chloroform–diethyl ether for 10 min at 20°, the mixture taken to dryness *in vacuo* and the products fractionated by counter-current distribution. After this treatment the two radioactive fractions were well separated. Fraction 3 was now located between the positions that had been occupied by phosphatidic acid and cardiolipin in the original distribution. Fraction 4 was located in the polar portion of the distribution.

These results are shown in Table 1, which describes one experiment in which the fractionation was more detailed than in six others, but there was full agreement in regard to the number and location of these four fractions. It is pertinent that the same fractions, in approximately the same proportions, have been observed in liver lipids extracted directly, as the possibility of breakdown in incompletely fortified homogenates cannot be excluded. When the labelling was accomplished by the injection of the phosphorus-32 into the living animal the radioactivity of these four fractions was very much less than that of the major phospholipids. In the *in vitro* experiments the specific radioactivity of the most active fraction (phosphatidic acid) was about 0·3 per cent of that of the inorganic phosphate, while in the *in vivo* case the specific radioactivity of the phosphatidyl ethanolamine was about 10 per cent of that of the inorganic phosphate.

Both phosphatidic acid and the phosphate esters of phosphatidyl inositol are known to exchange readily with phosphorus-32 (refs. 3 and 5) and in both cases mono-esters of phosphoric acid are in-

Table 1. RAT LIVER HOMOGENATE INCUBATED IN ISOTONIC SUCROSE, *tris* BUFFER (0·025 M *p*H 7·4) AND INORGANIC PHOSPHORUS (10^{-4} M. DISODIUM HYDROGEN PHOSPHATE, 5 × 10^6 COUNTS/MIN/μMOLE PHOSPHORUS) FOR 2 H AT 20°

Fraction	Partition coefficient	Phosphorus as percentage of total lipid phosphorus	Radioactivity as percentage of total radioactivity	Counts per min per μmole phosphorus
1	1·7–2·3 (heptane methanol)	0·4	22	9,300
2	0·03 (CCl_4—$CHCl_3$—CH_2Cl_2—CH_3OH—H_2O)*	0·4	5	2,200
3	2·4 (heptane methanol)	3·3	23	1,300
4	3·9 (CCl_4—CH_3OH—H_2O)†	0·8	14	3,200
Remainder of phospholipids		95·0	36	70

* (25 : 15 : 10 : 40 : 10 by vol.). † (62 : 35 : 3·15 by vol.)

volved. It is a reasonable assumption that in all four fractions described here the labelled phosphorus was present as a mono-ester.

Hokin and Hokin[5] have obtained rapid labelling in phospholipid fractions which correspond neither to phosphatidic acid nor to the major phospholipids, and these authors produce evidence[6] which appears to eliminate lysophosphatidic acid. Rapid labelling with phosphorus-32 of two unidentified phospholipids in mitochondria has recently been reported[7].

The metabolic significance of these minor components of the phospholipids cannot be assessed at the moment, but it is clearly of importance that their chemistry be elucidated.

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Incorporation of Molecular Oxygen into *Trans*-L-Epoxysuccinic Acid by *Aspergillus fumigatus*

SINCE its discovery in 1937[1] in appreciable yields in culture filtrates of two filamentous fungi, only a handful of studies, mainly by Sakaguchi, appear to have been made on the mycological formation of *trans*-L-epoxysuccinic acid[2]. The mechanism of its biosynthesis has been studied by Nomura *et al.*[2] using compounds labelled with carbon-14 to trace the origin of the carbon atoms.

An outgrowth of earlier studies in this laboratory[2] was the idea that the epoxide oxygen atom in the *trans*-L-epoxysuccinate molecule might be unique in being derived from molecular oxygen. This appears to be the case, and the following sheds some light on the pathway of biosynthesis of this uncommon dibasic acid.

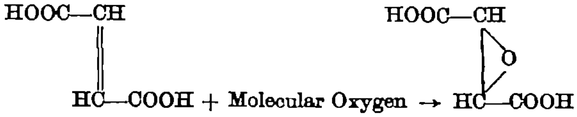
Shaken cultures of *Aspergillus fumigatus* mycelium were prepared as described previously[2]. The filtered mycelium, 5·6 g wet weight, was suspended in 200 ml. of 3 per cent glucose solution in a 2-l. gas-tight suction flask. The atmosphere in the flask was replaced with air containing 80 per cent oxygen labelled with oxygen-18, and the suspension was then shaken continuously for 87 h at 30° C. The epoxysuccinic acid was extracted from the culture filtrate with diethyl ether, yield *c.* 500 mg. It was purified by silica-gel column chromatography[3], and recrystallized from acetone–chloroform mixture. The crystals had the correct melting point for *trans*-L-epoxysuccinic acid (181° C).

The oxygen isotopic composition of the pure acid was determined by the method of Rittenberg and Ponticorvo[4]. The results (Table 1) indicate that one atom of oxygen in the epoxysuccinate was derived from molecular oxygen (assuming no exchange reaction). Since four of the five oxygen atoms in the acid are contained in carboxyl groups, we conclude that only one atom is labelled and that it must be the epoxide oxygen.

Table 1

Exp.	Oxygen-18, Atom % excess in		Percentage of oxygen in epoxysuccinate derived from molecular oxygen
	O_2	Epoxysuccinate	
I	5·466	1·086	19·87
II	4·906	1·056	21·52

At the moment it is not possible to identify the oxygenation reaction generating epoxysuccinate because we have been unsuccessful in obtaining synthesis of this product in cell-free extracts of the mould. Nomura *et al.*[2] showed that the carboxyl-carbons of epoxysuccinate produced in the presence of $^{14}CO_2$ had the same specific radioactivity as the mean values for carbons 1 and 6 in the citric acid formed in the same system, and oxalacetic acid was postulated as a common precursor of both acids. We have found that epoxysuccinate is formed from malic, succinic or fumaric acid as the sole carbon source in suspensions of living mycelium. Based on the available facts we postulate the following reaction:



Fumaric acid

Trans-L-epoxysuccinic acid

Such an oxygenase would be different from those described in the literature[5], and investigations directed towards its isolation are now being carried out.

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Oxidation of Vitamin A_1 and Vitamin A_2 Aldehydes to the Corresponding Acids by Enzymes from Pig and Rat Livers

VITAMIN A is stored in rat liver largely as its ester with small amounts of the alcohol, but is transported in the normal circulating blood in the latter form[1]. Although it was generally believed that the alcohol form is the more physiological state of the vitamin, since the work of Dowling and Wald[2], it is being recognized that vitamin A acid and not the alcohol may be nearer to the 'active vitamin A'. If this were to be so, it would be important to demonstrate that a mechanism exists in the rat for the production of vitamin A acid from vitamin A alcohol through the intermediate, the aldehyde. Regarding the formation of the aldehyde, it has been well established that the alcohol dehydrogenase can bring about the conversion of vitamin A alcohol to retinene[3]. The presence of an enzyme in rat and pig liver catalysing the oxidation of retinene$_1$ and retinene$_2$ to the corresponding acids has been demonstrated in the present work and the partially purified enzyme preparation shown to be completely devoid of alcohol dehydrogenase activity.

Retinene$_1$ was prepared by oxidation of synthetic vitamin A_1 alcohol with manganese dioxide[4]. Vitamin A_2 was isolated from high potency liver oil of the freshwater fish, *Wallago attu*[5], and retinene$_2$ was obtained by the oxidation of vitamin A_2 alcohol[6].

The enzyme from pig liver was prepared following the procedure of Mahler *et al.*[7] for the preparation of aldehyde oxidase. The same procedure was followed in the case of rat liver also. However, 45 per cent saturation with ammonium sulphate of the supernatant fraction obtained by centrifuging rat liver homogenate at 85,000*g* for 60 min was found to give a precipitate having all the oxidase activity free of the alcohol dehydrogenase activity.

The reaction mixture was made up of the following components: phosphate buffer (0·1 *M*, *p*H 7·1) 1·5 ml; 0·1 ml. of an ethanolic solution containing 50 μg of retinene$_1$ or retinene$_2$; enzyme solution containing 0·2–0·5 mg protein per reaction mixture; total volume was always made up to 2 ml. with water. Following incubation for 30 min at 37°, the reaction products were extracted with ethanol and petroleum ether and the disappearance of retinene added was estimated by the thiobarbituric acid procedure[8]. The acid formed, after isolation as described here, was estimated spectrophotometrically using $E^{1\%}_{1\,\mathrm{cm}}$ values in ethanol of 1,500 at 350 mμ and 1,395 at 370 mμ for vitamin A_1 and A_2 acids, respectively[9].

For the isolation of the acids from the reaction mixture, the petroleum ether (40–60°) extracts were chromatographed on 10 per cent deactivated acid washed alumina[10] and the unreacted aldehyde fraction was eluted with petroleum ether. The acid fraction was then eluted from the column by extruding the adsorbant and stirring it with a mixture of diethyl ether, ammonia and ethanol or methanol containing 30 per cent ammonia. The alkaline extract was acidified with glacial acetic acid and the vitamin A acid extracted with petroleum ether. The absorption maxima in ethanol of the enzymatic products obtained from retinene$_1$ and retinene$_2$ were at 350 mμ and 370 mμ respectively[9], and the ultra-violet absorption spectra closely agreed with the authentic samples of vitamin A_1 and vitamin A_2 acids. Furthermore, the enzymatic products had the same R_F values as those of vitamin A_1 and vitamin A_2 acids when chromatographed on Whatman No. 1 filter papers impregnated with 3 per cent 'Vaseline' and developed with ethanol/glacial acetic acid (3 : 1) or by the method of Mahadevan and Ganguly[11] using a mixture of methanol/ammonia/toluene (8 : 1 : 1) as solvent.

The enzymes from pig liver as well as from rat liver were able to carry out this oxidation. The specific activities of the rat liver enzyme with retinene$_1$ and retinene$_2$ were 83·37 μg and 28·64 μg, respectively, of the corresponding acids formed per mg protein. The enzymes from both the sources were active against acetaldehyde as well when assayed by the indophenol method[12]. In a preliminary report Dmitrovsky[13] had also shown that the non-specific aldehyde oxidase of pig liver can convert vitamin A aldehyde to vitamin A acid.

It is interesting that though there is a great deal of species difference in the distribution of vitamins A_1 and A_2 in Nature and though mammals, like the rat and pig, seldom have any vitamin A_2 in their tissues, the mechanism for the conversion of the aldehydes of both vitamin A_1 and A_2 to the corresponding acids seems to be the same in the livers of both the species.

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Growth-promoting Effect of the *B.S.* Factor and its Related Compounds

THE *B.S.* factor, tetramethylpyrazine, a product of *Bacillus subtilis*, is a growth-promoting factor for the bacterium[1,2]. This communication deals with qualitative characterization of the factor and discovery of more effective growth-promoting substances among compounds related to tetramethylpyrazine.

Bacillus subtilis was cultivated in a medium containing lysine as nitrogen source and the factor in several concentrations. After three days incubation, each tube was subjected to homogenization. Growth of the bacterium was determined qualitatively by means of photoelectric nephelometer. The result is given in Fig. 1, showing that the factor is effective in pretty high concentration such as 1,400 γ/ml.

For dihydro-tetramethylpyrazine, 3-amino-butanone-(2) and acetoin, which are plausible precursors[3] in the biosynthesis of tetramethylpyrazine, tests by the same procedure used here were carried out. Since dihydro-tetramethylpyrazine is very unstable and oxidizes into tetramethylpyrazine in air, tetramethylpyrazine was probably most used in the test. The results are shown in Fig. 1.

It is somewhat surprising that dihydro-tetramethylpyrazine and 3-amino-butanone-(2) were more effective than tetramethylpyrazine. This fact might suggest that the real growth-promoting factor is dihydro-tetramethylpyrazine. Therefore it is suggested that it would take an active part in transamination, as pyridoxine. To check this point, vitamin B_6 activities of tetramethylpyrazine and related compounds were examined using *Saccharomyces carlbergensis*. The results are given in Fig. 2. From this it can be seen that dihydro-tetramethylpyrazine has a little and tetramethylpyrazine and

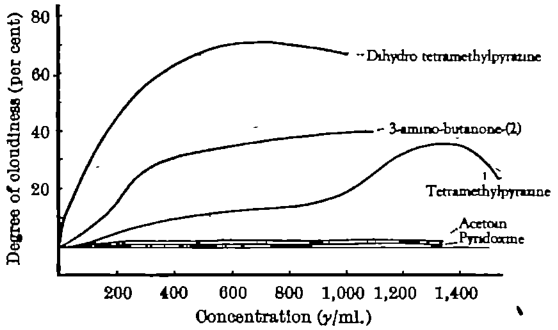


Fig. 1. Growth curves of *Bacillus subtilis*

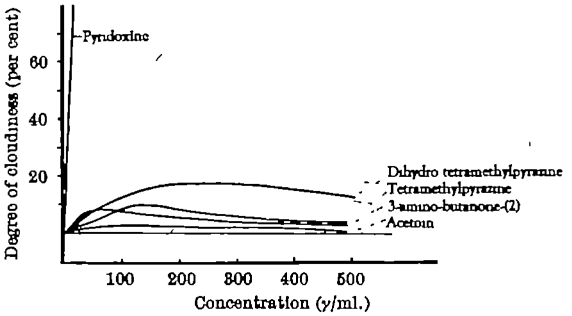
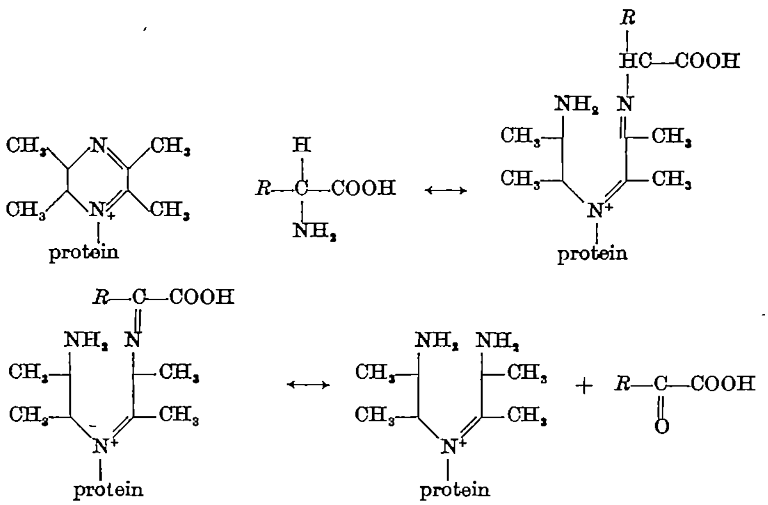


Fig. 2. Growth curves of *Saccharomyces carlsbergensis*

3-amino-butanone-(2) have very little vitamin B_6 activity, indicating that they would have somewhat resembled pyridoxine in their biological action and at least exert growth-promoting activity against *Saccharomyces carlsbergensis* too.

A mechanism of the action of dihydro-tetramethylpyrazine in transamination is proposed, as follows, by analogy with the interpretation of Schlenk and Fisher[4] for the action of pyridoxine:



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Possible Glucogenicity of Butyrate in Sheep

The virtual dependence of ruminants on endogenous sources of glucose and the relative abundance of short-chain fatty acids have stimulated much discussion on the possible existence in ruminant tissues of pathways leading to the net conversion of fatty acids to glucose. Butyrate, in particular, has been the subject of controversy since Potter[1] found that sodium butyrate injected intravenously relieved insulin convulsions. Several investigators have observed raised blood sugar concentrations following butyrate administration[2-6], and Shaw[7] has reported the net conversion of large amounts of butyrate to carbohydrate by the perfused goat liver. Kleiber *et al.*[8] obtained appreciable labelling of milk lactose following the intravenous administration of labelled butyrate in cows, but more recently, the absence of pathways which would account for the 'glucogenic' behaviour of butyrate in the lactating cow has been convincingly demonstrated[9]. Lauryssens, Verbeke, Peeters and Reinards[10] compared the incorporation into cow's milk constituents of butyrate labelled in the C-1 or C-3 positions and concluded that butyrate metabolism proceeded after cleavage into two-carbon fragments, a well-established pathway in other tissues.

Table 1. Labelling Patterns in Glucose produced by the Incubation of Sheep Liver Slices with Labelled Acetate and Labelled Butyrate

Substrate	Radioactivity recovered in glucose (mμc.)	Distribution of radioactivity in glucose carbon (per cent)					
		C1	C2	C3	C4	C5	C6
Acetate (1-^{14}C)	20	1	3	41	43	7	5
Acetate (2-^{14}C)	77	25	19	4	8	26	18
Butyrate (1-^{14}C)	17	2	4	43	48	2	1
Butyrate (2-^{14}C)	83	28	25	4	6	20	17
Butyrate (3-^{14}C)	13	5	5	39	44	5	2

1 g of tissue was incubated at 39° for 3 h in Krebs–Ringer bicarbonate (ref. 13) medium with each substrate (acetate, 160 μmole, 5 μc.; butyrate, 80 μmole, 5 μc.). The gas phase was 95 per cent oxygen, 5 per cent carbon dioxide.

As part of an investigation of glucose and fatty acid metabolism in sheep, the incorporation of short-chain acids into glucose by sheep liver slices has been examined using substrates labelled with carbon-14. Acetate labelled in the C-1 or C-2 positions was compared with butyrate labelled in the C-1, C-2 or C-3 positions. The distribution or radioactivity in glucose isolated from the incubation media was examined by step-wise degradation using *Leuconostoc mesenteroides*[11]. The results shown in Table 1 indicate the close similarity in the labelling pattern of glucose derived from C-1 labelled acetate with that from butyrate labelled in either the C-1 or C-3 positions and the pattern of glucose labelling produced from C-2 labelled acetate closely resembled that from C-2 labelled butyrate. The labelling patterns were consistent with incorporation into glucose through the tricarboxylic acid cycle[12], and were similar to those observed in other tissues incubated with C-1 and C-2 labelled acetate[12]. In particular, the close similarities in glucose labelling obtained with C-1 labelled acetate and C-1 and C-3 labelled butyrate showed the absence of major pathways leading to butyrate incorporation into glucose as a three-carbon unit. The liver is a major site of butyrate utilization in ruminants, and the present results strongly suggest the absence of net synthesis of carbohydrate from butyrate in this tissue.

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PHYSIOLOGY

Longitudinal Direct-Current Gradients of Spinal Nerves

IN a previous publication[1] some evidence was presented for the existence of a steady-state longitudinal charge carrier flow in the peripheral nerves of Amphibia. At that time this flow of current was considered to be unidirectional in the nerves with the necessary circuit completion path through some non-neural tissue. This concept required the presence of measurable longitudinal direct-current potential gradients along the peripheral nerves with a uniform polarity. The return path would then be evidenced by a gradient of opposite polarity in some other tissue. This communication presents the results of an evaluation of this hypothesis.

Large bullfrogs (*Rana catesbeiana*) were used as the experimental animals, and atraumatic surgical approaches were devised for exposure of the structures of the posterior thigh. Potential gradients were measured on the surface of intact structures, dissection being limited to gentle separation only. The electrodes were saline-agar filled silver–silver chloride with thin terminal cotton wicks. Potentials were amplified with either an electrometer amplifier (input impedance 10^{14} ohms) or a chopper-type direct current amplifier (input impedance 10^{8} ohms). Electrical isolation was secured at the measurement site by elevating the structure on a thin sheet of polyethylene.

In the first series of 12 animals, the direct-current gradients on the surface of muscle, blood vessel, bone and fascia were noted to be consistently distally negative. The gradients were generally uniform, although lower in magnitude than the surface direct-current gradients across comparable areas. Measurements on nerve utilized the sciatic nerve, which was exposed from its entrance into the thigh to the knee, a distance averaging 6 cm. The nerve sheath was left intact, the nerve being dissected from its bed sufficiently to secure electrical isolation. The gradient along the sciatic was found to be distally negative (−2 to −5 mV/cm) and linear down to the point of bifurcation. Distal to this point, the gradient either changed in value or in some cases reversed polarity. It was then found that the sheath could be partially removed without altering the gradient along the main trunk, thus permitting measurements of each of the branches separately. The tibial branch was found to be invariably distally negative with a gradient of greater amplitude (−4 to −15 mV/cm) than the main sciatic.

The peroneal branch, however, showed a direct-current gradient of opposite polarity with amplitudes always lower than those of the tibial (+3 to +10 mV/cm). On most occasions the algebraic sum of the gradients on the two branches was equal to the 1-cm gradient along the main sciatic. These gradients were noted to change with the depth of anæsthesia. If anæsthesia was intentionally very deep (prolonged exposure to high concentration of agents) the early potentials were reversed in polarity (peroneal distally negative, tibial distally positive). Within 5 min the gradients fell to zero and following this the normal polarities appeared. The amplitudes gradually increased as recovery from anæsthesia occurred, highest values being found after respiratory movements returned and before co-ordinated escape movements occurred. The remarkable consistency with which the differential polarization of the two branches was noted appeared to indicate some basic difference between the two rather than some artefact of dissection or measurement. Since each branch is a mixed nerve, an estimate of the percentage of motor versus sensory fibres in each was desirable. Fibre-size spectrum determinations were made (myelinated fibres only) since there is generally believed to be some correlation between fibre-size and function[2]. The tibial branch was found to contain a preponderance of large (12–18μ outside diameter) fibres while the peroneal branch revealed a preponderance of small (3–8μ outside diameter) fibres. It was then decided to measure gradients along spinal nerves of predominantly motor or sensory function. A second series of 12 frogs was then evaluated using the branch of the femoral nerve to the sartorius as the motor nerve and the nerves running between the dorsal body wall and the skin over the back of the animal as predominantly sensory nerves. Fibre-size determinations were made of these nerves and they were found to contain much greater percentages of large and small fibres, respectively, than did either the tibial or peroneal. In each animal of this series, one sciatic nerve and both of its branches were first exposed and their direct-current gradients measured. In all 12 animals the peroneal and tibial branches showed the same differential polarization as in the first series (that is, peroneal distally-positive, tibial distally-negative). A total of 33 predominantly sensory nerves were measured, with the gradients in each case distally positive and ranging in magnitude from 0·5 mV/cm to 6 mV/cm. Ten predominantly motor nerves were examined, with the gradient in each case distally negative and ranging in magnitude from 1 mV/cm to 4 mV/cm.

A final series of 8 frogs were obtained in the 'hibernating' state and kept at temperatures between 40° and 45° F until used. The same polarities of the direct-current gradients were obtained, but the magnitudes were considerably reduced and the changes with anæsthesia prolonged. The normal polarities (peroneal positive, tibial negative) were seen only late in recovery from anæsthesia and the reversed gradients (peroneal negative, tibial positive) persisted in each case until after the corneal reflex had returned. Tissue fragility appeared to be increased in these winter frogs and 3 of the 8 had to be discarded because of bleeding or nerve damage. For this reason, measurements on the motor nerve to the sartorius muscle were unsatisfactory. It was possible, however, to measure gradients along the dorsal dermal branches. Like the sciatic branches, these also showed a polarity reversal, being distally negative until late in recovery from anæsthesia when they became positive, remaining so up to the point when escape movements began. In no case, however, did these gradients exceed 1 mV/cm.

The differential polarization of the motor and sensory nerves suggests that both halves of the postulated electrical circuit are within neural tissue, with charge carrier movement in one direction in motor fibres and in the opposite direction in sensory fibres. It would be desirable to relate this, if possible, to some general neuronal mechanism. The possibility of longitudinal current flow within neural structures has been given little attention. However, in 1947 Lorente de No[3] postulated the presence of a longitudinally polarizable structure in the axoplasm, and recently Müller[4] has presented experimental evidence for this in isolated segments of peripheral axons. In intact neural structures Gerard and Libet[5,6] have reported a longitudinal or 'axo-dendritic' direct-current potential along the cerebral neurones of the frog. Gradients up to 10 mV in magnitude were measured and a relationship noted between the magnitude and polarity of the gradient and the activity of the neurones. Goldring *et al.*[7-9] afterwards extended these observations and advanced the thesis that the longitudinal direct-current gradient determined the level of irritability of these neurones. In all these studies, measurements were made between the pial and ventricular surfaces of the brain, that is, from the terminal dendritic arborizations across the cell bodies to a point on the outgoing axons. Anatomical considerations prevented us from measuring across the cell bodies of the spinal neurones. However, if an axono-dendritic direct-current gradient were present in these neurones, then our method of electrode placement would indicate a direct-current gradient of one polarity on motor nerves and a gradient of opposite polarity on sensory nerves. Other studies[10,11] have indicated that in the spinal, as in the cerebral, neurones the longitudinal direct-current gradient determines in part the level of irritability of these neurones. While the status and significance of the neural direct-current gradients in the intact, unanæsthetized frog can only be inferred at this time, it appears possible that the concept of a longitudinal direct-current polarization gradient may be extended to include the spinal as well as the cerebral neurones. The significance of the differential polarization as a means of circuit completion for current flow requires further experimentation.

Detailed technique. Large, adult bullfrogs (*R. catesbeiana*) were used as the experimental animal, and atraumatic surgical approaches were devised for exposure of the internal structures of the posterior thigh, including the sciatic nerve. Electrical measurements were taken only of intact structures, and cut tissue edges (particularly skin) were carefully retracted from any contact with either the electrodes or the structure to be measured. Damage to a structure was either visually apparent or evidenced by injury potentials and such preparations were discarded. Particular care was exercised to avoid open bleeding into the area because of the resultant shunting effect and electrode contamination. Electrical isolation was secured by slipping a thin sheet of polyethylene beneath the structure to be measured. Electrodes were silver–silver chloride, saline-agar filled with thin terminal cotton wicks. Electrode pairs were chosen for stability, resistance to polarization, and low inter-electrode potentials ($< 100\ \mu V$). Measurements were made over successive 1-cm segments of the structures with the reference electrode always placed proximally and the recording electrode distally. Potentials were measured with either a Kiethley 600*A* electrometer (input impedance 10^{14} ohms) or a Hewlett–Packard 425*A* chopper amplifier (input impedance 10^{8} ohms). Comparison measurements showed no difference between measured potential with either instrument. (If the variable input impedance of the 600*A* was utilized, potentials were maintained with little drop in magnitude over the range 10^{14}–10^{6} ohms, a 10 per cent drop was noted at 10^{5}, and 10^{4} ohms effectively shorted the potential.) All measurements were taken within a double-walled, grounded Faraday cage. The preparations were ungrounded although comparison measurements revealed no changes in recorded potentials if some remote portion of the preparation was connected through a similar electrode to circuit (not chassis) ground. Anæsthesia was obtained by titration with 'Tricaine' (Sandoz) and complete anæsthesia was arbitrarily designated as loss of corneal reflex, respiratory movements, and response to trauma. Following anæsthetization, the animals were rinsed in de-chlorinated tap water, drained and placed on 4-in. thick blocks of polyethylene for dissection and measurements. Potential readings were taken at intervals of approximately 5 min during subsequent recovery from anæsthesia. Small pledgets of saline-moistened surgical cotton were used to cover the incision when measurements were not being made, care being taken not to contact the nerve. The functional integrity of the sciatic, its branches, and the motor nerve to the sartorius was determined by their ability to transmit stimuli and produce a muscle contraction at the end of each procedure.

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Lipoprotein Nature of Red Cell Adenosine Triphosphatase

THE adenosine triphosphatase activity of stromata prepared from human red cells has recently been thoroughly investigated by Post *et al.*[1] and by Dunham and Glynn[2]. The authors were able to demonstrate that in this ATPase, which is activated by magnesium and shows optimal efficacy at *p*H 7, two fractions can be distinguished, one being activated by calcium and another being inhibited by calcium and activated by sodium and potassium. The part activated by sodium and potassium amounts to 30–50 per cent of the total, and is further characterized by being inhibited by cardiac glycosides. It thus shows striking similarity to the ATPase present in submicroscopic particles isolated from crab nerve sheaths as studied by Skou[3,4]. The dependence of these enzymes on sodium and potassium and their sensitivity towards cardiac glycosides strongly suggest that they play a part in the active transport of sodium and potassium through the cell membrane.

In 1950 Kielley and Meyerhof[5] had isolated from rabbit muscle homogenates a fraction sedimenting at 18,000*g* which displayed magnesium-activated, calcium-inhibited ATPase activity. This activity could partly be inhibited by treatment with an enzyme from *Clostridium welchii*, which removes phosphorylcholine from lecithin and likewise but at a slower rate from sphingomyelin.

In the experiments recorded here red cell ghosts, prepared from fresh human blood essentially according to the method of Post *et al.*, were subjected to a similar treatment. A volume of 0·2 ml. of stroma-suspension was added to 2·3 ml. of a fluid containing 12·8 mM imidazole-histidine buffer of *p*H 7·1, 16 mM potassium chloride, 64 mM sodium chloride and 4·8 mM magnesium chloride. 1 mg of a nucleic acid purified lecithinase from *Cl. welchii* type *A* culture filtrate was added to the samples, supplemented by 9·5 μmoles calcium chloride. (The enzyme preparation was a generous gift by Dr. I. Batty, Wellcome Research Laboratories.) After 20 min incubation at 38° C the tubes were chilled in ice-water and the free Ca^{++} was removed by addition of 10 μmoles of the tetrasodium salt of glycolether-diamine tetraacetic acid. Control samples showed that contact with calcium during 20 min and the presence of the complexing agent did not alter the ratio of glycoside-sensitive to glycoside-insensitive ATPase, the average ratio from 6 experiments being 0·57 in the treated samples as compared with 0·6 in the controls. Afterwards the samples were incubated for 70 min at 38° C in the presence of 4·5 μmoles ATP (disodium salt, Boehringer). In order to discriminate the fraction sensitive to sodium and potassium from the total ATPase activity $5 \cdot 5 \times 10^{-5}$ M g-strophanthin ('Ouabain', Merck) was added to one lecithinase-treated and one control sample respectively. After deproteinization with 2·5 ml. 10 per cent trichloroacetic acid the liberated phosphorus was determined by the method of Fiske and Subbarow. Since the lecithinase-treated samples gave a slight turbidity on addition of the molybdate reagent which was probably due to formation of an insoluble compound with the liberated phosphorylcholine, the colorimetric measurement was carried out in the presence of about 50 per cent acetone which dissolved the precipitate. Acetone, however, gives a blue colour with the reagent, increasing in intensity over a long time. Therefore the readings were taken exactly 2 min after addition of the acetone.

Table 1 summarizes 6 experiments performed with two different blood samples, giving the ATPase activity as percentage of the controls treated in the same way except for the addition of lecithinase. It can be seen that both the glycoside-sensitive and insensitive fraction are reduced to about the same level by attack with lecithinase. Whether complete abolition could have been achieved by prolonged exposure to the lecithinase was not investigated. These experiments show that the integrity of the lipid component of the stromata is essential for the ATPase activity. Skou[6] reported the abolition of the ATPase activity in crab nerve granules by treatment with phospholipase *A*, an observation pointing in the same direction.

Table 1

Blood No.	Age of ghosts (days)	ATPase activity of lecithinase-treated ghosts (percentage of control)	
		Glycoside sensitive	Glycoside insensitive
1	7	13·3	27·6
	10	10·0	37·5
	11	48·5	—
2	1	1·8	48·0
	8	33·3	39·2
	7	66·7	38·5
	Average	28·9	38·2
		$P = 0.001$	$P < 0.001$

On the other hand, both fractions of the red cell ATPase can be blocked by the organic mercurial mersalyl. In an experiment with blood No. 2 of Table 1 the concentration of mersalyl for 50 per cent inhibition of both fractions was found to be about 4×10^{-6} M. This brings out the fact that in the enzyme protein proper sulphydryl groups play a part, much as in the actomyosin ATPase[7].

The participation of the membrane lipids in the splitting of ATP can be visualized in different ways: (1) The lipid layer adjacent to the membrane proteins might help to stabilize the folding of the protein chains necessary for a good fit of ATP to the enzyme. (2) The characteristic phosphorylcholine group of lecithin and sphingomyelin, showing several negative charges in vicinity of the quaternary nitrogen, might fix the equally bipolar ATP molecule near the prosthetic group of the enzyme protein. (3) As suggested by Dunham and Glynn the enzyme responsible for the apparent splitting of ATP into ADP and phosphate might not liberate phosphate directly, but cause a transphosphorylation, the intermediary product undergoing subsequent hydrolysis. The phosphate-accepting substance might show liposolubility and be located in the lipid phase of the membrane. On destruction of the ordered array of the lipid molecules this acceptor might lose contact with the enzyme or even escape into the medium. It is an attractive hypothesis also advocated by Post[8] that some of the phosphorylated intermediary product might serve as a carrier molecule for transport of sodium out of the cell.

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Resorption and Glucuronide Conjugation of Salicylamide during Pregnancy

During the examination of human beings we have noted that when salicylamide was given orally during late pregnancy the blood concentrations of both the free salicylamide and glucuronide conjugate of it greatly differed from those found in non-pregnant control subjects. These changes might be caused either by disturbed resorption or impaired conjugation or by both. The possible role of an altered hepatic detoxication function during pregnancy also presents an interesting problem in this connexion.

Since it is quite impossible to perform such experiments on human beings which would more directly elucidate the effect of pregnancy on the resorption or glucuronide conjugation processes, we have carried out investigations on other animals.

The work was carried out on 11 female rabbits of which 6 were pregnant, 5 served as controls. The animals were anæsthetized by intraperitoneal administration of 0·4 ml./kg 'Sombutol' (Lääke Oy), a mixture of anæsthetic, of which 1 ml. contains 0·030 g penthobarbital, 0·0672 g sodium pentobarbital and 0·250 g urethane. The abdominal cavity was exposed by a midline excision, and blood samples were taken from the portal vein and from the caudal vena cava vein. After control blood samples were taken, a 300 mg/kg body-weight dose of salicylamide mixed into 0·5 per cent tragacanth suspension was introduced into the stomach with a gastric tube. Blood samples of both veins were taken 1 h and 4 h after the administration of the drug. Analyses of salicylamide and salicylamide glucuronide were performed following the same methods described in our earlier work. At the end of the experiment a 10 per cent aqueous homogenate was made of each liver. The salicylamide content of these samples was also determined.

Table 1. Blood Analyses of Free Salicylamide and its Glucuronide Conjugate in Pregnant and Non-pregnant Rabbits after Oral Administration of Salicylamide (300 mg/kg Body-weight)

	Free salicylamide		Glucuronide conjugate of salicylamide	
	1 h	4 h	1 h	4 h
Control group portal vein	2·6 ± 0·63	1·9 ± 0·52	3·1 ± 1·0	5·0 ± 1·3
Pregnant rabbits portal vein	0·64 ± 0·25	0·61 ± 0·20	2·3 ± 0·43	3·4 ± 1·1
Control group vena cava	8·9 ± 0·90	3·6 ± 0·85	6·2 ± 1·8	7·6 ± 1·4
Pregnant rabbits vena cava	1·4 ± 0·34	1·36 ± 0·27	5·5 ± 0·81	3·4 ± 1·2

The results are presented in Table 1. They clearly indicate that the concentrations in the portal blood of free salicylamide are greatly different in the pregnant and control groups. This difference is remarkably high after the first hour. The differences in the concentrations of the glucuronide conjugate of salicylamide are smaller, although all the values are higher in the control group than in the pregnant animals. Also in the peripheral blood the serum values in the control group are higher than in the pregnant animals although the differences are smaller.

These observations and results seem to indicate that the resorption and conjugation of salicylamide in the gastrointestinal tract are retarded during pregnancy. The results, especially those of the first hour, are also closely the same as those found in our work on human beings (peripheral blood samples). The 4-h values, however, did not show as great a decrease in the control group and increase in the pregnant group as was found in our work on human beings. These differences might be caused by species differences. They might also be due to the fact that in these animal experiments we used anæsthetized animals. The dosage of the drug was also higher for the animals, which also contributes to the difference. No accumulation of free salicylamide in the liver could be detected in either of the experimental groups.

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Changes in the Subcellular Distribution of Cardiac Catecholamines in Dogs dying in Irreversible Hæmorrhagic Shock

The work of Schlossberg and Sawyer[1] has established the fact that the reactivity of the sympathic adrenal system of an animal is involved in stress situations such as severe loss of blood. Indeed, vasoconstriction due to increased peripheral sympathetic tone and elevation of circulating catecholamines resulting from the stimulation of the adrenal medulla by the sympathetic centres are outstanding signs of hypovolemic hypotension. In experimental hæmorrhagic shock increased blood-levels of epinephrine and norepinephrine have most recently been reported by Greever and Watts[2] and Walker and co-workers[3,4].

A number of papers have lately pointed out the fact that rises in the levels of the circulating catecholamines bring about concomitant increases in the concentrations of these substances in the mammalian heart[5–7]. One would, therefore, expect that hæmorrhagic shock would similarly lead to intracardiac accumulations of these neurohormones. The results reported here indicate the reverse.

Mongrel dogs of both sexes were submitted to a standard shocking procedure described elsewhere[8]. Fig. 1 presents the changes in the circulating catecholamine-levels typical of our experimental animals: there were early rises primarily, if not exclusively, due to epinephrine followed by much less spectacular increases in norepinephrine. The latter appeared to reach something of a plateau concentration which was maintained until the death of the dog while the

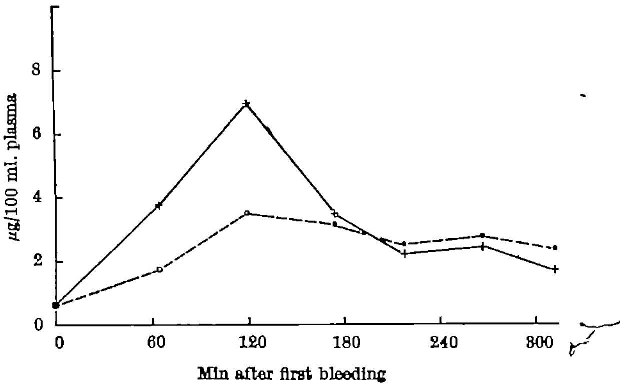

Fig. 1. Alterations in the blood catecholamines (ref. 9) in a dog subjected to the standard procedure resulting in irreversible hæmorrhagic shock. ——, Epinephrine; – – –, norepinephrine

epinephrine declined progressively although never to its original low value. Unbled controls, kept for 6–7 h under 'Nembutal' anæsthesia in the supine position with cannulæ in place, exhibited no significant variations from the original value.

In spite of these persistent high blood-levels, the heart of dogs dying in shock was found consistently to be severely depleted of catecholamines (Table 1). This depletion appeared to affect the atrium and the ventricle to about the same extent, 47 and 44 per cent respectively. Preliminary results of this type have led us to investigate the changes occurring at the subcellular level.

Table 1. CATECHOLAMINES IN THE VENTRICLES OF DOGS DYING IN SHOCK AND OF PAIRED CONTROLS GIVEN AS μG NOREPINEPHRINE/G WET WEIGHT (REF. 10)*

	Control	Shock
	1·20	0·45
	1·45	0·40
	1·40	0·40
	1·14	0·50
	1·26	0·81
	1·50	0·63
	1·17	0·48
Mean	1·30	0·52

* Results obtained by Dr. Woo Choo Lee shortly before his return to Seoul, Korea.

Recent observations have indicated that large amounts of catecholamines appear to be particle-bound in the heart of a dog[7,11]. Following the procedure described by Campos and Shideman[7] for the differential centrifugation of heart homogenates prepared in 0·075 M potassium phosphate at pH 7·5, we obtained three fractions, a 'coarse' or slow-speed sediment (5 min at 2,000g), a 'particulate' or high-speed sediment (60 min at 105,000g) and a 'soluble' or high-speed supernatant.

The results of the catecholamine concentrations in these three fractions obtained from dogs dying in irreversible hæmorrhagic shock and from controls are summarized in Tables 2 and 3. The most extensive depletion seemed to occur in the 'particulate' fraction, amounting to 68 per cent in the atrium and 63 per cent in the ventricle.

Table 2. DISTRIBUTION OF CATECHOLAMINES IN THE SUBCELLULAR FRACTIONS OF THE LEFT VENTRICLE, GIVEN AS μG NOREPINEPHRINE/G WET WEIGHT (REF. 10)

	Whole homogenate	Subcellular fractions				Recovery (per cent)
		Coarse	Particulate	Soluble	Total	
Control 1	1·92	0·58	0·85	0·36	1·74	91
2	1·08	0·33	0·46	0·21	1·00	93
3	1·66	0·45	0·51	0·29	1·25	75
4	0·92	0·37	0·34	0·16	0·87	95
Average	1·40	0·42	0·54	0·26	1·22	
Shock 1	0·64	0·15	0·19	0·22	0·56	88
2	0·96	0·19	0·34	0·16	0·69	72
3	0·85	0·21	0·15	0·27	0·63	74
4	0·68	0·18	0·12	0·05	0·35	51
Average	0·78	0·18	0·20	0·18	0·56	
Depletion (per cent)	44	57	63	31		

Table 3. DISTRIBUTION OF CATECHOLAMINES IN THE SUBCELLULAR FRACTIONS OF THE RIGHT ATRIUM GIVEN AS μG NOREPINEPHRINE/G WET WEIGHT (REF. 10)

	Whole homogenate	Subcellular fractions				Recovery (per cent)
		Coarse	Particulate	Soluble	Total	
Control 1	4·40	0·77	1·65	1·53	3·95	90
2	3·82	0·57	0·99	0·60	2·16	57
3	3·18	0·16	0·90	1·09	2·15	68
4	3·12	0·80	1·28	0·96	3·04	97
Average	3·63	0·58	1·21	1·05	2·82	
Shock 1	1·62	0·47	0·46	0·65	1·58	98
2	2·26	0·54	0·52	0·83	1·89	84
3	2·59	0·49	0·25	0·61	1·35	52
4	1·16	0·13	0·34	0·39	0·86	74
Average	1·91	0·41	0·39	0·62	1·42	
Depletion (per cent)	47	30	68	41		

One may speculate that this terminal depletion may be due either to over-stimulation of the sympathetic fibres terminating in the heart and/or to impairment of the mechanism responsible for the uptake of the catecholamines from the blood or to difficulties arising at the sites of neurohormone synthesis or metabolism in the heart. It is hoped that experiments using a variety of blocking agents will permit us to distinguish between these possibilities. Furthermore, we are concerned with the question of whether this type of cardiac involvement is an early or a late phenomenon in hæmorrhagic shock, that is, whether it is causatively related to the onset of irreversibility and the ultimate death of the animal.

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Cortisol Metabolizing Activity of Spleen and Liver of Erythroblastosis-afflicted and Normal Chickens *in vitro*

DOUGHERTY *et al.*[1,2] have shown that when performing *in vitro* experiments the lymphatic tissue of control albino mice does not metabolize cortisol-4-^{14}C in lymphatic leukæmia; however, it chemically changes a great part of cortisol. We have carried out comparative examinations *in vitro* with another kind of experimentally induced leukæmia, that is, with the virus erythroblastosis of chicken. Experiments were performed with equal parts by weight taken from the spleens and liver of normal and erythroblastosis-afflicted Leghorn chickens and their cortisol metabolizing activity examined.

Erythroblastosis was provoked by intravenous injection of erythroblastosis virus stock obtained from the Engelbreth-Holm Laboratory (Copenhagen). The normal chickens and those in the last stage of experimental erythroblastosis, weighing 150–360 g, were decapitated. The spleen and liver taken out fresh from the animals were put in a covered vessel, containing paper-cotton moistened with Krebs–Ringer solution. A quantity of about 0·5 g of the organs was quickly weighed and cut to slices. (Normally one whole spleen or the spleens of two animals were needed for this purpose.) The slices were incubated at 37° C for 3 h in 7-ml. Krebs–Ringer phosphate buffer, while a gas mixture, composed of 95 per cent oxygen and 5 per cent carbon dioxide, was bubbled through the solution. In addition the solution (pH 7·4) contained 200 mg per cent glucose, 1·22 mg

Table 1. METABOLISM OF CORTISOL BY SPLEEN-SLICES OF NORMAL LEGHORN CHICKENS *in vitro*

No. of chickens	Spleen-weight g/ 100 g body-wt.	Incubated spleen (g)	Cortisol (per cent) Unchanged Measured	Cortisol (per cent) Transformed Calculated
1	0·10	0·35	80·0	20·0
2	0·13	0·47	63·8	36·3
3	0·12	0·63	84·1	15·9
4	0·13			
5	0·17	0·58	75·0	25·0
6	0·21			
7	0·12	0·54	72·6	27·4
8	0·10			
9	0·12	0·50	72·7	27·3
10	0·10			
11	0·24	0·62	87·8	12·2
12	0·17			
13	0·12	0·41	81·8	18·2
14	0·15			
Average	0·14	0·51	77·2	22·8

Table 2. METABOLISM OF CORTISOL BY SPLEEN-SLICES OF ERYTHROBLASTOSIS-AFFLICTED LEGHORN CHICKENS *in vitro*

No. of chickens	Spleen-weight g/ 100 g body-wt.	Incubated spleen (g)	Cortisol (per cent) Unchanged Measured	Cortisol (per cent) Transformed Calculated
1	0·77	0·47	42·2	57·8
2	0·15	0·47	47·1	52·9
3	0·81	0·59	39·7	60·3
4	0·58	0·50	48·4	51·6
5	0·72	0·55	44·4	55·6
6	0·54	0·45	74·1	25·9
7	0·33	0·54	46·7	53·3
8	0·27	0·50	17·8	82·2
9	1·00	0·57	29·2	70·8
10	0·82	0·49	41·0	59·0
11	0·65	0·55	22·0	78·0
12	0·79	0·48	29·0	71·0
Average	0·62	0·52	40·1	59·9

Table 3. METABOLISM OF CORTISOL BY LIVER-SLICES OF NORMAL LEGHORN CHICKENS *in vitro*

No. of chickens	Liver weight g/ 100 g body-wt.	Incubated liver (g)	Cortisol (per cent) Unchanged Measured	Cortisol (per cent) Transformed Calculated
1	3·95	0·53	37·5	62·5
2	2·81	0·58	84·1	15·9
3	3·04	0·59	59·4	40·6
4	3·60	0·58	73·5	26·5
5	3·47	0·58	51·5	48·5
6	2·86	0·55	45·2	54·8
7	2·10	0·58	50·0	50·0
8	2·82	0·54	30·9	69·1
9	3·36	0·57	25·5	74·5
10	3·27	0·61	41·4	58·6
11	3·40	0·53	53·6	46·4
12	3·70	0·60	65·9	34·1
Average	3·19	0·57	53·0	47·0

Table 4. METABOLISM OF CORTISOL BY LIVER-SLICES OF ERYTHROBLASTOSIS-AFFLICTED LEGHORN CHICKENS *in vitro*

No. of chickens	Liver weight g/ 100 g body-wt.	Incubated liver (g)	Cortisol (per cent) Unchanged Measured	Cortisol (per cent) Transformed Calculated
1	5·75	0·57	52·0	48·0
2	3·62	0·52	52·0	48·0
3	7·69	0·54	37·6	62·4
4	4·07	0·54	30·9	69·1
5	4·74	0·54	45·4	54·6
6	6·22	0·54	43·6	56·4
Average	5·35	0·54	43·6	56·4

per cent nicotinic acid amide and 52·9 mg per cent sodium adenosine triphosphate. Finally, 80 μg cortisol dissolved in 0·01 ml. ethanol was added. Blank experiments with steroid were also set up without tissue slices. An extraction was performed with ethyl acetate. The material was shaken three times with double volume of ethyl acetate and washed once with 1 per cent sodium carbonate solution (1/5 vol.), and twice with distilled water, then kept in a refrigerator during the night. The ethyl acetate poured off from the frozen ice was evaporated dry in vacuum from a water bath at 50° C. The residue was dissolved in a mixture of methanol and chloroform (1 : 1) and, after having impregnated the papers with methanol of 90 per cent[3], a paper chromatograph was made at room temperature, passing through a system of toluene, methanol and water (10 : 7 : 3), continuously for 8 h[4]. Then, after having drawn it through a fresh mixture of 50 mg per cent solution of blue tetrazolium and 10 per cent solution of sodium hydroxide (1 : 1) it was subjected to reaction. Quantitative measurements were carried out according to Touchston and Hsu[5] by the dissolution of the formazan spots occurring at autentic series of cortisol standards.

When calculating the percentage of the cortisol values that changed or remained unchanged during our experiments, the quantities of the cortisol measured according to the foregoing method and the cortisol quantity recovered in the blank experiment were taken as starting-point.

The numerical results are shown in Tables 1–4.

As a result we found that in fully developed experimental erythroblastosis with Leghorn chickens a portion of predetermined weight of the hypertrophic spleen converts *in vitro* about twice as much cortisol as the normal spleen tissue of identical weight. In the case of the equally hypertrophic liver the metabolic ability observed with tissue preparations of identical weight seems to be unchanged.

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PHARMACOLOGY

Sub-cellular Distribution of Pharmacologically Active Substances in Guinea Pig Brain

RECENT investigations[1,2] have shown that acetylcholine in the central nervous system is located within cell particles which have been identified as pinched-off nerve endings. It was of interest to determine whether other substances which may act as transmitters, or as regulators of neuronal excitability, would have a similar sub-cellular distribution Therefore we have determined the sub-cellular distribution of acetylcholine-, substance *P*- and 5-hydroxytryptamine (5-HT)-like substances and also of glutamic acid and γ-aminobutyric acid (GABA).

Experiments were carried out on sucrose homogenates of guinea pig brain, prepared in a piston press homogenizer. Density gradient fractionation was carried out as described by Whittaker[1]. The total amounts of each of the substances were determined in the various fractions, nuclear (*P*1), crude mitochondrial (*P*2), microsomal (*P*3), cytoplasmic (*S*), and in the sub-fractions of *P*2, myelin (*A*), nerve ending (*B*) and mitochondrial (*C*). The particulate fractions, resuspended in isotonic saline, and the cytoplasmic fraction were heated for 10 min at *p*H 4 and centrifuged to remove the precipitated material. The supernatant fluids were adjusted to *p*H 7·5 and assayed directly for potassium, substance *P* and the amino-acids. For the acetylcholine and 5-HT assays the solutions were first incubated with chymotrypsin. Acetylcholine was determined as the bromide on the superfused guinea pig ileum treated with mepyramine and tryptamine. Substance *P* was estimated on the same preparation after the further addition of atropine to the perfusion fluid. 5-HT was assayed as the complex with creatinine sulphate on the isolated rat stomach strip in the presence of hyoscine. The specificity of the assays was verified by the use of atropine, chymotrypsin or '*BOL*-148' respectively.

GABA and glutamic acid were separated from most of the other components of the extracts by paper electrophoresis at *p*H 4 (pyridine–acetic acid–water buffer). After elution of the appropriate bands with water, the amino-acids were further purified by paper chromatography using isopropanol/water (7 : 3, v/v) and phenol/water (4 : 1, w/w) as the solvent systems for glutamic acid and GABA respectively. Eluates of the appropriate ninhydrin-stained spots were assayed spectrophotometrically[3].

The distribution of each substance was investigated in two to eight experiments. Table 1 summarizes the averaged results of two typical experiments in which assays for acetylcholine, substance *P*, GABA, glutamic acid and potassium were carried out on the same brain extracts, and the results of a further experiment in which fractionation was carried out in sucrose containing iproniazid. Iproniazid increased the amount of 5-HT in the extracts but had no effect on the distribution of acetylcholine or substance *P*.

Table 1. SUB-CELLULAR DISTRIBUTION IN GUINEA PIG BRAIN

	R_1* per g wet weight	% R_1				R_2†	% R_2		
		*P*1	*P*2	*P*3	*S*		*A*	*B*	*C*
Acetylcholine bromide	582 ng	11	75	4	10	45	14	85	1
Substance *P*	64 units	10	79	7	4	71	20	80	0
5-HT creatinine sulphate ‡	428 ng	0	36	26	29	105	16	31	53
GABA	225 µg	4	19	1	75	69	29	62	9
Glutamic acid	798 µg	7	28	1	64	46	25	75	10
Potassium§	62 µequiv.	6	29	2	62	82	43	48	9
Calculated 'free' distribution¶		3	12	2	83	9	44	49	7

* R_1 = total recovery in *P*1, *P*2, *P*3 and *S*.
† R_2 = total recovery in *A*, *B* and *C* expressed as a percentage of the amount found in *P*2.
‡ Sucrose homogenate contained 1 mM iproniazid.
§ Determined by flame photometry.
¶ See text.

The results are similar to those of Whittaker[1] in regard to the distribution of acetylcholine, but the observed distribution of 5-HT differs from that reported by Whittaker. Acetylcholine and substance *P* had a similar distribution, both being found predominantly in *P*2, and on subfractionation of *P*2, in the nerve-ending fraction, *B*. 5-HT was more evenly distributed between *P*2, *P*3 and *S* than was acetylcholine or substance *P*. On subfractionation of *P*2, 5-HT was concentrated in fraction *C*, in agreement with observations on rabbit brain[4]. However, in experiments on rat brain (Ryall, unpublished), fractionated by a modified technique[2], most of the 5-HT in *P*2 was recovered in fractions lighter than mitochondria, and termed fractions *B* and *C* by de Robertis *et al.*[2]. Potter and Axelrod[5] also located 5-HT in rat brain in a fraction lighter than mitochondria. These observations, together with the fact that a large proportion of the 5-HT in guinea pig brain homogenates was found in *P*3, and the possibility that *P*3 contains isolated synaptic vesicles[6], may indicate that dense 5-HT-containing nerve endings, sedimenting in fraction *C*, are easily disrupted, so releasing 5-HT-containing vesicles of lower density.

Most of the GABA (75 per cent) and also of glutamic acid (64 per cent) was recovered in the cytoplasmic fraction. These percentages are higher than those reported for cat cerebral cortex[7], but are of the same order as that found for Factor I in sucrose homogenates of rat brain[8]. Most of the amino-acids contained in the particulate fractions were associated with *P*2, which agrees with the observation of Tower[7] on the cat cerebral cortex, and about 60 per cent of the *P*2-content was recovered in the particulate subfractions. The lowermost line of Table 1 shows the distribution of a freely diffusible substance, calculated on the assumption that such a substance would be distributed between a sediment and its supernatant fluid approximately in proportion to their respective volumes. It is seen that the percentage recoveries of the amino-acids in *P*2 and its sub-fractions are greater than the calculated values, which indicates that the two substances are partially bound. The distribution of the amino-acids in *P*1, *P*2, *P*3 and *S* was similar to that of potassium, but there were differences in the sub-fractions of *P*2. Since very mild conditions were used for the extraction of potassium, the amount recovered probably represented mainly exchangeable potassium[9] and was therefore lower than the values for brain potassium usually reported in the literature.

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Maltoryzine, a New Toxic Metabolite produced by a Strain of *Aspergillus oryzae* var. *microsporus* isolated from the Poisonous Malt Sprout

Two cases of feed poisoning in dairy cattle were brought under observation in autumn, 1954. From the results of our survey the poisoning cases[1] were found to be due to the malt sprout supplied as food. After investigation of microflora in the poisonous malt sprout and toxicity of the isolated micro-organisms, the micro-organism responsible for poisoning was identified as *Aspergillus oryzae* var. *microsporus*[2].

This communication deals with the isolation, molecular formula, general properties and chemical structure of the toxic substance.

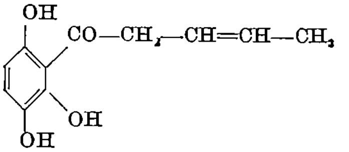
The procedure of the isolation and purification of the toxic substance was as follows. Toxicity was measured after each step by intraperitoneal injection in mice. A strain of *Aspergillus oryzae* var. *microsporus* was grown on a Czapek–Dox medium containing malt sprout extract in tank culture. After incubation for 2·5 days at 30° C, the cultured broth was collected by a filter press and concentrated *in vacuo*. After 18 h at 5° C the precipitate formed was collected by centrifugation and was extracted with hot absolute ethanol. The extracted solution was concentrated *in vacuo*, adjusted to *p*H 9·0 with sodium hydroxide, extracted with absolute ethyl ether and the basic aqueous solution was acidified with hydrochloric acid. The acidified solution was continuously extracted with absolute ethyl ether, dehydrated with sodium sulphate and finally concentrated *in vacuo*. The extract was chromatographed on a column of cellulose with *n*-butanol saturated with water as the solvent system. The toxic substance was found in tubes No. 20–40, and, after removal of the solvent, was crystallized from benzene.

Approximately 300 mg of the toxic substance were obtained as crystals from 150 l. of the cultured broth, which were pale brownish-yellow needles and melted with decomposition at 68·9°–69° C. The crystals had a toxicity in LD_{50} of 3 mg/kg in mice when given intraperitoneally, causing muscular narcotism.

The molecular formula of the toxic crystals was in agreement with $C_{11}H_{14}O_4$. $[\alpha]_D^{25} = 0$ ($C = 2·8$, in chloroform). Ninhydrin, Schiff and Gibbs[3,4] reactions were negative and ferric chloride, diazo, methyl red and 2,4-dinitrophenylhydrazine reactions were positive. Potassium permanganate solution was decolorized rapidly at room temperature. The toxic crystals were readily soluble in ethanol, methanol, ethyl ether and chloroform; slightly soluble or insoluble in pyridine, acetone, ligroin, ethylacetate and butylacetate. The toxic crystals were more readily soluble in basic solutions than in acid or neutral solutions, but were unstable in basic solutions. The ultra-violet absorption spectrum[5] showed a strong band (log ε 4·1) near 220 mμ, a shoulder band (log ε 3·1) near 280 mμ and a weak band (log ε 2·1) near 320 mμ. These results led us to assume that one benzoyl group existed in one molecule of the toxic crystals. The infra-red absorption spectrum indicated the presence of the hydroxy group (3,300 cm⁻¹), ketone group (1,700 cm⁻¹) and conjugated double band (1,600, 1,500 cm⁻¹).

The molecular formula of the toxic crystals methylated with dimethylsulphate was in agreement with $C_{14}H_{18}O_4$. Ozonolysis of the methylate of toxic crystals in chloroform with 1·3 per cent ozone at −10° C gave one mole of acetic acid and an acidic substance $C_{12}H_{14}O_6$. Ketonic hydrolysis of the acidic substance with 1 N sulphuric acid for 1–2 min at 120° C gave one mole of carbon dioxide and a ketone compound. Acid hydrolysis of the acidic substance with 1 N ethyl-alcoholic potassium hydroxide for 15 min at 75 ± 2° C gave one mole of acetic acid and 2,3,6-trimethoxybenzoic acid. Therefore, the acidic substance was shown to be 2,3,6-trimethoxybenzoyl acetic acid[8].

From these results, the chemical structure of the toxic crystals must be 1-(4-pentenoyl)-2,3,6-trihydroxy-benzene as shown in the following formula:



This new substance is different from hordenine[7], clavatin[8], etc., which have been previously reported as the toxic substance for dairy cattle. We propose to name the new substance 'maltoryzine'.

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Antitumour Properties of Phleomycin

PHLEOMYCIN, a water-soluble basic antibiotic, was discovered in Japan several years ago[1,2]. Recently, highly purified material prepared in this laboratory[3] was evaluated for potential antitumour activity by a new lysogenic induction technique[4]. Since positive results were obtained, further experiments were carried out in rodents to determine whether the material possessed *in vivo* antitumour properties against various transplanted tumours.

Twice-daily intraperitoneal injection of a saline solution of phleomycin for 7 days into mice bearing sarcoma 180 caused significant inhibition of tumour growth over a wide range of doses (Fig. 1). A similar pattern of inhibitory activity was observed with phleomycin therapy of mice with either adenocarcinoma 755 or Ehrlich ascites carcinoma. However, it was ineffective against *L*–1210 leukæmia. Phleomycin treatment of mice with adenocarcinoma 755 or

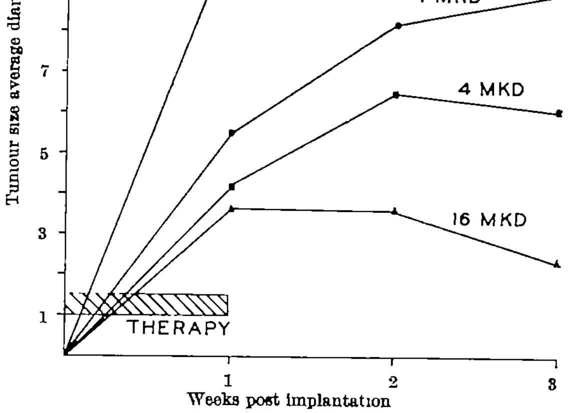

Fig. 1. Effect of phleomycin on sarcoma 180 *in vivo*. Saline solution injected intraperitoneally twice daily for 7 days starting the day after tumour implantation (by trocar into Swiss *HA/ICR* mice). Average tumour diameters determined by caliper measurement at the end of therapy and weekly thereafter. Results with doses 16, 4 and 1 mg/kg/day (MKD) are shown

sarcoma 180 frequently caused a significant increase in median survival-time over controls. Details of these and other pharmacological observations will be published in due course.

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PATHOLOGY

Tissue Eosinophilia in Hypersensitivity Reactions as revealed by the Human Skin Window

THE elusive eosinophil has been credited with a variety of diverse pathophysiological functions. It has been postulated as a carrier of histamine[1,2] though this has been disputed[3,4]. This cell has been shown to contain substances exhibiting anti-histaminic activity[5], and to possess phagocytic ability. It is capable of ingesting sensitized erythrocytes[6] and mast cell granules[7]. Eosinophil granules form the nidus and may supply the basic protein for the formation of Charcot–Leyden crystals[8]. It has also been suggested that the eosinophil plays a part in the sequence of events leading to secondary antibody formation[9,10]. This view has been challenged recently by Litt[11], who demonstrated that antibody in association with antigen initiates the aggregation of eosinophils, a view which is supported by the work of Cohen *et al.*[12].

Investigations of the eosinophil in man have been hindered by lack of a facile reproducible technique for the production of tissue eosinophilia. Rebuck and Crowley[13] have devised a simple method applicable to the study of leucocytes in acute inflammation in man, a technique now referred to by Sieracki and Rebuck[14] as the skin window technique. Eosinophils have been demonstrated in skin windows performed in new-born infants from two to twenty-one days after birth[15], and in the early hours of the normal inflammatory response in patients with blood eosinophilia[16]. Hu *et al.*[17] demonstrated an eosinophilic response commencing at 33 h in normal individuals exhibiting an allergic response to Rhus antigen but not in Rhus-sensitive individuals suffering from atopic dermatitis. In the present work this technique was utilized in the examination of tissue eosinophilia in clinically allergic individuals exhibiting positive skin reactions of the immediate type.

Tests were performed on the volar surfaces of the forearm of a total of eight allergic patients exhibiting strongly positive immediate intradermal reactions to either fish extract, grass or ragweed. Inflammatory areas about 5 mm in diameter were prepared by scraping away the epithelial layers with a sterile scalpel blade at 2–3 sites on the cleansed forearm until the fine bleeding points of the dermis were noted. Two drops of a water-soluble extract of either ragweed, grass or salmon (1,000 units per c.c.) were applied to each test site. Controls consisted of two drops of saline. Rounded cover-slips which were applied immediately following application of the test solution were replaced at 2, 4, 6, 12, 24, 36 and 48 h. On removal each cover-slip was stained with Jenner–Giemsa and mounted in permount on slides.

The control sites exhibited the normal progression of cellular responses as outlined by Rebuck[13]. At 2 h the cells consisted almost entirely of polymorphs. Mononuclear cells began to appear in appreciable numbers at 4 h, and afterwards increased in number while the polymorphonuclear response diminished. At 24 h, mononuclears were by far the most numerous cell. A few eosinophils and a rare basophilic leucocyte were also observed in some of the control windows.

In contrast, the cell responses in the test sites in all allergic individuals were characterized by the presence of large numbers of eosinophils. They were observed in greater number than in the control at 4 h, and they gradually increased in number so that at 24 h they were frequently the predominant cell, often comprising 60 per cent of the cells per high-power field (Fig. 1). The eosinophilia in the test sites was persistent, for appreciable numbers of such cells were observed at 48 h.

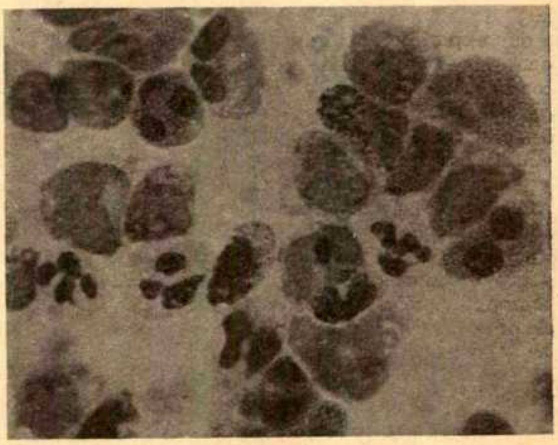

Fig. 1. 24-h skin window in grass-sensitive patient. Note the eosinophils throughout the field. A basophilic leucocyte with denser irregular granules is present at upper right. (× 500)

Attempts were made to transfer passively the eosinophilotactic activity. Eight non-allergic recipients were given intradermal injections of 0·1 c.c. of a highly potent reaginic serum from four allergic patients. A similar volume of serum from a non-allergic individual and saline, respectively, were injected into two other sites as controls. Sixteen hours later, after assessing the efficacy of the passive transfer by the conventional Prausnitz–Kustner test (P–K test), skin windows were prepared at these sites and ragweed applied as previously described. Eosinophilia was noted in the test sites prepared with allergic serum but not in the two control sites. In seven of the eight individuals tested, the eosinophilia differed from the intense and persistent eosinophilia observed when the test was done directly on allergic patients in that eosinophils were noted in only moderately greater numbers than in the control sites. In one individual, however, the eosinophilia was even more intense than in some of the allergic patients. Individual reaginic sera differed in their capacity to transfer eosinophilotactic activity in this latter non-allergic recipient, some sera producing a greater eosinophilia than others. Heating a reaginic serum to a degree sufficient to abolish the P–K activity also abolished the ability to transfer eosinophilotactic activity in this individual and in the one other instance in which it was tested.

Cohen *et al.*[12] have produced experimental eosinophilia in the popliteal lymph nodes following the administration of antigen (BSA) and anti-serum (anti-BSA) into the hind foot pad of rabbits. The eosinophilia was maximal when the antibody was given 30 min after the injection of the antigen. Simultaneous administration of antigen and antibody markedly reduced the eosinophilia while the injection of preformed immune precipitates and soluble complexes prepared in antigen excess produced no eosinophilia in the lymph node. These results supported by our own findings implicate sensitization of the tissue with specific foreign protein followed by antigen–antibody interaction as a prerequisite for eosinophilia.

Torre and Leiken[16] have observed increased numbers of eosinophils in the early hours in skin windows performed on patients with a blood eosinophilia. In our work, blood eosinophil-levels determined in several patients and normals were not raised. Thus, the persistent tissue eosinophilia in our experiments could not have been merely a reflexion of an increased blood-level of eosinophils.

Much work remains to be done on the quantitative aspects of the cellular responses in immediate hypersensitivity. Nevertheless, it is felt that the skin window technique may be applicable to the study of eosinophilia in man, the nature of the chemical mediators of eosinophilotactic activity and the role of eosinophils in hypersensitivities characterized by immediate skin reactions.

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A Phagocytic and Protein-forming Mesenchymal Cell in Human Cirrhosis

ALTHOUGH the derivation of serum protein secreting plasma cells and of macrophages from a common immature mesenchymal cell is accepted, it has become customary to differentiate sharply macrophages from lymphoid cells, particularly plasma cells[1,2]. Electron microscopically, the macrophage is characterized by numerous vacuoles of various sizes or phagosomes containing granular material with differing electron densities[3]. It also contains occasional profiles of endoplasmic reticulum with ribonucleoprotein particles[4]. In contrast, the plasma cell is filled with an elaborate system of lamellæ of endoplasmic reticulum lined by ribosomes[4,5]. This is considered a protein secretory mechanism and immunofluorescence studies have shown this to be γ-globulin[6].

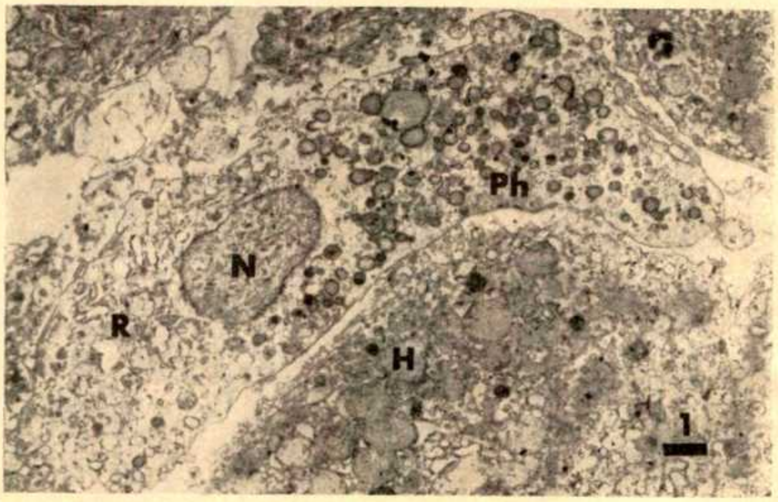

Fig. 1. Electron micrograph of mesenchymal cell neighbouring hepatocyte (*H*) in liver of patient with post-necrotic cirrhosis. Note portion rich in endoplasmic reticulum (*R*) on one side of nucleus (*N*) and portion with many phagosomes (*Ph*) on the other. ('Epoxy' embedded, uranylacetate stained. × *c*. 6,600)

Despite this usually accepted sharp division, in active post-necrotic cirrhosis of the liver with loss of single cells on the lobular periphery or 'piecemeal' necrosis[7], cells are found with phagosomes in one part of their cytoplasm and in the other part abundant profiles of endoplasmic reticulum appearing rough because of the presence of ribosomes. These two portions of the cell are generally separated by the nucleus. Gradations in the amounts of endoplasmic reticulum containing cytoplasm and the phagosome-bearing portion are seen from few lamellæ and many vacuoles to mainly endoplasmic reticulum and a few pigment granules. Amœboid extensions of the cell membrane are found along the surface of both portions. The cells are surrounded by a variety of mesenchymal cells characteristic of this lesion[8]. Both the secretory and phagocytic activities of these cells are supported by immunocytochemical studies of such lesions in which mesenchymal cells are found containing both γ-globulin in one part and spontaneously fluorescent lipofuchsin in the other[9].

The functional significance of these cells remains hypothetical, as is the possibility that the γ-globulin and antibody formation is stimulated by and directed against material incorporated by phagocytosis. This cell differs from that recently described in the rejec-

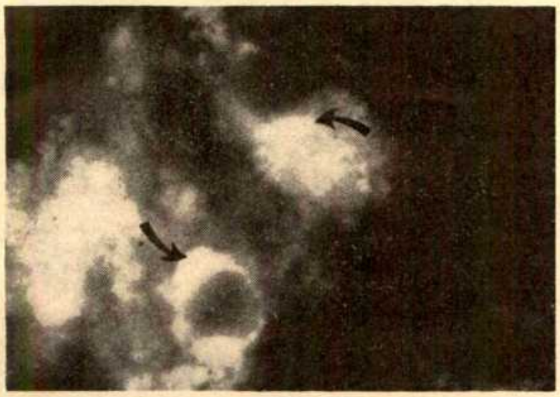

Fig. 2. Fluorescence micrograph of cell with much orange-brown lipofuchsin autofluorescence and green fluorescence (arrows) after treatment with fluoresceinated anti-human γ-globulin. (× *c.* 900)

tion following transplantation[10]. To include the cells described here among the immunologically competent group appears justified and their existence provides added support for immunological processes in the self-perpetuation characteristic of post-necrotic cirrhosis.

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RADIOBIOLOGY

Effects of 'Visible' Light on 5-Bromouracil-labelled DNA

INCORPORATION of 5-bromodeoxyuridine (BUdR) into the DNA of bacteriophages, bacteria and mammalian cells results in highly increased sensitivity not only to short-wave ultra-violet light[1–4] but also, and even more profoundly, to 'visible' light[2,4] or more probably to its long-wave ultra-violet component. An attempt was made to determine the photochemical mechanism of this phenomenon, as a logical extension of our earlier work on the mechanism of short-wave (2537 Å) ultra-violet light effects on BUdR-labelled cells and transforming DNA[5]. It was suspected that photochemical dehalogenation, a reaction already observed with short-wave ultra-violet light[6], accounts for most of the lethal effects of 'visible' light, especially of the 3000–3600 Å component. The experiments were performed either with intact cells or with protein- and RNA-free DNA isolated from genetically transforming strains of *Bacillus subtilis*. In a few experiments, a thymine-requiring strain of *Escherichia coli*, 15 T^-, was also used. The growth conditions resulting in massive incorporation of BUdR were described earlier[7]. 5-Bromouracil labelled with carbon-14 was used to label the cell DNA for the chromatographic experiments. A fluorescent lamp (General Electric, warm-white, 20 W) served as the source of 'visible' illumination. (Prolonged irradiation of frozen thymine solution with this lamp results in formation of small quantities of the thymine dimer, indicating that a minute fraction of its spectral output is a photochemically active ultra-violet component, which otherwise would scarcely be detectable by the usual spectrophotometric procedures.)

The photochemical changes produced by 36 h irradiation of carbon-14 BU-labelled DNA at a 2-cm distance from the fluorescent lamp were followed by (1) determination of the chromatographic behaviour of the carbon-14 label after 14 h (25° C) or 1 h (95° C) of 70 per cent perchloric acid hydrolysis of the non-irradiated and irradiated DNA; and by (2) measuring the effect of illumination on the buoyant density of BUdR-labelled DNA in the equilibrium cæsium sulphate gradient. As can be seen in Fig. 1, ^{14}C-BU in irradiated DNA is converted into material with a chromatographic behaviour indistinguishable from that of uracil. This apparent photochemical debromination reaction was observed only with DNA-incorporated BU; no photochemical changes were observed even after 7 days illumination of aqueous solutions of BU or BUdR. Similarly, the latter two compounds were scarcely affected by ultra-violet irradiation (2537 Å), while 5-iodouracil and BU dinucleotides were perceptibly sensitive to dehalogenation under similar conditions.

Similar experiments were performed using long-wave ultra-violet light (3130 Å and 3660 Å) obtained with a quartz monochromator with essentially

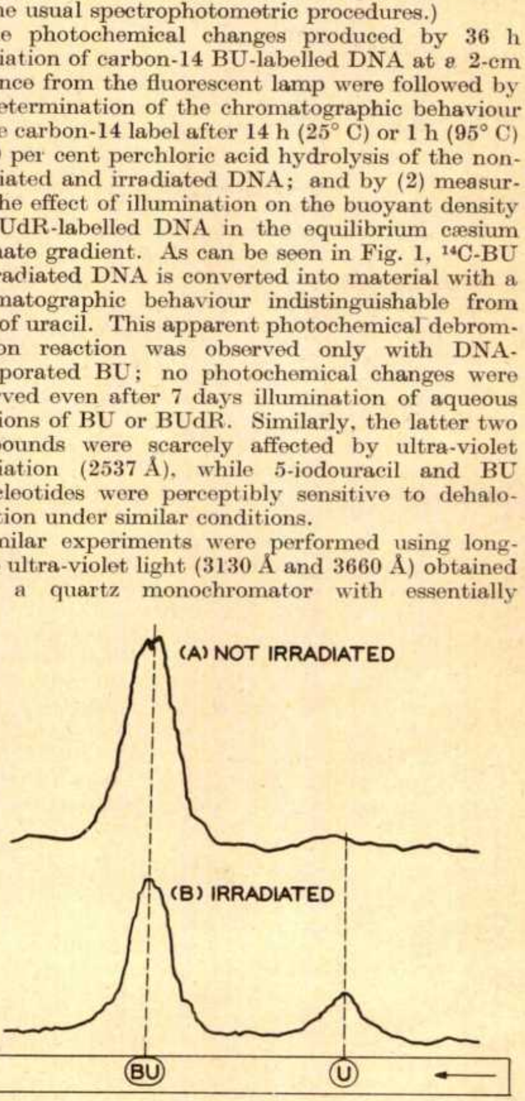

Fig. 1. Results of radioactivity scanning of paper chromatograms of perchloric acid hydrolysates of whole cells (*E. coli* 15 T^-) grown for 14 h in minimal medium in the presence of 1 μg thymine/ml. and 5 μg 5-bromouracil-2-^{14}C/ml. (specific activity 1 mc./mM), centrifuged, washed, suspended in original volume (7·5 ml.) of distilled water ('Pyrex' tubes) and non-irradiated (*A*), or irradiated (*B*) for 36 h with warm-white 20-W fluorescent light (20° C, 2-cm distance). For the ascending chromatography an *n*-butanol–water (86 : 14) mixture was used. BU and U indicate the position of 5-bromouracil and uracil in the control chromatogram

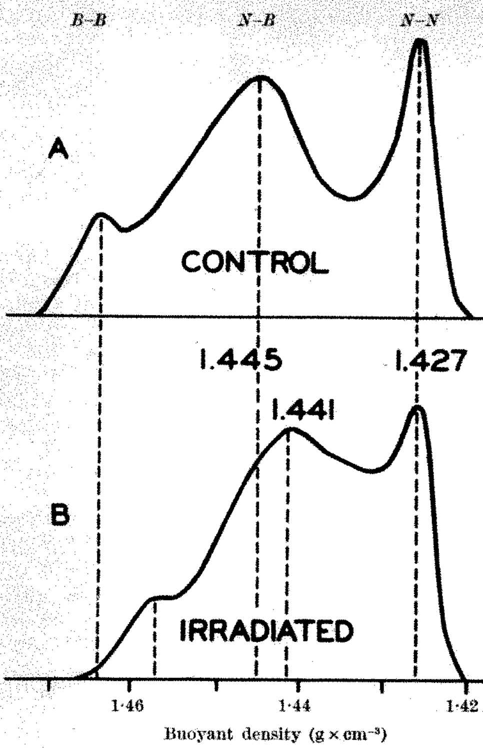

Fig. 2. Microdensitometer tracings of the cæsium sulphate-equilibrium density gradient photographs (20 h, 44·770 r.p.m.) of a mixture of unifilarly (*N–B*) and bifilarly (*B–B*) BUdR-labelled DNA extracted from *B. subtilis*, deproteinized and freed of RNA, non-irradiated (*A*), or irradiated (*B*) for 60 h under the conditions described in the legend for Fig. 1. Normal (*N–N*) *B. subtilis* DNA served as a density marker

identical results, indicating that the effects of 'visible' light are most probably attributable to its ultra-violet components, especially since monochromatic light above the wave-length of 4000 Å is essentially inactive. The question arises as to whether dehalogenation occurs during irradiation of BU-labelled DNA, or rather some latent reaction occurs, with the actual debromination taking place during acid hydrolysis of this 'activated' bromine-containing DNA. To answer this question, buoyant density measurements were performed on the halogenated DNA before and after irradiation. Since it is known that bromination increases strongly the buoyant density of DNA[3], debromination should cause a decrease in the density. Fig. 2*A* represents the sedimentation pattern of normal (1·4266 g × cm⁻³), unifilarly (1·4454) and bifilarly (1·4642) BUdR-labelled DNA isolated from *B. subtilis*. It can be seen (Fig. 2*B*) that after 60 h of illumination the peaks representing BUdR-labelled DNA moved toward normal DNA, indicating an approximately 20 per cent loss of bromine. An approximately 100-fold decrease in transforming activity was observed for halogenated DNA, the change in density of which is represented in Fig. 2*B*, while the same 60-h illumination resulted in only five-fold reduction in the transforming activity of non-labelled DNA. Several conclusions seem to be justified on the basis of these observations. (1) The replacement of thymidine, probably the most ultra-violet-sensitive (thymine dimerization[8]) DNA component, does not result in increased ultra-violet resistance of the DNA. Conversely, the ultra-violet and 'visible' light sensitivities increase, since debromination of BU (and perhaps some correlated reactions[9]) seems to be a still more efficient photochemical process. Obviously, such DNA inactivated by dehalogenation will not be susceptible to reactivation phenomena[2,4], which seem to depend on the splitting of the thymine dimer[6]. (2) Since debromination of BU most probably leads to a uracil-for-thymine substitution in DNA, this reaction must be lethal in view of the lethal effects of illumination on BU-labelled DNA and cells. BU dimerization and secondary reactions, including water addition or uracil dimer formation, were not observed with model compounds, and seem to be highly unlikely during irradiation with the long-wave ultra-violet component of 'visible' light, since this is not absorbed either by labelled DNA[10] or by BU and uridine.

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Photoreactivation after Division

IN a recent review of the literature of photoreactivation, Jagger[1] indicates that photoreactivation of the lethal and mutational effects of ultra-violet radiation occurs only up to the time of the first post-irradiation division. Brandt[2] reports a photoreversal of the division-delaying effect of ultra-violet radiation in two protozoa, *Didinium nasutum* and *Paramecium caudatum*. This photoreversal occurs after the first post-irradiation division, but not after the second.

It is not uncommon for ciliate protozoa, given a lethal dose of ultra-violet light, to divide twice before death. In a strain of *Tetrahymena pyriformis* grown in this laboratory, division before death is observed following ultra-violet irradiation in doses ranging from 900 ergs/mm² (LD_{50}) to 4,000 ergs/mm².

This strain of *T. pyriformis* was tested for photoreactivation after division in the following manner. A dose of 2,000 ergs/mm² of 2537 Å ultra-violet light was administered, reducing survival to a low level (1 per cent). Ninety-eight animals were isolated singly in depression slides, and were grown in the dark for two days. During this time, some of the isolated animals divided twice, others divided only once and a few failed to divide. Half the progeny (one animal) from those animals that had divided only once and two animals from those lines that had divided twice were placed in individual test-tubes. These animals were then photoreactivated at room temperature with two separate doses of photoreactivating light of high intensity, 2·5 million ergs/mm² total (3600–4000 Å). The other half of the progeny were held in the dark and observed until recovery or death occurred.

Each photoreactivated animal was isolated in a depression slide and observed periodically until it had either disintegrated or resumed division and produced a mass colony of several hundred animals. Daughters of ultra-violet-irradiated animals could be compared, and those receiving the same treatment were counted as one animal. If either or both sisters recovered, they were scored as one survivor; if both died, then they were scored as one non-survivor.

For comparison, a group of non-irradiated animals and a group of animals irradiated with the same ultra-violet dose and receiving immediate but lower intensity of photoreactivation are included in Table 1.

Table 1

Dose		No. of divisions at time of photoreactivation	Lines isolated	Surviving lines	Survival-rate (%)
Ultra-violet light (ergs/mm²)	Photoreactivating light (ergs/mm²)				
0	0	—	14	14	100
2,000	1 million	0	14	14	100
2,000	0	—	98	1	1
2,000	2·5 million	1	48	11	23
2,000	2·5 million	2	37	19	51

The apparently greater ability to reactivate animals after two divisions than after one division is probably caused by a greater delay of division of the more damaged animals (those that had accomplished only one division at 2 days, the time of photoreactivation) and thus would not be significant. The efficiency of photoreactivation falls considerably after the animals divide; however, one can conclude that the type of ultra-violet damage which causes these animals to die persists in a state which can be photoreactivated even after an irradiated individual has divided once or twice.

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BIOLOGY

Gibberellin-like Substances in Plants

THE universal, or even widespread, occurrence of gibberellin-like substances in the plant kingdom has yet to be demonstrated. Such substances are known to occur in numerous angiosperms[1], the brown alga *Fucus vesiculosus*[2], and certain fungi[1]. Previous attempts to demonstrate the presence of gibberellin-like substances in gymnosperms[3,4] and ferns[4] have been unsuccessful. This communication reports the first evidence for the presence of gibberellin-like substances in the latter two groups.

The plants investigated were a gymnosperm, *Juniperus chinensis* L.; a fern, *Alsophila cooperi* F. Muell.; a liverwort, *Marchantia polymorpha* L.; a green alga, *Chlamydomonas reinhardi* Dang.; a brown alga, *Macrocystis pyrifera* (L.) C. Agardh.; and a dinoflagellate, *Gonyaulax polyedra* Stein. In each case, 50–1,000 g (fresh weight) of cells or tissue were collected and extracted with 3 batches of acetone/water (80 : 20 v/v) for a total of 72 h at 1° C. The extracts were combined, and the acetone and most of the water removed in a flash evaporator. This concentrate was adjusted to pH 8 and extracted with ethyl acetate. The aqueous phase was readjusted to pH 3 and extracted with 3 equal volumes of ethyl acetate. The combined ethyl acetate extracts obtained at pH 3 were washed with water, dried over sodium sulphate, and evaporated to dryness. Aliquots of this residue were dissolved in acetone/water (30 : 70 v/v) and applied to seedlings of dwarf mutants of *Zea mays* L. according to methods described by Phinney and West[1]. All experiments, including collecting, extraction, purification, and assay, were repeated at least three times with similar results.

The results of a number of experiments are summarized in Table 1. Extracts of 50 g (fresh weight) of berries of *Juniperus chinensis* proved active in promoting the growth of the *dwarf*-3 and *dwarf*-5 mutants of *Zea mays* (that is, the growth response in the bioassay exceeded that of untreated controls by 25 per cent or more). Extracts of 50–150 g (fresh weight) of the same material were inactive in promoting the growth of the *dwarf*-1 mutant. This pattern of mutant specificity is also characteristic of gibberellins A_5 and A_9 (refs. 5 and 6). Extracts active on the *dwarf*-1, *dwarf*-3, and *dwarf*-5 maize mutants were obtained from 50 g (fresh weight) of immature fronds of the tree fern *Alsophila cooperi*. This represents the first evidence for the occurrence of gibberellin-like substances in gymnosperms and ferns.

Table 1. GIBBERELLIN-LIKE ACTIVITY IN PLANT EXTRACTS

Source	Bioassay response (as percentage of control)		
	Dwarf-1	*Dwarf*-3	*Dwarf*-5
Gymnospermae			
Juniperus chinensis	92 (−)	128 (+)	148 (+)
Filicinae			
Alsophila cooperi	145 (+)	170 (+)	146 (+)
Bryophyta (Hepaticae)			
Marchantia polymorpha	...	...	90 (−)
Chlorophyta			
Chlamydomonas reinhardi	...	...	102 (−)
Phaeophyta			
Macrocystis pyrifera	...	...	105 (−)
Pyrrophyta			
Gonyaulax polyedra	...	...	98 (−)

Results based on eight days of growth following treatment. Each figure represents thirty controls and six treated plants.

No evidence of gibberellin-like substances was obtained from numerous extracts of the following materials: *Chlamydomonas reinhardi*, *Gonyaulax polyedra*, growing regions of *Macrocystis pyrifera*, or thalli and antheridiophores of *Marchantia polymorpha*. Aliquots of each of these inactive preparations were further purified by paper chromatography using *n*-butanol saturated with 1·5 N ammonium hydroxide as solvent[7]. The resulting chromatograms (57 cm wide × 35 cm long) were cut into ten equal strips (corresponding to R_F values) and eluted with acetone/water (30 : 70 v/v). The eluates were applied to dwarf maize seedlings. Even after this further purification, no activity was observed.

In the light of these results and others cited here, it may be concluded that gibberellin-like substances are of widespread occurrence, being reported in angiosperms, gymnosperms, ferns, brown algae, and certain fungi. Failure to find these substances in other plant groups may result from their absence from these groups or, quite possibly, from shortcomings in the extraction, purification, or bioassay techniques thus far used.

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Action of some Acenaphthene Derivatives on Mitosis in *Pisum* Roots

IN a previous communication[1] I reported the c-mitotic effect of 3-(5′-acenaphthyl)-propanol and 4-(5′-acenaphthyl)-butanol on onion root-tips. This work formed part of a programme of investigations of the structure-activity relationships in the acenaphthene series. The work of Wilson and co-workers[2–6] on the use of *Pisum* as test material for chemically induced abnormal mitosis has led me to attempt its use for evaluation of the acenaphthene derivatives.

In my method a group of seedlings having equal root development of about 5 cm was selected; these were placed in the appropriate solutions and their root-growth and root-tip squashes examined at intervals.

I first used saturated aqueous solutions of the various derivatives listed in Table 1. In most cases (Fig. 1) these severely inhibited root growth, noteworthy exceptions being the 3- and 5-acenaphthyl carbinols. Possibly due to this growth-inhibition, the tests with colchicine and the carbinols were the only ones in which root-tip squashes (aceto-carmine) showed the presence of dividing cells. All mitoses in the carbinol-treated seedlings were apparently normal, whereas typical 'clumping' and root-tip swellings were observed with colchicine.

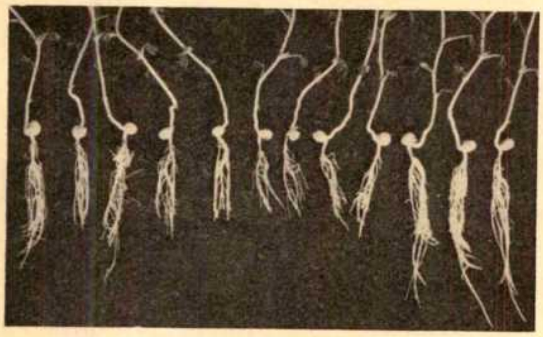

Fig. 1. Inhibition of root growth of *Pisum*, after five days in saturated solutions of (left to right) 3-acenaphthyl carbinol, 1-(5′-acenaphthyl)propane-1:3 diol, 5-acenaphthyl carbinol, 3-(5′acenaphthyl)allyl alcohol, colchicine, acenaphthene, 3-(3′acenaphthyl)propanol, 3-(5′acenaphthyl)propanol, 3-(3′acenaphthyl)allyl alcohol. The last three specimens are controls

Table 1

Substance tested	Root growth inhibition (saturated aqueous solution)	Mitotic abnormalities (half-saturated solution)
3-Acenaphthyl carbinol	−	−
5-Acenaphthyl carbinol	−	−
3-(3′-Acenaphthyl)propanol	+ +	+
3-(5′-Acenaphthyl)propanol	+ +	+ +
3-(3′-Acenaphthyl)allyl alcohol (trans)	+	−
3-(5′-Acenaphthyl)allyl alcohol (trans)	+	−
1-(5′-Acenaphthyl)propane-1,3 diol	+	−
Acenaphthene	+ +	(+)

The duration of the tests was 3–4 days.
The (+) in the second column indicates the appearance of abnormalities only after six days treatment.

Using half-saturated solutions, growth in all cases took place at a rate which permitted at least a limited examination of dividing cells, and the results given in Table 1 were obtained.

Only with the acenaphthylpropanols and acenaphthene are abnormalities observed. In Fig. 2 are some of the relevant chromosome configurations. The remaining substances, while growth-inhibiting, are apparently without marked effect on the pattern of mitosis. In my previous work, I suggested that when a hydrophilic side-chain is attached to the acenaphthene nucleus, activity is retained only when the chain is long (more than 3 carbon atoms) and 'flexible'. That

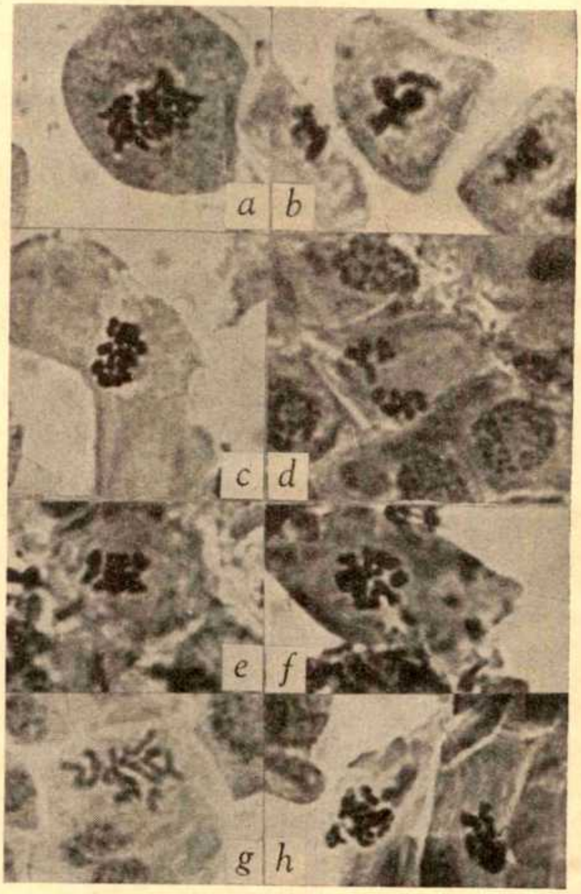

Fig. 2. Chromosome configurations in treated *Pisum* root tips. *a* and *b*, Acenaphthene (6 days); *c*, 5-acenaphthylpropanol (2 days); *d*, 5-acenaphthylpropanol (3 days); *e*, *f* and *h*, 3-acenaphthylpropanol (4 days); *g*, acenaphthene (normal metaphase, 4 days). All photographed at the same magnification

suggestion is confirmed here, for the activity of acenaphthylpropanol is destroyed either by the introduction of a second hydroxyl group adjacent to the ring, as in acenaphthyl propane-diol (effective chain shortening), or by unsaturation, as in the acenaphthyl allyl alcohols (restriction of rotation about a C—C bond).

Nevertheless, it appears that *Pisum* is of less value than *Allium* in evaluating acenaphthene derivatives. Schmuck and Guseva[7] found that peas were unaffected by acenaphthene derivatives; although I have not confirmed this precisely, acenaphthene certainly affects *Pisum* roots only slowly. Abnormalities may be observed in *Allium* roots treated with acenaphthene within 24 h, but *Pisum* requires at least six days. It appears that the *Pisum* root is relatively impermeable to acenaphthene. By gas-chromatographic analysis of root-extracts[8], I find that roots of *Pisum* and *Allium* grown in saturated aqueous acenaphthene absorb 10 and 750 μg/g root, respectively, in 11 days. 0·1 μl. of root extract in 'Decalin' was placed on a 4 ft. 10 per cent 'Apiezon *L*' column ('Celite' 80–120 mesh) at 200° C, gas flow-rate 75 c.c./min (Pye argon chromatograph). The acenaphthene retention time was 7½ min. I conclude that *Pisum* is not a sufficiently sensitive test material in the acenaphthene series. The higher acenaphthene concentration in *Allium* roots is probably due to their oil content.

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Osmoregulation in Chimæroid Fishes

The ability of elasmobranchs to keep the salt concentration of the body fluids lower than that of the environment is probably in part due to the activity of the rectal salt gland. In the spiny dogfish this gland secretes sodium chloride at about twice the concentration of the plasma[1]. In most dogfishes and rays the rectal gland is a single appendix-like organ, but in *Hexanchus* and *Heptanchus* it is built up of separate lobes. A still more primitive condition exists in *Chimaera* with several small clusters of grape-like glands in the wall of the hindmost part of the intestine[2,3]. The glands consist of branched secretory tubules surrounded by blood capillaries. The microscopic structure is simple and little specialized[4], and supposedly these glands make a less-efficient salt excretory system than the type of rectal gland met with in the dogfish.

A chimæroid fish, *Hydrolagus colliei*, is stated to have a blood, which is slightly hypo-osmotic to sea-water (freezing point depression = 1·49), but this needs to be re-investigated[5]. We have analysed blood plasma of *Chimaera monstrosa* for sodium, potassium, chloride and urea. The fishes were caught from depths of 250–700 m. The blood samples were from animals kept alive for a few hours in running sea-water at 10° C. In Table 1 our results are compared with values from the literature regarding the blood of a hagfish (*Myxine*) and a dogfish (*Squalus*).

Table 1. Concentrations in m.mol./l.

Animal	Na^+	K^+	Cl^-	Urea	Ref.
Squalus acanthias (spiny dogfish)	254–320	4·4–7·0	239–252	347–356	(1)
Chimaera monstrosa (ratfish)	341–384	10·2	364–396	244–287	Present investigation
Myxine glutinosa (hagfish)	558	9·6	576	2–4	(6)

As seen in Table 1 the blood of *Chimaera* probably is iso-osmotic with sea-water, most of the osmolarity being caused by sodium chloride. With regard to osmoregulation, *Chimaera* takes a position between the hagfish and the dogfish, showing more resemblance to the latter. However, its blood plasma has more sodium and chloride and less urea than that of the dogfish. The high percentage of sodium chloride may be the consequence of a weakly developed salt excretory system. The varying development of extrarenal salt glands in different groups of elasmobranchs might have implications on questions concerning the phylogeny of elasmobranch fishes, especially as regards the possibility of fresh water ancestry.

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New Records for Distribution of the Frilled Shark

The frilled shark, *Chlamydoselachus anguineus*, Garman, 1884 (Chlamydoselachidae–Selachii), is a living representative of a primitive order of sharks, fossil remains of which are known from the Pliocene of Europe and the Miocene of the Caribbean[1]. Much of the interest of this shark lies in its being a living representative of an otherwise fossil order; but it has in addition an interesting distribution. The purpose of this communication is to place on record some new occurrences of this species in the north-eastern Atlantic.

The first known specimens originated in deep water off Japan about 1880[2,3], and later workers on the anatomy and embryology of *Chlamydoselachus* obtained all their material from this area[4,5]. In March 1889, however, an immature female (610 mm total length) was caught off Madeira by local fishermen[6]. This first record for the Atlantic was soon followed by a second when a female (1,910 mm) was caught in a net set for saithe (*Pollachius virens* (L.)) at 100–150 fathoms at Bugonaes, in Varanger Fiord, Norway, on August 4, 1896[7].

Subsequent records have been mainly between the latitudes 32° and 45° North, in deep water off the Iberian and North African coasts. Listed in chronological order they are as follows: (3) 1900, July 22, a male of 920 mm off Cezimbra[8]; (4) 1904, off Cezimbra in 820 m (no details of sex or length)[9], and possibly another, a male of 1,240 mm from 850 m now in the

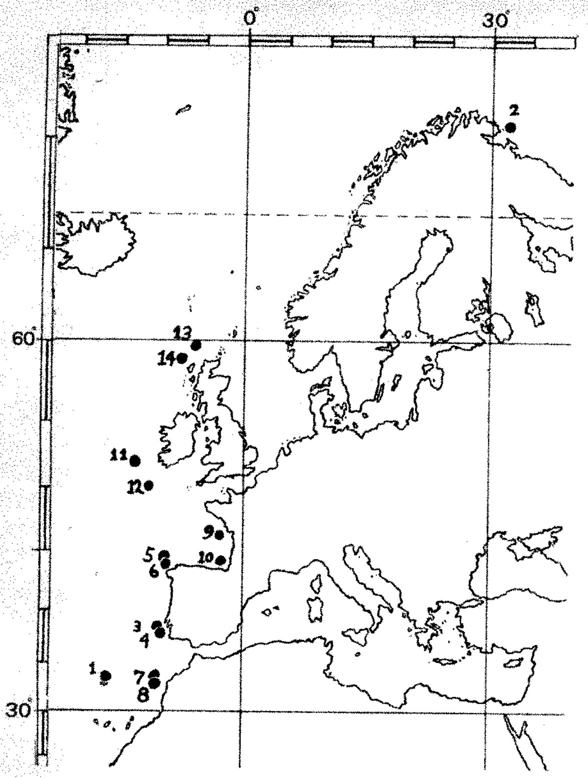

Fig. 1. Records of the frilled shark in the north Atlantic. The figures refer to numbered records in the text

collection of the British Museum (Natural History); (5) 1906, off Corunna, sex unknown, 1,440 mm[10]; (6) 1909, July 28–August 2, off Corunna, a male of 1,240 mm[11]; (7) probably in 1909, off Morocco, a female of 1,600 mm[12]; (8) probably in 1913, off Morocco, sex unknown, 1,300 mm[13]; (9) 1913, Gulf of Gascogne, off La Rochelle, sex unknown, 1,490 mm[9]; (10) 1925, December 15, 13 miles off San Sebastian, at 160 fathoms, a female of 1,860 mm[10].

In addition to these occurrences in the Atlantic there are four records from British waters, two of them previously unpublished. In 1935 (possibly in January) a single frilled shark was taken off the west coast of Ireland (53° N., 13° W.). This specimen, which was briefly recorded by Jenkins[14], is now in the collection of the British Museum (Natural History); it is a female, 1,350 mm in length. Another specimen, a male of 1,448 mm, was caught in the same area (50° 10′ N., 11° 10′ W.) in early April 1936, in 350 fathoms; it is now in the collection of the National Museum of Wales. Two recent specimens, hitherto unrecorded, have been sent to the British Museum (Natural History) by Mr. D. R. McCulloch, of Fleetwood. The first, caught in 300 fathoms at 59° 50′ N., 6° 30′ W., in May 1961, is a male, 1,520 mm in length; the second, caught in early July 1962, by the F. V. *Boston Neptune* in 280 fathoms, 40–45 miles off the Butt of Lewis, is a female of 1,825 mm.

The viscera of both these recent fish have been examined. The gut of the male was empty above the spiral valve. The stomach of the female contained a quantity of semi-fluid matter, some pieces of flesh, two lenses and a paired cartilaginous structure. This latter has been doubtfully identified as of selachian origin but further identification has not been possible. The testes of the male are comparatively small, and do not suggest sexual activity. The gonads of the female were likewise apparently inactive, the ovarian eggs being uniformly developed and approximately 10 mm in diameter. A similar condition of sexual activity can be assumed for Collett's Norwegian specimen, which he described as having "ten large eggs, about the size of the yolk of a small hen's egg", as well as a number of smaller eggs in the "uterus", which as has been argued by Gudger[4] was written in error for "ovary". Fertilized uterine ova in this species are relatively immense; one egg from Japanese material figured by Nishikawa[15] measured 100 × 65 mm, and from this it seems safe to assume that in the Norwegian specimen the ova were far from maturity. In so far as it is safe to generalize from these three examples, it seems that, in British and northern waters, *Chlamydoselachus* is sexually inactive. In this the frilled shark invites comparison with the blue sharks (*Prionace glauca* (L.)) found in British waters in summer and autumn, which all seem to be either immature or sexually inactive, probably in many cases a post-parturition inactivity.

I thank Mr. D. R. McCulloch, chief port health officer, Fleetwood, for sending both the recent (1961 and 1962) specimens to the Museum; and also Mr. K. Beavers, skipper of the *Boston Neptune*, for recognizing this second specimen as a rarity and preserving it whole for study. I also thank Dr. Colin Matheson, of the National Museum of Wales, for information on the specimen in his charge.

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Specific Gravity of the Cases of the Larvæ of *Limnephilus* and *Triaenodes* (Trichoptera)

The larvæ of many caddis flies build cases which they carry about with them wherever they go. In some recent work on two species of these insects, it was necessary to measure the specific gravity of their cases. The irregular case of *Limnephilus* was determined by displacement of water in a tube a little larger in diameter than the case. The tube was filled to a datum line with water; some of the water was removed from the tube by a micropipette and the case placed in the remaining water in the tube. The micropipette was now allowed to empty until the tube was refilled to its datum line. Any water still in the pipette equalled the volume of the case. Readings were to the nearest 0·01 ml.

The cases of *Triaenodes* were so small that this method was too inaccurate. For these cases a narrow tube just wide enough to admit a case was closed at one end and filled with distilled water until no more could be added. The meniscus of water on the open end of the tube was removed by a piece of filter paper placed flat across the top of the tube until it would absorb no more water. A case was now inserted and the displaced water absorbed by a piece of filter paper of known weight. The paper plus the absorbed water was now weighed and thus the weight of water displaced was found. With a little practice this method gave consistent results.

The main precautions were: (1) the filter paper should be handled with forceps, not fingers; (2) the case must not touch the filter paper when sinking into the tube otherwise capillarity upsets the results; (3) the filter paper should be kept in a closed container between taking up the water and being weighed.

Previous drying of the filter paper is undesirable because it would then take up moisture from the ambient air during use and weighing. It is assumed that the paper is at equilibrium with the ambient humidity throughout the experiment, and that this does not alter significantly, assumptions which are fully justified over the short time the experiments last.

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A Giant Form of *Chlorella pyrenoidosa*

Hunter, J.[1], and Hunter, J., and Burk, D.[2], have shown that the intraperitoneal injection of the thermophilic alga *Chlorella pyrenoidosa* into the mouse results in hypoglycæmic action.

On this basis a series of similar experiments were carried out on this problem. When *Chlorella pyrenoidosa*, in the proportion of 25–100 mg dry weight of alga per 100 g of rat, is intraperitoneally injected to 4-months-old diabetic white Wistar rats, the following phenomenon was observed: from 48 h after injection until about two months, the diabetic rats show green nodules of variable size all around the abdominal cavity, the nodules being encapsulated into the connective and adipose tissues.

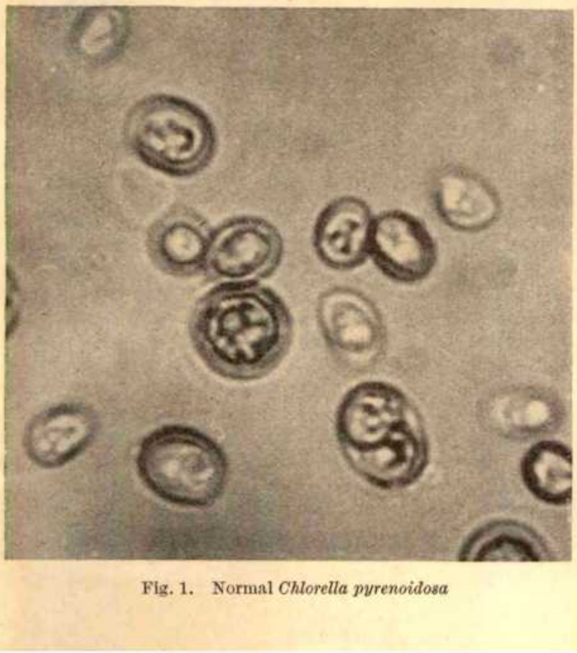

Fig. 1. Normal *Chlorella pyrenoidosa*

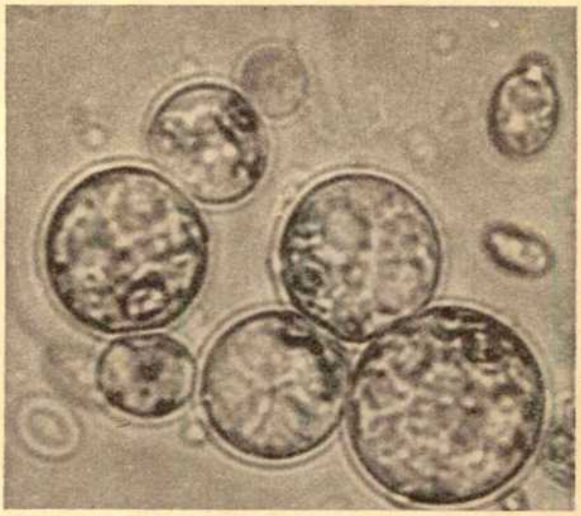

Fig. 2. Giant *C. pyrenoidosa* from a nodule in the abdominal cavity of the rat

Most of the cells in the nodules are of giant size (15μ diameter) in comparison with the normal *Chlorella pyrenoidosa* forms (8μ diameter). The chloroplasts that fill the cell show a strong green colour in spite of the long time spent inside the animals. Sporulation was not observed under the light microscope.

Investigations of the cytological and physiological properties of the isolated giant forms are in progress and results will be described elsewhere.

We thank Dr. D. H. Northecote and Dr. J. R. Villanueva for advice.

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MICROBIOLOGY

An Increase in Sensitivity for the Assay of Penicillin in Milk

THE paper disk assay method is frequently used for the detection of penicillin in milk. An overnight test with the cylinder-plate method using *Sarcina lutea* as the test organism can detect the presence of 0·005 I.U. of penicillin/ml. of milk[1]. Another method using *Bacillus subtilis* and a thin layer (6 ml.) of seeded agar can detect 0·05 I.U./ml. within 2½–3 h (ref. 2).

Various attempts have been made to increase the sensitivity of the disk assay method. Cerny and Morris[3] modified the disk-plate assay method by using two 0·5 in. diameter filter paper disks (No. 740 *ES* and *S*) one on the top of the other for absorbing the test sample. They also used a thin layer of agar to enlarge the area of the inhibition zone. By this means, 0·01 I.U./ml. of penicillin can be detected after 8 h incubation. Kosikowski and Mocquot[4] used dried milk tablets made from the test milk sample instead of filter paper disks. In this modification 2-ml. portions of the test milk sample were dried overnight in a vacuum freeze-dryer and the dried milk was compressed with a glass rod in a strong glass tube and forced out on to the surface of the seeded agar. By this method, it was possible to detect 0·01 I.U. of penicillin/ml. of milk.

The following method, which involves separation of the whey by rennet coagulation and concentration by a rotary film evaporator, has also increased the sensitivity of tests for antibiotics in milk.

A sample of 100 ml. of rehydrated skim milk powder (Difco) containing a known concentration of penicillin *G* sodium (Nutritional Biochemical Corporation, Cleveland, Ohio) was heated at 82·2° C (180° F) for 5 min and cooled to room temperature. One ml. of cheese rennet was added, and the test sample put into a water bath at 40° C for about 10 min. The whey was separated by filtration. Ten ml. of whey was pipetted into a rotary film evaporator (Rinco Instrument Co., Greenville, Illinois) connected with a vacuum condenser. The whey was condensed at 55° C (131° F). A 10:1 reduction in volume can be obtained in 7–10 min depending on the level of vacuum.

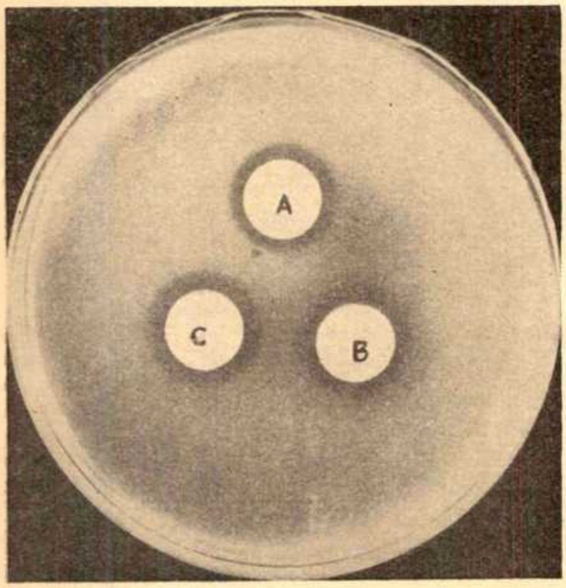

Fig. 1. A comparison of standard plate assay. *A* (0·02 I.U. penicillin/ml. added to 1 : 9 reconstituted skim milk powder) with the modified test procedure; *B* (20 : 1 whey concentrate from 1 : 9 reconstituted skim milk powder to which 0·0015 I.U./ml. penicillin had been added) and *C* (10 : 1 whey concentration from 1 : 9 reconstituted skim milk powder containing 0·003 I.U./ml. of penicillin)

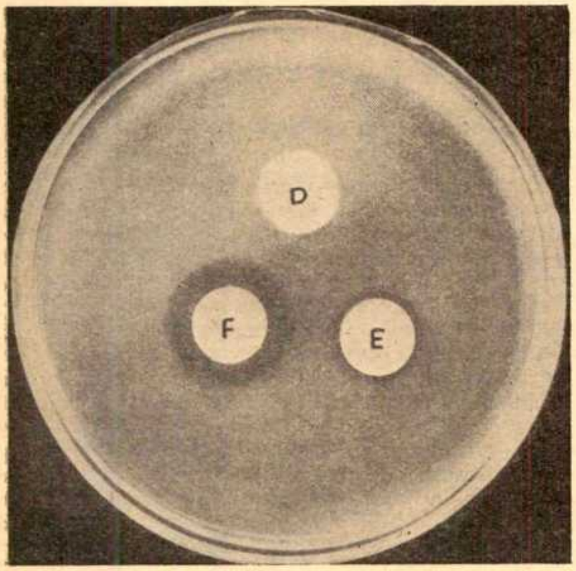

Fig. 2. Tests for penicillin in a known brand of whole milk powder. *D* (1 : 3 reconstitution); *E* (whey separated from *D*); *F* (10 : 1 concentration of *E*) (powder containing approximately 0·0015 I.U./g penicillin)

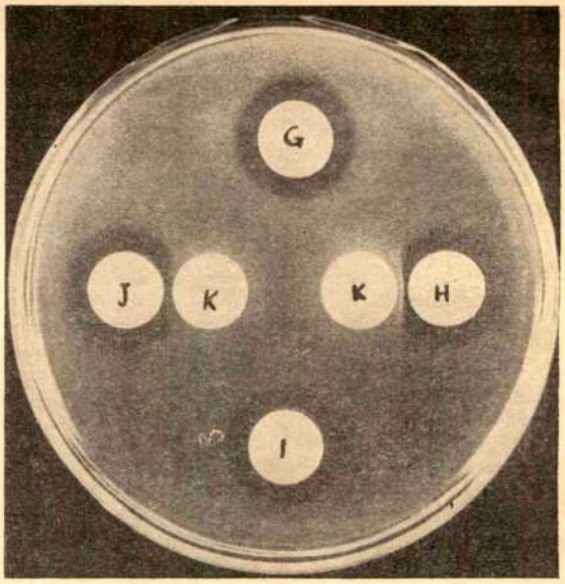

Fig. 3. Identification of penicillin with standard and modified tests. *G* and *H* (1 : 9 reconstituted skim milk powder to which 0·05 I.U. penicillin/ml. had been added, showing effect of penicillinase disk *K*); *I* and *J* (10 : 1 whey concentrate from 1 : 3 reconstitution of known brand of whole milk powder containing approximately 0·0015 I.U./g penicillin showing similar action of penicillinase disk *K*)

In testing milk powder, 30 g milk powder are added to 90 ml. of distilled water. The preparation of the sample is the same as for liquid milk except that the test sample is not heated.

The test organism used was *Bacillus subtilis* A.T.C.C. 6633. One ml. of the *Bacillus subtilis* spore suspension (Difco) was added to 100 ml. of sterile melted whey agar at 55° C. Plates were prepared by pouring 6 ml. of seeded agar into each 15 × 90 mm Petri dishes. Two 13 mm diameter filter paper disks (Difco) were saturated with the concentrated whey and put on the surface of the inoculated agar and pressed lightly with sterile forceps. Another two disks were saturated with Difco penicillinase solution and placed 2 mm from the test sample disks to determine if the inhibitory substance in the test sample was penicillin. The plates were inverted and incubated at 37° C for $2\frac{1}{2}$–3 h. After incubation, the plates were examined for complete or partial zones of inhibition, the latter identifying the inhibitory substance as penicillin.

The maximum sensitivity of the thin layer disk assay method is 0·02 I.U./ml. With the improved method 0·003 units/ml. of penicillin in milk can be detected either in milk heated at 82·2° C for 5 min or in unheated samples (Fig. 1).

Fig. 2 shows the zones of inhibition with 1 : 3 re-constitution of a known brand of milk powder, indicating that the concentrated whey showed a bigger zone of inhibition.

The inhibitory substance contained in the whole milk powder was identified with penicillinase solution as penicillin (Fig. 3).

The technique described in this communication is at least ten times more sensitive than the standard assay method, though 30–40 min extra is required in preparing the test sample. Greater sensitivity could be obtained by concentrating 20 ml. of whey to 1 ml. In this case, an additional 7–10 min for evaporation would be needed, but this would detect penicillin in a concentration as low as 0·0015 I.U./ml.

The effect of heat on the potency of penicillin plays an important part. Some investigators consider penicillin to be relatively heat-resistant. Foster and Woodruff[5] showed that aqueous penicillin heated at 140° F for 30 min resulted in a 5–30 per cent inactivation, while Storrs and Hiett[6] suggested that there was no effect on the potency of penicillin in milk which had been heated to 145° F for 10 min or 190·4° F for 30 min. We found that heating milk containing 0·05 I.U. of penicillin/ml. at 55° C for 20 min caused 3·0 per cent inactivation of the antibiotic.

A further refinement is needed in the degree of concentration of whey in the evaporator and the volume of concentrate absorbed by the filter paper disks before the technique can be regarded as an accurate assay method for the presence of penicillin in milk.

We thank the Department of Industry and Development, Government of Alberta, for taking the photographs.

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Production of Protoplast-like Structures from Various Species of Fungi

IT has been shown that the juice of the gut of the snail *Helix pomatia* contains enzymes which attack the cell-walls of moulds, and has been used to prepare protoplast-like structures from strains of *Neurospora crassa*[1,2]. These structures have been described as relatively stable, being able to grow and regenerate under appropriate conditions. However, it was found that some of these 'protoplasts' could be obtained only from particular strains, making them of somewhat limited value. Meinecke[3] obtained structures resembling free protoplasts from *Penicillium glaucum* as a result of cultivation on thickened cell-free guinea pig serum. This communication describes an enzymatic method using the "suc digestif d'*Helix pomatia*" obtained from L'industrie Biologique Française[2], which has produced a high yield of protoplast-like structures from a number of species of fungi. However, during most of this work we used *Fusarium culmorum* and *Aspergillus nidulans*, which appear to be the most sensitive to snail enzyme among the organisms tested, giving rise to the formation of protoplasts in about 2-h incubation. These preparations resemble in many respects the protoplasts formed by digestion of the cell-wall of bacteria with lysozyme[4] and yeasts, either with snail enzyme[5] or *Streptomyces* enzyme[6–8]. Observations were made using a phase-contrast microscope on living material either on wet-mount preparations or in moist chambers.

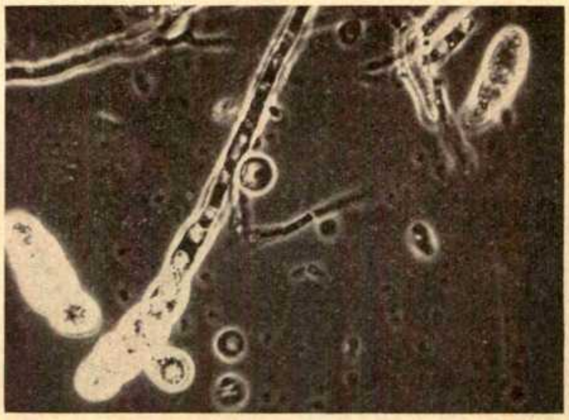

Fig. 1. 'Protoplasts' of varying sizes from *Fusarium culmorum*

Protoplast preparations were obtained using young hyphæ as starting material. Young cultures of the fungi species were obtained by inoculating a small volume (1 ml.) of a heavy suspension of conidia, collected from a slant culture, into 100 ml. Czapek medium and shaking the culture vigorously at 28° C. The cells were collected after about 18–20 h when a young culture was wanted. After washing in distilled water three times, the mycelium was suspended at low density in 0·005 M McIlvaine's sodium citrate–phosphate buffer at *p*H 5·8 containing 0·8 M mannitol. One-tenth volume of 1 per cent cysteine hydrochloride solution was added followed by 1 mg/ml. of the freeze-dried snail enzyme. After about 2 h at 28° the hyphæ of the most-susceptible species began to be converted into protoplast-like structures, emerging first through pores in the hyphal walls, which are slightly changed. Very often it was observed that all the contents of a hypha between septa was extruded as a single, large 'protoplast' between 15 and 25μ in diameter containing several nuclei. Their appearance was granular with a density equivalent to the contents of the original hyphæ.

The susceptibility of different species of fungi to cell-wall digestion by the snail enzymes is variable. While some species will yield protoplasts readily, others, under the same conditions, produced no protoplasts even after prolonged digestion. Protoplasts have been obtained by this method from the following moulds: *Penicillium italicum*, *Aspergillus nidulans*, *Fusarium culmorum*, *Alternaria citri*, *Helminthosporium gramineum*, *Verticillium albo-atrum*, *Trichotecium roseum*, *Mucor sphaerosporum*. With susceptible species of fungi it is generally found that it is easier to observe formation of protoplasts from young cultures (20 h old). In old cultures protoplasts were observed in much lower proportion and the length of time taken to emerge was considerably extended.

The free 'protoplasts' are sensitive to osmotic shock, and lyse immediately when placed in distilled water. The osmotic structures retain their morphological integrity for several hours when stored in a cold room in the incubation mixture in which they are formed.

In a further experiment attempts were made to purify the preparation of *Fusarium* 'protoplasts' obtaining osmotic bodies essentially free from whole hyphæ or debris, but recovery was generally low because of settling of heavier protoplasts.

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CYTOLOGY

New Evidence on the Cytotaxonomy of *Vaccinium* Species as revealed by Stomatal Measurements from Herbarium Specimens

In an earlier paper[1], we described two groups of lowbush blueberry plants, one diploid and the other tetraploid, the taxonomic status of which was in confusion. Both groups resembled *Vaccinium angustifolium* Ait., yet were different enough from each other to warrant separation as species. Four characters were considered to be of value in separating the two species : chromosome number, corolla-length, degree of branching, and pollen grain size. On inquiry, we found that the type specimen of *V. angustifolium* consists of two unbranched shoots without flowers and with unknown chromosome number. Therefore, none of our characters could be used to compare the type with our two species. Moreover, since both species occur in the locality where the type specimen was originally collected, it was impossible to identify positively the type with either of them. Although it was recognized that vegetative characters of lowbush blueberries vary greatly under different environmental conditions, such characters were the only ones that could be used in making the comparison. Since the leaves of the type specimen were similar in size to those of the tetraploid plants and larger than those of the diploid ones, we considered the tetraploid species to be *V. angustifolium* and the diploid one was named *Vaccinium boreale* sp. nov.

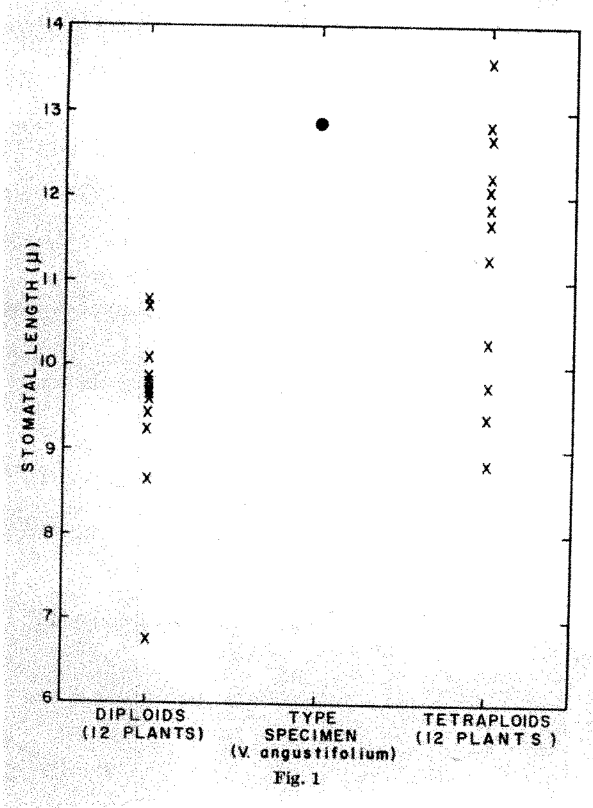

Fig. 1

Dr. W. G. Dore, of the Plant Research Institute, Ottawa, has suggested that stomatal measurements from leaf peels of herbarium specimens might be used as an indication of chromosome number. Dr. J. F. M. Cannon, of the British Museum (Natural History), kindly supplied us with cellulose acetate peels taken from the type specimen of *V. angustifolium*. We prepared peels from herbarium specimens of 12 plants of each of our two species using the same technique[2]. We examined all 25 preparations, taking 20 measurements from each of our preparations and 40 from the preparation of the type specimen. Although there is some overlapping between plants of the two chromosome groups, it is clear that measurements of the type specimen fall within the range of the tetraploid plants and outside the range of the diploid plants (Fig. 1). This evidence strongly supports the conclusion that the type-specimen belongs in the tetraploid group and not in the diploid one. Thus, on the basis of present data, the tetraploid, common lowbush blueberry of eastern Canada must be called *V. angustifolium*, and the name *V. boreale*, proposed previously by us for the diploid species, remains valid. Moreover, the name *Vaccinium lamarckii* Camp, which was proposed for the tetraploid group on the assumption that *V. angustifolium* was diploid[3], must be considered invalid because the two names refer to the same group of plants, and the name *V. angustifolium* takes precedence.

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Deoxyribonucleic Acid Content of Syncytial Nuclei treated with Papain and Trypsin

Syncytial nuclei, following isolation and staining by Feulgen's reaction, form an irregular pattern consisting of clumps, with single nuclei scattered in between. It is possible to estimate the deoxyribonucleic acid (DNA) content of single syncytial nuclei by microspectrophotometry, but not those in clumps, due to overlapping and lack of definity. It was considered that proteolytic enzymes could break up the clumps, but it was not known whether they would also damage the nuclei. In this investigation, papain and trypsin were used to break up the clumps, and their effect observed on nuclear size, shape and DNA content.

Placentæ were obtained following 23 deliveries at term and the syncytial nuclei isolated by a modification of the method previously described[1]. The method was modified by reacting at room temperature

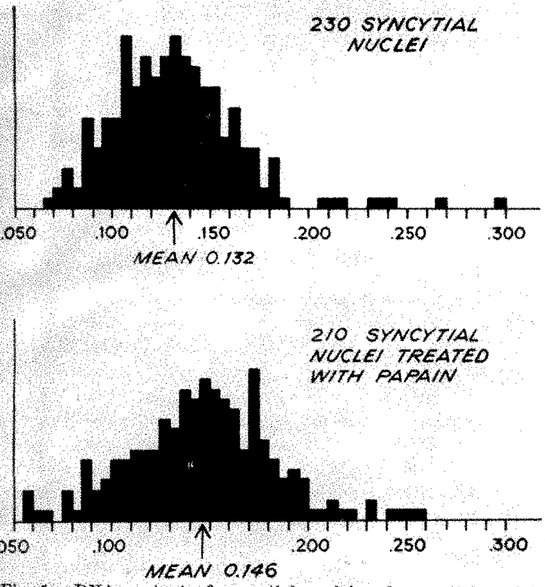

Fig. 1. DNA content of syncytial nuclei and syncytial nuclei treated with papain

2 ml. nucleus suspension with 2 ml. of 5 per cent papain (Merck) for 5, 10, 15 and 30 min before centrifugation with water, and for 5, 10, 15 and 30 min after centrifugation with water. The papain-treated nuclei were stained by Feulgen's acid fuchsin reaction and compared with untreated syncytial nuclei from the same placentæ, and with lymphocytes obtained from the blood of five donors.

The nuclei treated with papain before centrifugation in water all showed stippling due to DNA being unevenly distributed. The clumps were broken up, but the nuclei were unsuitable for microspectrophotometrical examination.

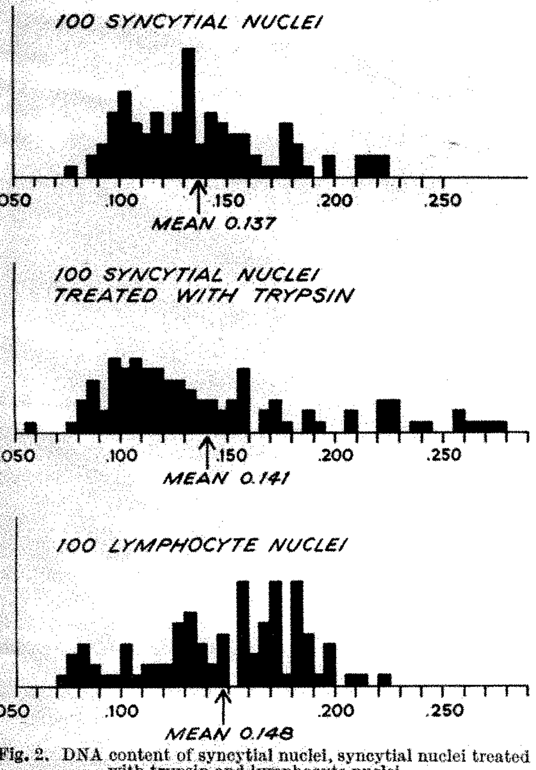

Fig. 2. DNA content of syncytial nuclei, syncytial nuclei treated with trypsin and lymphocyte nuclei

The nuclei treated with papain after centrifugation in water showed an even distribution of DNA. The clumps were broken up more after 15 min reaction than after 5 or 10 min. Reactions of 30 min did not reduce the size of the clumps further. After 15 min reaction, there appeared to be no damage to the nuclei as evidenced by the size, shape and DNA content. The mean arbitrary DNA value for 210 papain-treated syncytial nuclei was $0{\cdot}146 \pm 0{\cdot}037$ and that for 230 untreated syncytial nuclei $0{\cdot}132 \pm 0{\cdot}0332$ (Fig. 1). These figures are statistically similar ($t = 0{\cdot}0338$).

One ml. suspensions of syncytial nuclei from 5 placentæ were reacted with 2 ml. solutions of 2·5 per cent trypsin (Difco) for 15 min after centrifugation in water, and stained by Feulgen's reaction. Most of the nuclei were damaged as evidenced by loss of DNA and irregularity of the nuclear shape. The few undamaged nuclei contained normal amounts of DNA (Fig. 2).

A 5 per cent solution of papain reacted for 15 min with an equal volume of syncytial nuclear suspension after centrifugation in water, broke up the nuclear clumps without damaging the nuclei, and without affecting the DNA content. It was considered that the application of papain in this manner was a useful addition to the technique for examining syncytial nuclei and estimating their DNA content.

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GENETICS

X-linked Inheritance of Developmental Timing in Man

FOLLOWING genetical theory, one might expect full sisters to be more similar than full brothers or siblings of opposite sex. Since sisters share the paternal X-chromosome in common, while only half of brother–brother or cross-sexed siblings share the same maternal X-chromosome, sister–sister pairings should be more alike where X-chromosome inheritance is involved.

In reviewing extensive sibling comparisons involving a total of 318 sister–sister, brother–brother and brother–sister pairs, a tendency for greater sister similarity in developmental timing was clearly evident. As summarized in Table 1, the number of ossification centres of the hand present at exact half-year intervals from 1·0 to 7·0 years, and the age at appearance of 28 individual ossification centres of the hand, proved more alike in full sisters than in the other pairings considered. Similarly, the age at appearance of specific stages of calcification of 5 mandibular posterior teeth was more alike in sisters than in brothers or brother–sister pairs.

The discrete events compared, ossification-rate (number of centres present), ossification timing (age at appearance of individual centres), and calcification timing of the posterior permanent teeth, appear to be primarily gene-determined within the well-nourished population segment studied. This is demonstrated in various ways, including monozygotic twin compari-

Table 1. SISTER, BROTHER, AND BROTHER-SISTER SIMILARITIES IN DEVELOPMENTAL TIMING

	Correlations involving					
	Sister–Sister pairings		Brother–Brother pairings		Brother–Sister pairings	
	N	r*	N	r*	N	r*
Ossification-rate †	494	0·62	688	0·39	1,223	0·40
Ossification timing †	854	0·54	1,260	0·40	2,171	0·47
Tooth calcification †	140	0·41	246	0·22	486	0·24

* From weighted mean Z transforms (ref. 7) of r and normalized T scores.

† Rate = No. of centres present at exact half-year intervals (refs. 1 and 2). Timing = age at appearance of 28 individual centres (refs. 1 and 2). Calcification = age at appearance of particular stages of tooth calcification (ref. 3).

sons[1-3]. Thus, the greater sister similarities pertain to genetically determined aspects of developmental timing.

The sample size in this investigation is large ($N=318$ pairs) and the number of pairings larger still ($N=7,562$) since serial longitudinal observations are involved. Moreover, the correlational data summarized in Table 1 are condensed from a total of 158 age-, sex-, stage- and centre-specific correlations. Accordingly, it is felt that the greater similarity of sisters in developmental timing, as compared with brothers or siblings of unlike sex, is not merely a chance manifestation.

Moreover, sex differences in absolute variability (σ^2) may be rejected as a possible explanation for the findings. All the raw distributions were normalized, by McCall's method, before the correlations were computed, using the I.B.M. 602 calculator of the Fels Computer Facility. Variance was thus made equal by the normalizing transforms and the sex-specific T-scores had identical means and standard deviations.

There is, moreover, other evidence for sex-chromosome influence on developmental timing. In all developmental events, even those which precede steroid-hormone differentiation, girls are ahead of boys[4,5]. In addition, recent examination of osseous maturation in the chromosomal types XO and XXY further confirm the hypothesis of sex-chromosome involvement[6]. Thus, the findings reported here, involving two independent studies (by separate raters), and completed more than three years apart, provide further confirmation of the general hypothesis.

Additional evidence for X-chromosome influence on hand-wrist ossification and dental calcification could come from the comparison of parents and their children at identical ages. Here, the greatest degree of similarity might be expected for father–daughter pairings. Unfortunately, our two-generation studies, involving some 72 pairs to date, are as yet inconclusive in view of the small number (16) of fathers and daughters for whom we have comparative data at identical ages during childhood.

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Somatic Reduction of Tetraploid Sorghum to Diploid Mutants following Colchicine Treatment

COLCHICINE treatment of seedlings of certain sorghum lines has resulted not only in the expected polyploids, but also in diploid plants which are mutant for many characters. Some of these diploid mutants breed true immediately while others give segregating progenies[1-3]. The original hypothesis to explain their appearance suggested that a somatic reduction of the chromosomes (similar to that observed by Huskins[4]) occurred and was followed by duplication. These mechanisms could result in the concentration of chromatin from ancestors of a putative allopolyploid. However, investigations have produced either no or little cytological evidence for duplication or relocation of chromatin[5,6]. In addition, mutant characters which have been studied have shown Mendelian inheritance[7-10]. The alternative hypothesis was then proposed that, in addition to a balanced somatic reduction of the chromosomes after application of colchicine, mutations at many loci also occurred prior to duplication back to the diploid number.

Early attempts to demonstrate somatic reduction and doubling of chromosomes by treatment with colchicine of seedlings heterozygous for cytological or gene markers met with only a few successes (corn, Ross unpublished results, and flax[11]). Possible explanations for lack of success were found when it was demonstrated that genotype[12] and environmental factors[13] influenced the induction of true-breeding diploid mutants in sorghum by colchicine treatment. A laboratory method has now been developed which produces a fairly high proportion of such mutants among surviving plants in the sorghum line, Experimental 3[14,15].

This report deals with the results of treating tetraploid Experimental 3 seedlings, an investigation suggested by the appearance of one diploid plant in each field-grown progeny of two separate C_1 tetraploid Experimental 3 plants (C_0=colchicine-treated plant). One appeared to be normal diploid Experimental 3 except for a dominant height gene which mutates quite frequently (in which case the tall plant segregates 3 tall: 1 short), and the other differed from Experimental 3 in many characters. It had juicy stalk and awns (Experimental 3 is dry and awnless), lacked Experimental 3 leaf blotches, and was taller. Both plants produced uniform self progenies in 1961. Since height did not segregate in the progeny of the tall diploid, and since the tall factor did not show up in any of its 1960 sib plants, it could not have arisen from tetraploid Experimental 3 by just a simple chromosome reduction plus the mutation of a single height gene. The progeny of the apparent multiple mutant showed that its parent had green seedling base (Experimental 3 is red) in addition to the other changed characters.

Cytological examinations were carried out to see whether there was any evidence of chromosomal irregularity. Both plants showed an increase over normal Experimental 3 diploids in pollen mother cells containing univalents at diakinesis and metaphase I. Examination of 356 cells of normal diploid Experimental 3 gave 2 per cent cells with univalents. The tall diploid contained 17·5 per cent abnormal cells, and the multiple mutant, 30·4 per cent. The latter also showed anaphase aberrations, 8 cells with lagging chromosomes to 22 without. Some

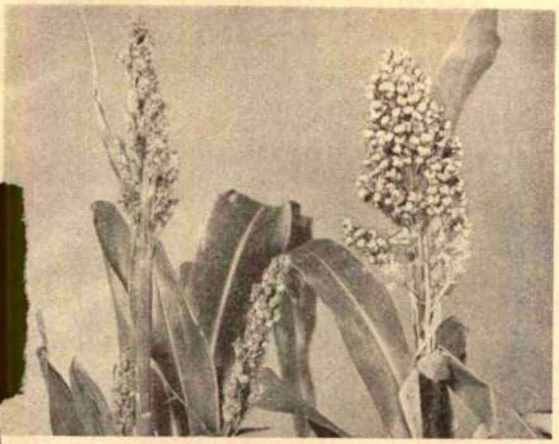

Fig. 1. Left, sterile heads from an untreated tetraploid plant of the sorghum line, Experimental 3; right, the fertile head of a diploid awned mutant derived from such a tetraploid by treatment with colchicine

indications of chromosomal irregularity have also been found in studies of non-true-breeding colchicine mutants[6].

The occurrence of these diploid plants in tetraploid progenies raised the question as to whether a tetraploid cell (duplication) could be the first step in the mechanism resulting in diploid mutants after treatment with colchicine, and somatic reduction a later step. To investigate the possibility of somatic reduction in tetraploid cells and the result if it occurred, tetraploid seedlings of Experimental 3 were treated with colchicine using the laboratory method for induction of true-breeding diploid mutants in diploid Experimental 3. After germination in the dark at 70° F, 20 seedlings were treated by coating the coleoptiles with melted lanolin 0·5 per cent colchicine when they were about 1/3 the diameter of the seed. These were placed in a jar on the surface of sand saturated with water and covered with a glass plate. Nineteen untreated seedlings were set up in the same manner. Both jars were maintained under a 'Heat Ray' bulb (infra-red 250 W) at 68° F in a controlled temperature room for 4 to 6 days with continuous illumination. For the next 13–14 days, the jars were kept under a combination of incandescent and fluorescent bulbs at temperatures varying from 68° F to 75° F during alternate periods of dark and light. During this time glass plates were removed. The seedlings were then transplanted into soil in a greenhouse maintained at approximately 80° F.

Chromosome examinations of these plants were made at meiosis in pollen mother cells using the propionic carmine squash technique. Examinations of field plants were made at mitosis in root tips from seedlings using the same technique except for treatment with aqueous 0·1 per cent colchicine before fixation.

Nine treated plants and 15 untreated plants survived. Four of the treated plants are diploid mutants ($2n=20$) with obvious changes in many characters such as awn condition, seed colour, height, leaf and panicle type; one of these is male sterile. A fifth plant, also a mutant for many characters, is a mixoploid made up of cells with diploid and different polyploid numbers. These plants have not yet been progeny tested. The remaining four are unchanged Experimental 3 tetraploids. Panicles of tetraploid Experimental 3 (normally highly sterile) and of a derived diploid awned mutant are shown in Fig. 1 and illustrate the striking restoration of fertility in the mutant. All 15 surviving untreated plants were phenotypically tetraploid Experimental 3, and cytological examination of 13 of them indicated the exact tetraploid chromosome number ($4n=40$). From the same seed source, 27 other plants grown in the field were also all phenotypically tetraploid; 24 of these were identified as tetraploid by cytological examination. Altogether, 42 untreated $4n$ plants have been grown and 37 of them have been examined cytologically without finding any deviation from the tetraploid chromosome complement. The possibility of diploids being present originally in this tetraploid population would appear to be ruled out.

Cytological examination of approximately 50 pollen mother cells in each of the four diploid mutants indicated that three (including the male sterile plant) are normal with 10 bivalent chromosomes. In the fourth plant, 48 cells had 10 bivalents, and 4 cells had 9 bivalents and 2 univalents, four times the number of such cells observed in Experimental 3 diploids, but fewer cells with univalents than in the 2 diploids which appeared spontaneously in progenies of Experimental 3 tetraploids. For comparison, 53

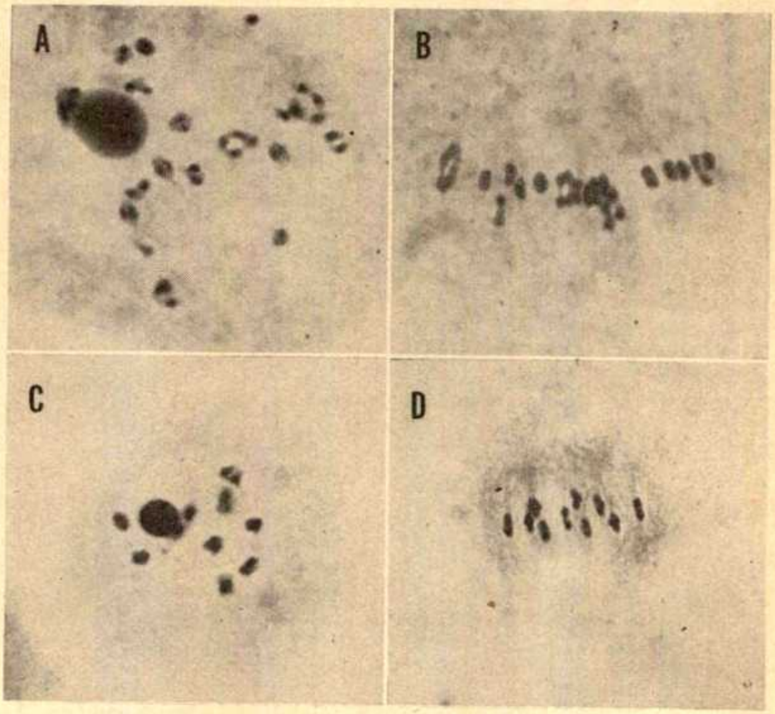

Fig. 2. Pollen mother cells of tetraploid sorghum line, Experimental 3, and of one of the diploid mutants derived from such tetraploid material by colchicine treatment. *A*, Diakinesis in $4n$ Experimental 3 showing 3 IV's (1 attached to the nucleolus), 12 II's and 4 I's; *B*, metaphase I in $4n$ Experimental 3 showing 2 IV's and 16 II's; *C* and *D*, diakinesis and metaphase I in a diploid mutant, each showing 10 II's

pollen mother cells of an untreated Experimental 3 tetraploid gave means of 3·3 IV's, 0·04 III's, 12·8 II's and 1·2 I's. Diakinesis and metaphase I of an untreated tetraploid and of one of the derived regular diploids (the mutant in Fig. 1) are shown in Fig. 2. The IV's, II's and I's in the tetraploid and the 10 II's in the diploid are evident in addition to the chromosome numbers of 40 and 20, respectively.

These results provide real evidence that chromosomes may undergo reduction in somatic cells following colchicine treatment and so give support to the hypothesis that somatic reduction is one of the mechanisms in the process which gives rise to colchicine-induced true-breeding diploid mutants in certain diploid lines of sorghum. If the induced diploid mutants from the tetraploid seedlings prove to be true-breeding like the spontaneous mutant, it would also suggest that chromosome duplication may precede reduction in the formation of such mutants from diploid seedlings. It would seem that the origin of colchicine mutants must involve both duplication and reduction of the chromosomes. The relationship between the two mechanisms needs to be clarified along with the question as to how the mutations occur.

This research was supported in part by U.S. National Science Foundation grants *G*5489 and *G*14990. The chromosome counts were made by C. H. Chen.

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PSYCHOLOGY

Sensory Perception: Use of a Musical Notation

RECEPTOR units of the peripheral nervous system are distributed in space either compactly, as in the retina, or diffusely, as in the skin and musculature. From these units, afferent fibres enter, again more or less diffusely, a portion or portions of the central nervous system, and with their terminals define within the tissue a series of more-or-less sharply circumscribed volumes, with varying degrees of overlap, into each of which volumes the primary afferents of a sensory modulus come into contact with neurones of the second order. So far, then, we have a point-to-point or point-to-volume mapping of receptor units into a series of territories within the central nervous system[1].

The territories contain dendritic fields, each field centering on a nerve cell body and giving rise to a single axon. Of the axons, some end at short d tances from their cell bodies, while others leave t 'centres' of origin to project into more distant p of the central nervous system. Characteristically projection of fibres is into regions where the matter is arranged in sheets, as in the cerebral cerebellar[2] cortices of higher vertebrates, and in tectum of lower forms. These anatomical features in favour of an arrangement whereby neurones block of tissue project on to something resemblin geometrical surface. A three-dimensional neuron array can thus be mapped into a two-dimensional fiel and it is in consequence possible to map a successi of points in a cross-section of a neuronal array, on a line.

We may then visualize our nerve centre, whi receives afferent impulses and transmits by means o radiation of fibres to a distant sheet of grey matt as a compact body made up of cubical units and suspended in space that its interior can be screened of the passage through it of straight line radiations in any direction. The nerve centre has its own axes of symmetry.

A single point in each unit of the nerve cent linked to a point on a line in the grey matter surf In planes parallel to the (x,y) plane of the 'centre', units are mapped one-to-one on to the line in se fashion. As we pass along the z axis from one pl to another, the pattern of representation is repea For the entire projection on to the surface, theref there will be a series of adjacent lines linked t succession of planes in the 'centre'. Activation of excitable units at an angle θ in the (x,y) plane give rise to a pattern of impulses such that $P = f(\theta$ where L is some quantity which indicates the pattern of linkage. Similarly in the (x, z) and (y, z) planes there will be a pattern-dependence on θ.

In each compartment of the lattice the neuronal projection is supposed to come from the point of intersection of the two diagonals. It is as though a thread was laid across the surface of a chess board, or a line drawn so as to pass through each compartment of the grid once and once only. The line drawn through each one of the two lattices shown (Fig. 1) represents one of two possible modes of projection from a small neuronal array; one is in a series of loops, the other in a spiral. The neuronal array is supposed to be excited by afferent impulses, represented by arrows numbered 1–4. It is evident that the pattern of firing, P, of the neuronal array, will depend on the angle θ through which the array is approached. A convenient means of expressing this dependence of P on θ is by using a simplified musical notation, similar to that used in electronic computer composition[3]. Here the key of G major has been selected, the scale of 25 notes ranging from low G to high C. Time signatures and rests are omitted.

Patterns of impulses in a sheet of grey matter to which a neuronal array projects, that is, pitch, interval between notes and shape of phrase, will depend on: (1) spatial relations beween the afferent inflow and the neuronal array; (2) the mode of linkage between the neurones of the array and the grey matter to which they project. Thus in the model system considered, a looped method of linkage gives to the system a sensitivity to changes in direction of approach such that transition from position 1 to position 2 implies a reversal in direction of impulses along the corresponding linear projection. On the other hand, a spiral arrangement gives the system a greater stability, in that the shape of the phrase does not depend on direction of approach. In both instances, should the array be approached in a direction normal to the plane of the paper, the result will be a reiterated pattern of single notes, since we are supposing that the same linkage pattern is repeated throughout the block.

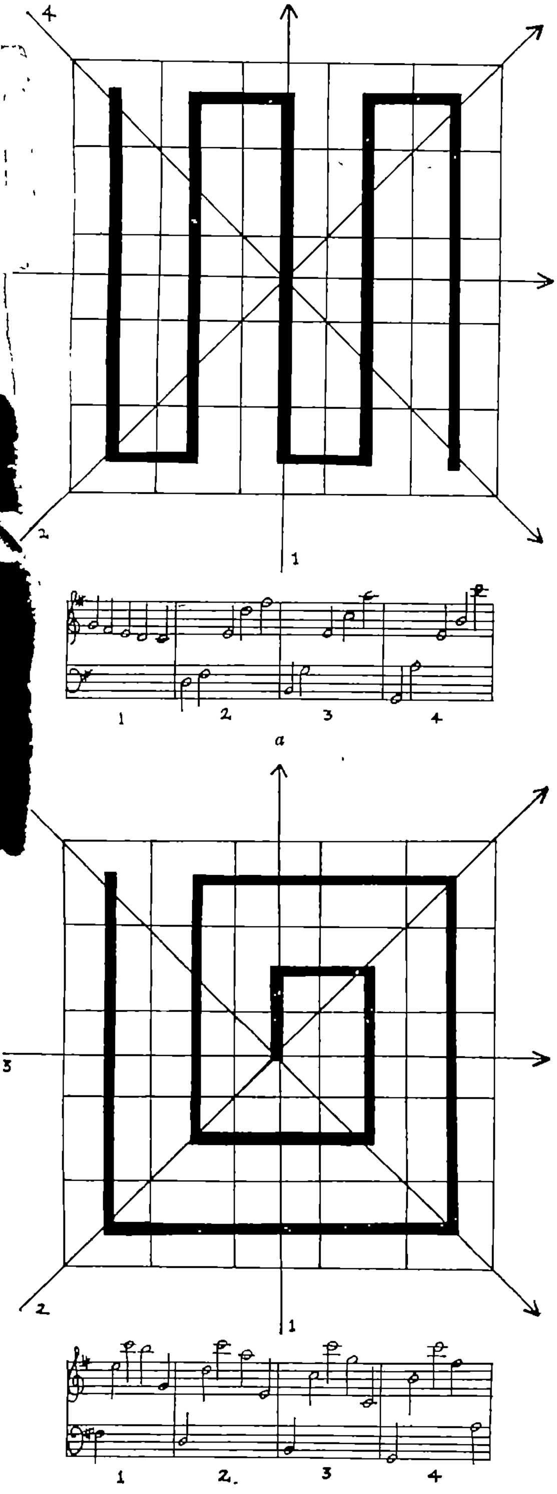

Fig. 1. (a) Looped. (b) Spiral pattern of solid-surface linkage Each bar of music corresponds to the activation pattern obtained by scanning in the direction of the numbered arrow

The analysis of excitation patterns within neuronal arrays, the patterns depending on modes of linkage between elements of a solid and of a surface, would lead into relational mathematics, since we are dealing with n-dimensional manifolds. Musical notation, however, is a simple and universally understood system whereby changing linkage patterns may be expressed and examined in a manner which is potentially quantitative. We are provided here with a simple and unified means of depicting sequences of events that may take place in the sensory modalities —not necessarily auditory—and which are the physical basis of perception.

In a theoretical model such as is described, it is more convenient to regard L as constant and to allow θ to vary. In the central nervous system, however, where nerve centres are as a rule approached by bundles of afferent fibres running parallel to one single line of direction, values for θ would remain fixed, while L varied.

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Threshold Differentiation of Drive and Reward in the Olds Effect

ACCORDING to Deutsch's theory[1], the Olds effect[2] of electrical self-stimulation is produced as a result of the concomitant simulation of both drive and reward pathways. If two such systems, one mediating drive and one reward, are indeed involved, the threshold stimulus intensities required to activate each might differ. If the drive system has a lower threshold than the reward system, then electrical stimulation of lesser intensity than that necessary to produce learning (where reward is also requisite) should prolong a habit which has already been learned, irrespective of the timing of the stimulation with respect to the performance of the habit. That is, the stimulation should prolong, say, bar-pressing, even if its administration is non-contingent on the act of pressing. If, on the other hand, such non-contingent stimulation is administered at an intensity above the threshold intensity necessary to produce learning, then a habit which the animal has already learned for intracranial stimulation should become weaker, since the animal will also be accidentally rewarded for other acts and so come to repeat these instead. Such learning of alternative habits should not occur when the non-contingent stimulation is of sub-threshold intensity for learning, and so there should be less interference with the original habit.

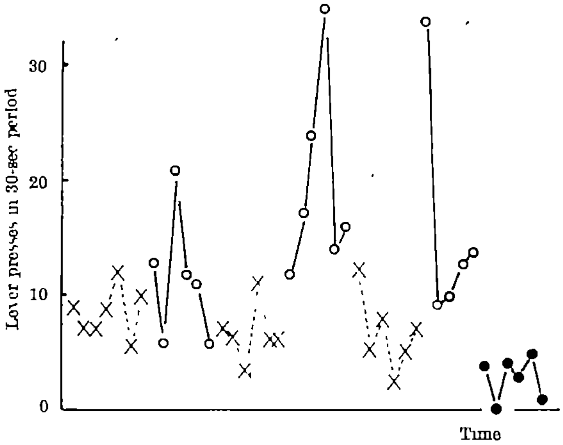

Fig. 1. Results of preliminary experiment on one animal. Crosses show the number of lever presses to extinction in successive 30-sec periods of normal extinction. Circles represent the number of lever presses in 30 sec of extinction during which a low intensity of repetitive stimulation was given, and solid circles show the same for 30-sec periods when full intensity repetitive stimulation was used

In a preliminary experiment, rats were trained to touch a lever for intracranial stimulation. The number of trials it took to 'extinction' when the current was switched off was recorded. Further, the number of trials to 'extinction' was also measured when the stimulating current was suddenly changed from the animal's own control to that of a pulse generator, adjusted to run roughly at the rate at which the animal was obtaining the stimulus through its own responding. In one condition the repetitious non-contingent pulse was at about half threshold strength (threshold strength being defined as that value necessary to produce learning and sustain performance); in the other the repetitious pulse was at threshold. The results of the first animal tested appear in Fig. 1. It is evident that a repetitious sub-threshold stimulus has a large effect on maintaining performance, whereas a threshold stimulus when administered in the same way actually produces a decrement.

Unfortunately such a design relies too much on the individual style of each animal. The animal emitting its responses with a metronome precision does not give the pulse generator a chance to reward it for anything but the response it has already learned. As the stimulus introduces drive also as well as reward, it was found that with other animals elevated rates of responding could be maintained as a superstition.

Consequently, the experiment was redesigned. We decided to compare the animals' response to non-contingent stimulation (delivered at roughly the same rate as its normal rate of responding) with its response to contingent stimulation, each under two conditions, (*a*) stimulus intensity below threshold for learning, and (*b*) stimulus intensity at threshold for learning. What interested us was comparing the two ratios 'contingent-threshold : non-contingent-threshold' and 'contingent-sub-threshold : non-contingent sub-threshold'. For we reasoned that if in the sub-threshold condition drive alone were introduced the animal would perform at least as well in the non-contingent as the contingent situation, since it would be unable to learn new interfering habits. However, in the threshold condition if reward were also introduced the animal should perform less well on the non-contingent than on the contingent condition, since it would quickly learn to perform new interfering habits.

As in the previous experiment, the animal was taught a bar-touching response. Each response triggered 1/10 sec train of 60 c.p.s. at the beginning and end of each response. The current was adjusted to the minimum necessary to produce and sustain learned behaviour. It was administered through an isolation transformer and a 200*K* series resistor.

In the first half of the experiment 1 min training trials were terminated alternately with 1-min periods during which the animal received either contingent sub-threshold stimulation or non-contingent sub-threshold stimulation. Of the eight tegmental animals used, five showing a good effect in the first condition were tested in the second. In the second half of the experiment, 1-min training trials were alternated with 1-min periods during which the animal received non-contingent threshold stimulation. The same threshold stimulation was used throughout the experiment in training and in the second half of the experiment in the non-contingent condition. The rate at which non-contingent stim lation was delivered was the same in the sub-thresho non-contingent and threshold non-contingent co dition. Results are given in Table 1. Furthermor in the 'sub-threshold condition' when cessation responding occurs, no systematic reaction to the non-contingent stimulation can be detected. The animal sits, grooms, wanders around a little, but does acquire any superstitious habits in spite of the am opportunity it has to do so, as it spends a great dea time away from the lever. This is in marked cont with its behaviour in the non-contingent thresh situation, where any twitch or mannerism is ampli even while the animal is executing its old response a cyclic superstitious sequences of behaviour are s established.

Table 1

Rat	Significance of difference between contingent low and non-contingent low	Ratio between contingent low and non-contingent low	Significance of difference between contingent high and non-contingent high	Ratio between contingent high and non-contingent high	μam Low	High
1	0·03	0·74	0 001	1 89	40	100
2	0·30	0 83	—	—	85	75
3	> 0·0001	0 31	0 20	1·13	100	175
4	0 025	0 53	> 0 0001	16·73	150	300
5	0 05*	1 56	—	—	100	225
6	0 14	0 67	—	—	55	100
7	0·02	0·74	> 0 0001	6 98	110	175
8	0·0005	0 50	> 0 0001	2 36	35	70

* Significant difference in direction opposite to predicted

These results make it plausible to believe that in the areas from which self-stimulation is obtainable there are two closely adjacent or intermixed systems, one subserving functions of reward and the other producing drive, in the manner postulated by Deutsch's theory[1].

This work was supported by U.S. Public Health Service grant No. *M*–4563 and National Science Foundation grant No. *G* 21376, while one of us (C. I. Howarth) was a National Institutes of Health Special Fellow. We thank Dr. David A. Hamburg for his help.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public

Monday, November 19

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Prof. D. W. McKenzie: "The Twisting Land".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "The Future of Global Communications" opened by Mr. R. J. Halsey.

Society of Chemical Industry, Pesticides Group (joint meeting with the Agriculture and Fine Chemicals Groups, at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Mr. J. F. Scott: "The Design and Analysis of Experiments".

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. W. R. Mead: "The Geographical Tradition in Finland (Inaugural Lecture).*

Royal Meteorological Society (in the Conference Hall, County Hall, Westminster Bridge Road, London, S.E.1), at 6 p.m.—Dr. R. Frith: "The Upper Atmosphere" (Popular Lecture, to be repeated on November 20).

Royal Institute of Chemistry (joint meeting with the Dartford and District Branch of the Pharmaceutical Society, at the North-West Kent College of Technology, Miskin Road, Dartford), at 7.45 p.m.—Dr. R. E. Bowman: "Some Aspects of Research in the Pharmaceutical Industry".

Tuesday, November 20

Institution of Mechanical Engineers, Automatic Control Group (in conjunction with the Institution of Electrical Engineers, at 1 Birdcage Walk, Westminster, London, S.W.1), at 10 a.m.—Informal Discussion on "Components Used in Automatic Control Systems".

University College, London (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. W. Feldberg, F.R.S.: "A New Pharmacological Approach to the Brain".*

University of London (at the Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. P. T. Matthews: "Some Particles are more Elementary than Others" (Inaugural Lecture).

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. Mary Barber: "Synthetic Penicillins" (Eleventh of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

Wednesday, November 21

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. F. B. Smith and Mr. J. S. Hay: "The Expansion of Clusters of Particles in the Atmosphere"; Mr. P. G. Saffman: "The Effect of Wind Shear on Spreading of an Instantaneous Ground Source".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Dr. J. O. Irwin: "The Place of Mathematics in Medical and Biological Statistics".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Microminiaturization".

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. J. A. Painter: "Changing Influence in the Planning of the Grid System".

Institution of Electrical Engineers, Electronics Division P.G.E5 (joint meeting with the British Institution of Radio Engineers Medical Electronics Group, at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Automatic Biochemical Analysis and Particle Counting".

Institute of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Sir Harrie Massey, F.R.S.: "Engineering Problems in Space Research" (Thomas Hawksley Lecture).

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Prof Sir Lindor Brown, F.R.S.: "Science in China"*.

Institute of Biology, London Branch (at 41 Queen's Gate, London, S.W.7), at 7 p.m.—Annual General Meeting. 7.30 p.m.—Prof. A. Haddow, F.R.S.: "Aspects of the Biology and Chemistry of Cancer".

Royal Institute of Chemistry, London Section (joint meeting with the South-West Essex College of Technology Chemical Society, at the South-West Essex College of Technology, Forest Road, London, E.17), at 7 p.m.—Prof. F. S. Dainton, F.R.S.: "The Simplest Charge, Atom and Molecule".

Thursday, November 22

University College, London (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. R. E. Bradbury: "The Ogwega Oracle—an Index to Benin Culture".*

Plastics Institute (at the National College of Rubber Technology, Holloway, London, N.7), at 2.30 p.m.—Mr. J. M. J. Estevez: "The Cure of Thermo-setting Plastics" (Students' Lecture).

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Meeting on "Computers in Biology". Dr. G. C. Ainsworth: "Storage and Retrieval of Information by Computer"; Dr. P. H. A. Sneath: "Computers in Taxonomy"; Prof. W. T. Williams and Mr. M. B. Dale: "Special Difficulties in the Analysis of Taxonomic and Ecological Data".

University College, London (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Dr. J. S. McKinley-McKee: "The Mechanism of Enzyme-Pyridine Nucleotide Coenzyme Reactions".*

Royal Institute of Chemistry (joint meeting with King's College Chemical Society, in the Anatomy Lecture Theatre, King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. R. O. C. Norman: "Quantitative Aspects of Aromatic Substitution" (Meldola Medal Lecture).

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. H. Belcher: "Radioactive Tracer Methods in Clinical Medicine".* (Twelfth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Steam Plant Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Troubles in Steam Plant and Remedial Measures".

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 6.30 p.m.—Informal Discussion on Education.

Institution of Mechanical Engineers, Nuclear Energy Group (at the College of Science and Technology, Manchester), at 6.45 p.m.—Mr. K. H. Dent and Mr. D. W. Williams: "Burst Cartridge Detection in British Gas-cooled Reactors".

Thursday, November 22—Wednesday, November 28

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2)—International Conference on "Satellite Communication".

Friday, November 23

Royal Astronomical Society (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "Geomagnetic Studies". Chairman: Prof. J. M. Bruckshaw.

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. I. G. Gass: "Tristan da Cunha".

Saturday, November 24

Botanical Society of the British Isles (in the Botany Department, Imperial College, Prince Consort Road, London, S.W.7), from 2 p.m.to 5.30 p.m.—Annual Exhibition Meeting.

London County Council (at Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. A. F. Shore: "Ancient Egyptian Painting".*

Monday, November 26

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. Brian Ackner: "Alcohol Addiction".*

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. J. Wilson and Mr. A. W. Comer: "Transistorized Television Camera Chain".

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Pump Test Codes".

Institute of Metals (at the Royal School of Mines, South Kensington, London, S.W.7), at 7 p.m.—Prof. R. F. Mehl: "A Few Problems about Pearlite and Bainite".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Eric Shipton, C.B.E.: "Land of Fire".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer (with an engineering or equivalent qualification with suitable teaching and industrial experience) to teach Electrical Engineering to Higher National Certificate and Diploma classes—The Principal, Royal Radar Establishment College of Electronics, Malvern (November 21).

Senior Physicist, to be responsible for clinical radiation physics and serve as deputy to the Director of the Department of Physics—The Secretary, Board of Governors, Hammersmith Hospital, Du Cane Road, London, W.12 (November 23).

Assistant (with an honours degree in biochemistry or equivalent) in Agricultural Biochemistry—The Secretary, The University, Aberdeen (November 30).

Junior (Assistant) Lecturer (with a good honours degree in physiology or a qualification in medicine) in the Department of Physiology—The Registrar, King's College (University of London), Strand, London, W.C.2 (November 30).

Lecturer in the Department of Physics—The Registrar, The University, Liverpool, quoting Ref. No. CV/204/N (November 30).

Lecturer or Assistant Lecturer (with a good honours degree in geology and research and teaching experience in geochemistry and petrology) in the Department of Geology—The Registrar, The University, Keele, Staffs (November 30).

SENIOR LECTURER or LECTURER (preferably with special interests in ionospheric physics) IN PHYSICS—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 30).

ASSISTANT IN BOTANY—The Secretary of University Court, The University, Glasgow (December 8).

LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Hull (December 8).

LECTURER/SENIOR LECTURER IN APPLIED MATHEMATICS at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 10).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY—The Secretary, The University, Edinburgh (December 14).

LECTURER IN GEOGRAPHY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 15).

CHAIR OF STATISTICS in the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

LECTURERS/SENIOR LECTURERS (2) IN BIOCHEMISTRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

SENIOR FELLOW; a SENIOR RESEARCH FELLOW; FELLOWS; and RESEARCH FELLOWS IN THE DEPARTMENT OF NUCLEAR PHYSICS, Institute of Advanced Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

ASSISTANT EXPERIMENTAL OFFICER (male graduate), for work on field behaviour of bees, including pollination—The Secretary, Rothamsted Experimental Station, Harpenden, Herts.

ASSOCIATE PROFESSOR OF PHARMACOLOGY—Prof. M. F. Murnaghan, University of Ottawa, Ottawa, Canada.

BOTANY GRADUATE (woman, 25–50, with a good honours degree in botany and a postgraduate diploma in education), to teach botany to "A" level and general science to lower forms at Queen's College, Lagos, Nigeria—The Recruitment Attache, Nigeria High Commission, Nigeria House, Northumberland Avenue, London, W.C.2.

SCIENTIFIC INFORMATION OFFICER (Abstractor) (with a degree in agriculture or natural science and able to translate from German and/or Russian)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire.

SENIOR LECTURER IN CORROSION IN THE DEPARTMENT OF METALLURGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

TECHNICIAN (preferably with a knowledge of biological techniques or previous experience of micropalæontology) IN THE DEPARTMENT OF GEOLOGY, for specialist responsibilities in a micropalæontology laboratory—The Bursar (B.34), The University, Sheffield 10.

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SUPPORT FOR THE HUMANITIES AND SOCIAL SCIENCES IN BRITAIN

IN a report issued last year on research in the humanities and the social sciences, based on a survey by the British Academy 1958–60 and published with the authority of the President and Council of the Academy in the hope that appropriate action would follow, the establishment of a Council for Research in the Humanities and the Social Sciences was once more strongly recommended. The British Academy was concerned not only with the inadequacy of funds for such research, including the provision for study leave and travel grants for research workers and with the uneven distribution of such funds, but equally with the absence of effective means for ensuring the development of new lines of research. It was not considered that the expenditure of more than a few hundred thousand pounds a year was needed, but the Academy was very clear that some new central body the members of which, appointed, for example, by the Lord President of the Council, possessed knowledge and experience in the relevant fields of research was essential for effective administration of existing funds and to ensure that there was neither overlapping nor neglect of promising or important fields.

Although the Minister for Science has indicated that he is prepared to consider the establishment of a new research council, though not necessarily at the moment, the piecemeal approach to research in the social sciences has nevertheless continued. The Minister of Education has established a research fund and a grant of £5,000 has been made to the National Institute of Economic and Social Research, with further grants up to £1,900 over three years for research into growth in the British Economy. Still more recently, an Exchequer grant of £25,000 to the British Academy for research in the humanities has been announced. The last annual report of the Council for Scientific and Industrial Research showed that the Council was steadily increasing its support for the human sciences, for which, in 1961, 41 awards totalling £316,910 were made, compared with 19 totalling £93,105 in 1959 and 23 totalling £199,305 in 1960.

Nevertheless, the chapter on the humanities in the subsequent report, *D.S.I.R., Universities and Colleges, 1956–60,* fully substantiates findings of the survey made by the British Academy and could be causing the Minister for Science and his Advisory Council to think further about support for research in the social sciences and its administration.

The Human Sciences Committee of the Council for Scientific and Industrial Research was appointed in 1957 with the general function of keeping under review the development of the human sciences in relation to industrial needs and with the specific function of recommending the award of research grants in this field. Surveying specifically the position of research in psychology and sociology applied to industry on the disbanding of the two Joint Committees with the Medical Research Council on Human Relations and on Individual Efficiency, the Committee recognized the difficulty of distinguishing between pure and applied science and that the first requirement was a steady flow of research funds in step with the problems requiring solution, the expansion of facilities and the availability of well-trained research workers. It also recognized the dependence of success in this field on team-work and the importance of security of tenure if, once a successful team has been established, it is not to be scattered when the initial project has been completed.

The Committee accordingly started with the intention of devoting a major proportion of its limited funds to the support of five or six proved centres, provided they continued to put forward projects of high standard, and to use the balance to support worthwhile investigations among the wider field of applicants. This policy was frustrated partly by the limitations of the research grant scheme itself, notably in respect of the provision of accommodation and partly by the unexpectedly large number of very good applications from elsewhere. In consequence, a larger proportion of grants than intended went to support projects at emergent centres of research.

After conferences, first with representatives from industry, academic and other research groups to discuss research and training needs in ergonomics, secondly with psychologists and sociologists from universities to discuss the areas of research most valuable to industry, and thirdly with teachers of management subjects from industry, the universities and technical colleges to discuss the background of research to management studies, and after other studies and conferences, four focal points of potential growth were specially noted. These four—industrial recruitment, control skills, the impact of change, and the analysis of, and training in, conventional skills—have served to assist both in assessing the priorities of grant applications and, as a background, in formulating the Department's own future programme of research in the human sciences, now the responsibility of the Warren Spring Laboratory. Nevertheless, in fitting into the pattern already established for the Department's research grants and training awards in other sciences, difficulties were encountered which, in spite of the much more limited sector of the human sciences envisaged by the Committee, fully corroborated what was noted in the wider survey of the British Academy.

There is, for example, the scarcity of senior teaching posts at universities, and indeed of teaching posts generally, which, coupled with insecurity in research, makes a career in this field unattractive. Experienced research assistants are thus often hard to find and more flexibility is needed, and, furthermore, the Committee has not yet been able to support representative projects in all the fields of psychology and sociology within its interest. This is in spite of the fact that the capacity for research in the human sciences is manifestly growing in the universities as well as interest in the application of human sciences to industry, and that there is an increased interest in the colleges of technology which has permitted the transfer of projects from university departments to the colleges. All these objectives have a potential practical value for industry, and some of the problems concerning skills and ergonomics call for an increased knowledge of human capacities and of human function through laboratory investigations, sometimes in industry itself.

The magnitude of support to the universities and technical colleges from the Department of Scientific and Industrial Research for what, in spite of its importance, is a relatively limited range of social research, may not compare favourably with that given in the physical sciences, but its proportion may not be very dissimilar and much university research in psychology and sociology now has an industrial orientation. The possibility of distortion cannot be ignored and the Human Sciences Committee has recently been considering the possibility of a lack of balance in the public support of the human sciences. Whether or not the terms of reference of the Committee are a contributory factor, it is obvious that the piecemeal approach is the real danger, and this report once again points to the *prima facie* need to consider afresh the whole administration of research in the human sciences, without prejudice to the question whether a Social Science Research Council is needed, or whether the existing structure would suffice if adequate use were made of powers which the Advisory Council on Scientific Policy already possesses.

On balance, however, on the evidence of this survey by the Human Sciences Committee of the Council for Scientific and Industrial Research, it would seem that the establishment of a new and comprehensive body would offer substantial advantages and remove some of the difficulties which might arise if one body were responsible for making awards in the humanities and the sciences. It does not seem that the awards which the Council for Scientific and Industrial Research at present makes should necessarily in future be made through the new body, though that possibility should be examined impartially if it is decided to create a new body, in which unquestionably should be vested the responsibility for making all grants from public funds for fundamental research in the social sciences. The difficulty in distinguishing between pure and applied research in this field is minor compared with those which might be encountered if one body had to administer grants for nuclear physics research and for research in archæology or the classics, for example.

The argument for a research council for the social sciences is convincingly presented in a memorandum, by Prof. D. V. Glass and Prof. M. Gluckman, on the social sciences in British universities which has been submitted to the Robbins Committee by the Council of the British Association on behalf of Section N (Sociology)*. This Section, which met for the first time at Cardiff in 1960, covers sociology, social anthropology and social psychology, and no other group unites these disciplines in quite the same way. It is concerned both with the full contact with other university disciplines essential for fruitful work in the social sciences and with the further provisions required for development of the social sciences as university disciplines. The scope of the memorandum is thus sufficiently different from that of the Human Sciences Committee to give added weight to the common findings regarding the needs of the social sciences.

The memorandum begins by directing attention to the great need for further expansion in the social sciences at British universities in spite of what has been done since the Clapham Committee reported on June 3, 1946 (Cmd. 6868. London: H.M.S.O.). In several universities the subjects are not taught at all; frequently departments are small and it may be almost impossible to find reasonable time for research. Many major gaps in our knowledge of social conditions in Britain which the Clapham Committee suggested sociologists, social anthropologists and social psychologists should be encouraged to fill still exist, and the lack of consistent policy in developing the social sciences within the university framework has added greatly to the difficulties of building up a body of knowledge and a methodology suitable for the study of rapidly changing social conditions in Britain and in many other parts of the world.

The three social sciences covered in this memorandum have suffered acutely from a lack of the kind of research funds which would permit the systematic building up of knowledge and valid generalizations, and in social anthropology special difficulties have been encountered. While between 1930 and 1955 social anthropologists in Britain built up a substantial body of knowledge about, and understanding of, the social life of the peoples of the Commonwealth and Empire, the process has been interrupted by the granting of independence to various territories and the closing down of the Colonial Office funds for research in these areas. So much is this so, that under present conditions, even with the new fellowships provided for research under technical aid, the study of the developing societies is becoming increasingly the preserve of universities in the United States, in spite of the importance, both for Britain and in helping students from less developed countries, that Britain should expand her knowledge of what is happening within such countries in the new circumstances.

A striking example of the inadequacy of present research funds cited in the memorandum is in the field of demography, but Prof. Glass and Prof. Gluckman have in mind documentary studies as well as new field investigations. They are well aware, too. of the support given by the Medical Research Council and the Council for Scientific and Industrial Research, but it will be noted that they do not mention the Colonial Research Council or its successor, the Overseas Research Council. All such support is, in any event, limited by the terms of the charters of the respective Research Councils, and while the Nuffield Foundation, for example, has provided sizable support for social research, such support is normally for pioneer ventures and not for the support of research projects which it is reasonable to expect the universities themselves will steadily finance.

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* *The Advancement of Science*, 19, 154 (1962).

Such steady finance is a primary need, and the memorandum emphasizes that there is still insufficient realization by the University Grants Committee—and often by the universities themselves—that social fact-finding, by means of intensive and extensive field and documentary research, is both essential and very expensive. Without this social fact-finding, little effective development can be expected in the realm of validated theory and thus in the build-up of a firmly based set of disciplines. Moreover, the more the social sciences concern themselves with comparative studies, the more the costs of research will rise, and these will also increase as Britain is able to train mature and responsible research workers and provide the higher proportion of senior posts which is also an important element in the expansion of undergraduate as well as postgraduate teaching in sociology generally.

Essential as is the provision of larger financial resources, the memorandum emphasizes that the methods of financing research are also important. It urges that a much larger proportion of research finance should come from the normal university income, and it strongly deprecates heavy dependence on *ad hoc* grants. Much of the strength of the argument for establishing a human or social sciences research council derives from this consideration. Nevertheless, the memorandum still urges that provision of something in the nature of earmarked grants, analogous to those awarded in consequence of the recommendations of the Clapham Committee, is still required to enable the social sciences to catch up with the older disciplines.

It is obvious that there are innumerable questions of collaboration and administration which the creation of a new research council would not by itself resolve. Its relations with the other Research Councils as well as with the other authorities responsible for public expenditure in this field would require very careful consideration, as would the responsibility of the council itself; for example, whether, like the University Grants Committee, it should come under the Chancellor of the Exchequer or under the Minister for Science. Nevertheless, the case for the establishment of such a council in principle seems very strong, and, apart from any advantage it might possess in expanding public provision for such research and its more equitable and effective distribution, its existence should tend to discourage conditions and an outlook which in some universities has limited the provision of either facilities or finance for such research. In describing recently the School of Social Studies at the new University of Sussex, Prof. Asa Briggs remarked: "The lack of adequate research in the social studies in this country—and of the financial means to pursue it—is one of our worst national shortcomings".

That remark is fully substantiated by two recent inaugural lectures in this field. Prof. R. K. Kelsall, speaking on sociological research in Britain when taking up his duties as professor of sociological studies in the University of Sheffield, made the case rather by pointing to what had already been done as illustrative of the wide opportunities for such research in Britain. Prof. François Lafitte, on the other hand, dealing with social policy in a free society in his inaugural lecture[†], to what he described as the first chair in a British university to have the words "social policy" joined in its title with the older title "social administration", looked rather to see the way in which research in the social sciences could provide a practical basis for social policy.

Prof. Lafitte pointed out that the founders of the Department at Birmingham, W. J. Ashley and J. H. Muirhead, believed that State and municipality could and should be used as instruments to enlarge freedom and promote social betterment. He affirmed his own belief that in Britain's kind of society, while social policy was not 'essentially' interested in economic relations, it was very much concerned with the extent to which economic relations and aspirations should be allowed to dominate other aspects of life. Social policy addressed itself to a whole range of needs—material, cultural, emotional—outside the satisfaction which could conveniently be left to the market, and protest against economic domination of human values was a main strand in social policy. The economic inefficiency of social results of *laissez faire*, as well as their imperial and military inefficiency, were other strands, and a fifth derived from the transformation of Britain's way of life by science and industry. The themes of social policy hitherto accepted, however, required rescoring with improved harmony in a composition dedicated to working out the implications of citizenship in a free society so as to give responsibility, freedom, equality and fellowship a richer and less confused content.

Prof. Lafitte in this context had the nature of the social services specially in mind. He concluded his address by stressing the importance of social sciences in Britain's free society, the challenges they offered, and some of the needs which social research could meet—a sociology and social psychology of medical care institutions, for example, of relations between doctors and other health workers, and between them all and patients, a sociology of the medical profession to establish, among much else, the essentials of professional freedom in the new society. In this emphasis on the practical contribution of sociology to the welfare of society, Prof. Lafitte established an impressive case for the expansion of social research and, on the basis of his paper alone, it is clearly time for a fresh look at the instruments we possess to assist the productive reaction of society and science. Science itself is truly a social activity and accordingly has its own social problems, at least in respect of the social institutions, whether research councils, research institutions or other bodies essential to provide the means and conditions for effective work and to formulate projects and programmes which are worth while and practicable. The evidence that establishment of an appropriate research council might be of decisive value grows, and the case presented by Profs. Glass and Gluckman should not only receive the most serious consideration by Lord Robbins and his Committee but also equally merits attention in the meanwhile by the Minister for Science and his Advisory Council.

[†] *Social Policy in a Free Society.* By Prof. François Lafitte. (An inaugural lecture delivered in the University of Birmingham on 18th May, 1962.) Pp 17. (Birmingham The University, 1962.) 2s. 6*d*.

APPLICATIONS OF X-RAYS IN SCIENCE AND TECHNOLOGY

Advances in X-ray Analysis

Proceedings of the Sixth, Seventh, Eighth and Ninth Annual Conferences on Applications of X-ray Analysis. (Sponsored by University of Denver, Denver Research Institute.) Vol. 1: Pp. 494. 75*s*. net. Vol. 2: Pp. 359. 75*s*. net. Vol. 3: Pp. viii+376. 90*s*. net. Vol. 4: Pp. v+568. 115*s*. net. (London: Sir Isaac Pitman and Sons, Ltd., 1960 and 1961.)

PROGRESS in the development of X-ray methods in physics, chemistry and technology continues at an ever-increasing rate. Responding to the challenge of this situation, the University of Denver in Colorado has, since 1951, organized a conference each summer on the applications of X-ray analysis. Starting as a very small meeting in 1951, these conferences gained so much in popularity and support that, with the advent of the sixth, in 1957, book-form publication of all the papers was decided on. The books under review contain the material presented at the Denver conferences of 1957, 1958, 1959 and 1960, respectively: about 120 papers in all.

In fact, these books comprise the first numbers of what is in effect a new journal, the title of which should perhaps be, "Applications of X-rays in Science and Technology". The papers are each complete in themselves and independent of one another. Their interest is primarily technological with the accent on metallurgical science—an understandable bias. Every paper involves X-rays; this is the only unifying theme. Otherwise their scope is very wide and varied. The word 'analysis' used in the title of the volumes covers both chemical analysis and solid-state physical investigations. X-ray emission, absorption, diffraction and fluorescence are represented; the design of instruments is extensively discussed; measurements of particle size, of film thickness, of diffusion, of phase relationships in alloys or in mineral systems are reported, together with technical engineering applications such as the measurement of steam voids in superheated water flowing through steel tubes at 1,000 lb./in.^{2}. The only aspect conspicuous by its infrequency is the determination of molecular structure.

Volume 1 has the curious feature of the inclusion of the conference banquet speech—on *Vanguard* satellites! Volume 4 has the special feature of recording some of the verbal discussion following the papers; this is sometimes rather inconsequent (Dr. X: "Do you form a carbon film in there at all?") and would be better omitted, but it does add a human touch. Volumes 3 and 4 include the contributions of invited European scientists. All four volumes are comparatively well produced, though misprints are not uncommon; plates are of poor quality, but diagrams are clear.

One disadvantage of this sort of production of conference papers is that it can, and sometimes does, duplicate existing publications: that is, there is nothing to prevent papers appearing twice over. Further, the expense of these volumes is high, presumably owing to their limited distribution. Nevertheless, we may congratulate the University of Denver on these annual conferences and wish them continuing success.

J. H. ROBERTSON

MANPOWER AND MATERIALS

Organization, Automation and Society

The Scientific Revolution in Industry. By Robert A. Brady. (Publications of the Institute of Business and Economic Research, University of California.) Pp. xiv+481. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1961.) 8.50 dollars; 68*s*. net.

THIS book is an economist's assessment of the need to rationalize the economic structure of our industrial society. The theme is the maximizing of production combined with conservation of resources, by large-scale economic planning.

The author, who is emeritus professor of economics in the University of California, writes vividly on the impact of the "second industrial revolution" and suggests that because of the part played by science and scientists in bringing it about it should be looked on as "the scientific revolution in industry".

The book is a stimulating one, and it must be agreed that much more wide-scale planning or "rethinking through", as Brady frequently puts it, is urgently needed in the field of industrial economics. This book contributes to this by assembling a considerable quantity of economic facts and suppositions, particularly on the supply of raw materials, electricity production, standards formulation, retail distribution of goods and telecommunications. References are made to books, articles and pamphlets from both American and European academic, industrial, magazines and Government sources. Regrettably, no reference is later than 1956. Further, the references are immersed in 634 annotated notes covering 40 pages immediately following the 424 pages of the thirteen chapters, and there is no separate bibliography.

Prof. Brady admits that to achieve the necessary high order of industrial organization there must be a world-wide system of standards of form and dimension of products, that many services such as transport and telecommunications will have to be internationalized and that the large manufacturing companies will have to at least submit to a centralized internationally controlled planning organization. The idealism is refreshing but regrettably unrealistic.

The words 'science' and 'scientific' are used very loosely throughout the book, usually to flatter rather than define. The thinking that has gone into the book is clearly far-reaching and far-sighted, but it is to be regretted that the 'scientific' solutions that are suggested do not include any controlled investigations into the effectiveness of these or alternative solutions, nor are there suggested any social criteria for evaluating the proposals, the sole criterion being implied in the text is that of maximum output with minimum waste.

The facts contained in this book make it historically useful to many kinds of readers. It would be of particular interest to industrial economists and sociologists. It could be of use to the 'technologist-administrator' in upholding his long-term budget proposals in discussions with 'political-economic' planners.

G. A. RANDELL

TWELVE HUNDRED YEARS OF ADULT EDUCATION

A History of Adult Education in Great Britain

By Dr. Thomas Kelly. Pp. xii+352. (Liverpool: The University Press, 1962.) 42*s*. net.

DR. KELLY has provided the first comprehensive history of adult education in Great Britain. *A History of Adult Education*, by J. W. Hudson, was published as long ago as 1851, but it was concerned mainly with the adult schools and mechanics' institutes of the early nineteenth century. Dr. Kelly covers the period from the Middle Ages to 1939, with a last brief look at post-war changes and problems.

One fact that emerges very clearly is the considerable effect the growth of science has had on adult education. From the Anglo-Saxon period until the seventeenth century adult education was mainly a function of religious institutions and movements. The course of development was from the instruction provided by the parish priest in pre-Reformation times to the preaching and Bible study of the Puritan lecturers. The first cause of the appearance of secular adult education was the geographical discoveries of the sixteenth century, which, through the stimulus they gave to the study of navigation, caused classes in mathematics and astronomy to be offered to adults. By the eighteenth century, in Dr. Kelly's words, "Public lectures on science became so widespread that a detailed record is impossible". Later the drive to spread scientific knowledge proved a major influence in two nineteenth-century developments, the foundation of mechanics' institutes and the beginning of university extension. In what are now accepted as the first university extension lectures in 1867, James Stuart insisted on taking the history of science as his subject, and in 1890–91 courses in natural science constituted more than one-third of the 457 courses provided by the universities of Oxford, Cambridge and London.

Dr. Kelly's book is not a narrowly conceived history of adult education but a contribution, from a particular point of view, to the history of British culture. He casts his net wide to show the general social, cultural and educational background against which developments in adult education took place. Readers with special knowledge of certain aspects of adult education will notice the broad generalizations and omissions inevitable in a survey of such a long period of history. They should also notice, however, the evidence of much painstaking research into obscure aspects of British educational history, for example, the extent of illiteracy in the early nineteenth century and the contribution made to national education by the night schools of the early Victorian period. It seems likely that Dr. Kelly's book will stand alone in its particular field for a long time.

W. E. STYLER

COMPARATIVE ANATOMY AND TAXONOMY OF THE PRIMATES

Primates

Comparative Anatomy and Taxonomy. By W. C. Osman Hill. Vol. 5: Cebidae, Part B. Pp. xxi+537+31 plates. (Edinburgh: At the University Press. Distributed by Thomas Nelson and Sons, Ltd., 1962.) 189*s*. net.

THIS volume covers the sub-families Alouattinae and Atelinae and brings Dr. Osman Hill's work on primate anatomy to a stage of having treated all the prosimians and New World monkeys. The monograph follows a pattern now familiar to readers of its predecessors: a short historical introduction leading to summary accounts of general and external features and followed by more detailed descriptions of individual anatomical systems. Sections dealing with behaviour, development and reproduction then lead to accounts of the geographical distribution and detailed taxonomy of each genus and, in the case of the Alouattinae, of the fossil genus *Homunculus*.

In contrast to earlier volumes where lack of detailed information necessitated the frequent use of descriptions compounded from several genera, the data have been sufficient for this general descriptive pattern to be followed for each genus, extensive gaps in the literature for the rare group *Brachyteles* being, so far as possible, filled by original observations of a specimen that became available when the book was in preparation. The result is a more balanced overall account than was possible in earlier volumes.

But within sub-sections, there is still an unevenness imposed, one can infer, by the still enormous gaps in knowledge—for example, whereas the space allotted in standard texts of human anatomy to descriptions of the external genitalia is about one-fortieth of that accorded to the central nervous system, in Dr. Osman Hill's monograph the two, as in earlier volumes, rate equally. Again, in the descriptive sections, even when sufficient data are available, considerations of space have prohibited the inclusion of accounts of the variability of each feature. In fact, short of consulting the copious references quoted in the text, it is usually impossible to determine how many observations have formed the basis for each statement—a criticism which often, although not always, also applies to the presentation in these volumes of Dr. Hill's original observations. Consequently, although workers in this field cannot fail to admire Dr. Hill's sustained enthusiasm that has resulted in the publication of five volumes of this work in less than ten years, their gratitude cannot but be tempered by the realization that such qualifications materially reduce the value of these monographs as a basis for more detailed studies of form and function in individual anatomical regions and systems.

The anatomical descriptions lead to the taxonomic sections and these—presented in a classical style—make it only too clear that, at present, only a fraction of the topographical material is sufficiently well known to enable the extent to which different characters vary between species and sub-species to be defined. But differences in many features are well established between higher taxonomic groups, and a critical reader leaves the volume with the impression that a selective treatment of these would have provided a more immediate contribution to primate biology.

E. H. ASHTON

HIGHER EDUCATION IN AFRICA

By DR. J. W. COOK, F.R.S.
Vice-Chancellor, University of Exeter

A CONFERENCE on the "Development of Higher Education in Africa" was held, under the auspices of Unesco, in Tananarive, Malagasy Republic, during September 3–12. This Conference was a sequel to the Conference of African States on the Development of Education in Africa held in Addis Ababa during May 1961 and its stated objectives were:

(1) To identify possible solutions to: (*a*) problems of choice and adaptation of the higher education curriculum to the specific conditions of African life and development, and the training of specialized personnel for public administration and economic development techniques; (*b*) problems of administration, organization, structure and financing encountered in the creation or development of institutions of higher education both from the point of view of the institutions themselves and from the wider angle of national policy.

(2) To provide data to the United Nations, its specialized agencies, and to other organizations and bodies concerned with international co-operation and assistance, for the development of their programmes in aid to and use of institutions of higher education in Africa.

'Higher education' was defined as education of an institutional nature for which the basic entrance requirement is completion of secondary education, the normal entrance age is about eighteen years, and in which the courses lead to the giving of a named award (degree, diploma or certificate of higher studies). In fact, attention was concentrated on university education, and although the need for the development of other forms of higher education was recognized and some tentative conclusions reached as to the desirable extent of such development the detailed discussions which took place at the Conference were concerned very largely with the development of universities, which were well represented.

About two hundred people attended the Conference. There were delegations from thirty-one African countries (excluding the Republic of South Africa and the Portuguese territories, but including Madagascar and Mauritius), and observers from fourteen non-African states. The United Nations and its specialist agencies (for example, the World Health Organization) were represented, as were sixteen international non-governmental organizations and six private foundations, together with a very efficient complement of Unesco secretariat and staff. Among the non-governmental organizations invited to send observers was the Association of Universities of the British Commonwealth, which it was my privilege to represent. Those present included nineteen principals or vice-chancellors of African university institutions and nine vice-chancellors or presidents of European or American universities.

The Minister of Education of the Malagasy Republic, Mr. L. Botokeky, was elected president of the Conference, and after two plenary sessions at which representatives of each delegation were invited to speak, the Conference broke up into three Commissions for detailed consideration of the matters on the agenda. Commission I, concerned with problems of staffing, had as its chairman Mr. Doo Kingue, of the Cameroons Ministry of Education. Dr. O. A. Ajose, principal and vice-chancellor of the University of Ife, was chairman of Commission II, concerned with the finance of higher education, and Dr. D. H. S. Nicol, principal of the University College of Sierra Leone, was chairman of Commission III, which was concerned with curricula. Dr. Ajose had attended the Association of Universities of the British Commonwealth Executive Council meetings in New Zealand this year, and Dr. Nicol had attended last year's meetings in Hong Kong. Commission III found it convenient to operate through four working parties which considered respectively: (*a*) African studies; (*b*) economic development; (*c*) natural resources; and (*d*) public administration. The deliberations and findings of the Commissions were finally discussed and adopted at plenary sessions, the last of which, on September 12, approved a comprehensive report of the Conference which will be published by Unesco in the course of the next few months. One of the key personalities of the Conference was Dr. M. S. Adiseshiah, the assistant director-general of Unesco, who guided its work with great adroitness and tact. It was in large measure due to his skill and efficiency that the Conference was able to complete its business and produce its massive report within the allotted time.

The work of the Conference was also greatly assisted by the prior preparation of a number of background papers, of which the most significant were two reports which set out the results of extensive studies on "The Staffing of Higher Education in Africa" by a team under Sir Alexander Carr-Saunders and "The Financing of Higher Education in Africa" by the Netherlands Economic Institute, Rotterdam, under Dr. J. Tinbergen. These two reports, which set out the detailed implications, in terms of staffing and finance respectively, of that part of the Addis Ababa plan concerned with higher education, modified somewhat in the light of trends which have since become clearer, were fundamental to the discussions of the two Commissions concerned with these topics.

The plan for the development of higher education in Africa, agreed in outline at Addis Ababa and worked out in greater detail at Tananarive, is for a very large expansion covering the period until 1980. The needs, both in terms of manpower and of finance, for the implementation of the plan are of such proportions as to demand the most careful attention to economy and efficiency. It was such considerations that influenced many of the recommendations of the Conference, on such matters as size of institution, limitation of numbers of institutions, staff/student ratios, regionalization and co-operation between countries. The Conference recognized the uncertainties inherent in long-term planning in rapidly developing countries, and it was made clear that the plan which emerged will be subject to periodic review.

It was recognized at the outset that the situation in North Africa is very different from that in the countries south of the Sahara, but excluding the Republic of South Africa—an area conveniently termed 'Middle Africa'. For example, whereas the present student enrolment in all the countries of Middle Africa is some 31,000, that in North Africa is no less than 134,000, mostly in the United Arab Republic. This was the principal reason which led the Conference to deal separately with North Africa and Middle Africa. The broad recommendations of the Conference are a planned expansion of student enrolment in Middle Africa to 46,000 by 1965, 80,000 by 1970, 144,000 by 1975 and 274,000 by 1980. These figures represent approximately the following percentages of the estimated relevant age-groups of the populations—0·35, 0·55, 0·89 and 1·51. In the case of North Africa the proposal is an expansion to 176,000 students by 1965 (4·1 per cent of the relevant age-group) and thence to 365,000 by 1980 (5·9 per cent).

Many of the implications of planning on this scale are self-evident, and the Conference served a very valuable purpose in enabling the representatives of the African countries to state their needs and their ambitions in the presence of representatives of countries and foundations to which they look for assistance and support. There was no tendency to minimize what had already been achieved, which was in fact admirably illustrated by the quality of the contributions made to the discussions by those who have been educated in the new African institutions. Representatives of the 'aiding' countries and organizations came away with a much clearer picture of the magnitude of the task which has yet to be carried out. One very valuable feature was the bringing together of many concerned with the planning of higher education in the two major groups of English-speaking and French-speaking African countries. There was a clear recognition of the need to break down the language barriers which have tended to keep these two groups apart. To some of the English-speaking observers at the Conference it was a revelation to learn that the French Ministry of Education still accepts full responsibility for the organization, staffing and finance of the universities in the former French Territories which are now independent. This must be an appreciable burden on the French tax-payer.

There was general agreement that the foreign systems of university education which have been developed in African countries should now be adapted to suit national needs. Africanization of staff and curricula were strongly advocated, although it was recognized that care will be needed to avoid the danger of isolation. It was insisted that there should be no sacrifice of the international standing which is so important to a university. This, however, is not inconsistent with the extensive development of African studies, and it was pointed out that even in specialized scientific fields text-books and syllabuses could be devised which would use illustrations drawn from sources which would be familiar to students in African countries rather than from sources entirely foreign to them. Although a progressive reduction of the proportion of expatriates on university staffs is envisaged (from 80 to 10 per cent) the actual numbers needed will show a sharp increase during the next few years, concurrently with a speed-up in the training of Africans for academic posts.

It was suggested that there should be standard conditions of service for academic staff based on African levels of remuneration in comparable professions, and that inducements to expatriates should be provided by a topping-up of salaries either by the employing institution or by the sending country. A somewhat unrealistic suggestion was that such supplementary payments to seconded staff should be retained in the sending country. It was pointed out that research institutes compete for the same type of staff as universities, and this led to a recognition of the need for a closer association of the research institutes with the universities. The Conference was agreed that it was desirable that so far as possible undergraduates should study at institutions in their own countries, and that where facilities are available at home such students should be increasingly discouraged from studying abroad at this level. It was also agreed that postgraduate study abroad was a desirable objective, and that this could be an important ingredient in the training of teachers for the universities in the students' home countries. However, it was thought that overseas study should not be on such a scale as to hinder the development of postgraduate studies and research schools in the African universities. It was suggested that graduates studying overseas could with advantage be given some teaching experience and some insight into departmental administration. Stress was also laid on the need to make provision for the training of supporting staff (librarians; registrars and other administrative staff; laboratory technicians).

Although it was recognized that recruitment of expatriate staff would be needed on an increasing scale for some years it was not felt that this could best be done through centralized international agencies such as Unesco. The valuable part played by the Inter-University Council in Britain was widely appreciated and it was urged that similar national agencies should be set up in other countries which are in a position to supply staff. The role of Unesco would be that of a co-ordinating body and an agency for the dissemination of information. The scale of the recruitment effort needed can be gauged from the estimate that in the period up to 1980 it will be necessary to recruit for higher education in Middle Africa some 7,000 expatriates and 14,000 Africans.

The estimated total capital and recurrent expenditure for the proposed expansion of higher education rises from 98 million dollars in 1965 to about 534 million dollars in 1980 for Middle Africa alone. If North Africa is included these figures become 357 million dollars and 1,043 million dollars respectively. The prospect of expenditure on this scale points to the need for the most economical and efficient uses of resources. It was recommended that for the period under consideration the number of universities in Middle Africa should be restricted to the present twenty-eight institutions together with the four others planned and projected. A minimum enrolment of 5,000 students was suggested as an estimate of the optimum size of a university, with a staff/student ratio of 1 to 15 (1 to 20 for non-university institutions of higher education). It was thought that the output of non-university institutions should be about four times that of universities, and it was also suggested that the present ratio of 40 per cent of students in scientific and technological faculties (including agriculture and medicine) to 60 per cent in other faculties should be reversed. In order to make the most effective use of resources it was recommended that close co-operation be established between university institutions in African countries and in particular that expensive faculties such as medicine and veterinary science should be limited in number and should provide a service on a regional basis.

It was agreed to set up a permanent conference of vice-chancellors of African Universities, and there was an interesting discussion about the principles of academic freedom and the steps which should be taken to safeguard these principles. There was complete agreement that African universities must have the fullest freedom to teach, to advance the frontiers of knowledge through research and to disseminate as widely as possible the results of their research. They must defend these rights as may be necessary.

The Conference was an educational conference, and its discussions took place in an atmosphere of harmony and co-operation. The one or two slightly jarring notes which crept in were concerned purely with political considerations, as in the somewhat spiteful references by members of the Nyasaland delegation to the 'so-called' University College of Rhodesia and Nyasaland and the twice-repeated assertion of the Gambia delegate that educational backwardness in his country was a deliberate act of British policy. There was general agreement that the Conference had served a most valuable purpose. It had pinpointed with all their starkness the higher educational needs of the African countries. Between now and 1980 the universities and other higher educational institutions of Middle Africa will require an additional teaching staff of 21,000 and an investment of more than 1,500 million dollars. Even with the successful completion of the programme only 1·5 per cent of the relevant age-group will be receiving higher education. African countries alone cannot deal with the problem. They will require external aid on a large scale.

TECHNOLOGY IN THE UNIVERSITIES*

By SIR KEITH MURRAY

Chairman of the University Grants Committee

IT will very soon be ten years since, on January 29, 1953, the British Government made its first major decision on its proposals for the development of technological education in the British universities. It is perhaps therefore a good time to look back on the intervening decade and to discuss the background to this decision, which was a momentous one for the Imperial College of Science and Technology, to outline the expectations and hopes that lay behind the decision and, finally, to look at what has happened and is happening in ten years, a truly short period in the life of a university institution. This can in no sense be a full history of the decade; but the survey may throw some light on the more obscure beginnings of the story: it may even help to belie Carlyle's cynical dictum that "History is a distillation of rumours".

Mr. Boyd Carpenter, who was then Financial Secretary and who is now chief secretary to the Treasury, the Minister responsible for university affairs, stated in 1953: "After consulting the University Grants Committee, the Government propose to put further resources at the disposal of the University of London for the purpose of major expansion of the Imperial College of Science and Technology. . . . It is desirable that the work of Imperial College shall be so co-ordinated with that of the other schools of the University that full advantage can be taken of the whole resources of the University. For this reason and in order that the current work of the College may be disturbed as little as possible, my right honourable Friend is advised that the expansion should take place around the existing site of the College in South Kensington. . . . The College will be regarded as having first claim on other parts of the area (between Prince Consort Road and Imperial Institute Road) as it becomes possible to release these from their present use".

No one with any experience of Government departmental affairs would expect such a precise announcement to have been formulated without an immense amount of preliminary thought and discussion, and this was indeed the case in this instance.

The post-war years had seen the publication of the Percy Report on Higher Technological Education in 1945, which established the National Advisory Council on Education for Industry and Commerce, and of the Barlow Report on Scientific Manpower in 1946, which concerned the universities more directly and more immediately. The latter advocated the doubling of the university output of scientists and technologists in the next ten years—a target which was in fact reached in about half the time.

By 1948 the Government's Advisory Council on Scientific Policy was urging further expansion and stressed the need for an institution, within the university system, where science and technology would be developed in association and to a high level; they advocated the grounding of technologists in the fundamental sciences followed by courses in an applied science. Then followed some four years of widespread discussions by interested bodies, such as the Federation of British Industries and the professional institutions, as well as by Government committees and councils. Two years later, in a debate in the House of Lords in March 1950, Government spokesmen first referred to the possibility of building up the Imperial College as the germ of a technological university.

There was almost complete unanimity about the need for more technologists; there was also general recognition of the urgency of this need. There were, of course, gloomy forebodings of excessive expansion. *Nature* reported in May 1950 that the Ministry of Labour statistics indicated that the demand for scientists and technologists did not greatly exceed the present supply and showed no signs of increasing substantially—early evidence of the great danger of underestimating the long-term demand for graduates.

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* Substance of an address delivered, as Special Visitor, before the Imperial College of Science and Technology on October 25 (see also p. 721 of this issue).

But there was controversy—sometimes, and in some places, heated controversy—about the kind of technologists needed and consequently on where they should be produced. The embers of this conflagration are fortunately long since cold, especially since the conception and planned development of the colleges of advanced technology, but the underlying issues are still important.

There was reputed to be a feeling among the employers of university graduates that there was something missing in them as compared with the counterpart products on the Continent or in the United States. As *The Times* noted in a leading article, "the product of the continental schools has been educated as well as taught . . . he has a grasp of affairs . . . he has a knowledge of economics that lets him see the world of industry as a whole . . . he is potentially an administrator . . . and . . . has a knowledge of other nations' history, traditions, arts and politics". This is probably another marked exaggeration of the virtues of education in other people's countries and institutions; there is a danger in looking at overseas examples without also looking at a country's complete educational systems, both in their lower and in their higher reaches. But there was certainly sufficient justification in *The Times* quotations for sitting up and taking notice of the criticism. Others questioned whether the products of the sixth-form system in British schools and of the three-year British university course, and a further two years of industrial experience, were so inferior to the five-year Continental products. The majority seemed, however, to think that professional training in the universities was too vocational; that a knowledge of how society worked and of human relationships was an important part of a technologist's education; and that the acquisition of these should not be left to chance. The main controversy settled, however, on whether this immediate expansion and change in the content of technological education should take place without or within the university system; on whether technological education at the highest level should be given in an institution limited to technology or in a university, an institution in which a wide range of subjects—the arts, social studies, pure science and other forms of applied science—were found.

The arguments in favour of going outside it were in the main three-fold. It would be easier to put right the reputed shortcomings of the British university courses, namely, that they were too narrow in scope, in a new institution where there might be less resistance to change. If the full range of technology were to be covered and if the heavy investment in equipment were to be fully utilized, it would be necessary to envisage an institution of some 2,000–3,000 students; such a development might imperil the 'balance' within a university and inhibit the universities in the adequate development of their departments of technology. Finally, the brilliant example of the Technische Hochschulen on the Continent and the Massachusetts Institute of Technology in the United States dazzled the outlook of a number of those involved in the issue.

One of the strongest arguments of the proponents of the opposite view, that immediate expansion should take place in an existing institution, was the time-factor. It was pointed out that the planning of a new institution, building and staffing it, would take at least seven years and that it would therefore be about ten years before the first graduates appeared; if the new institution was to play an influential part through its development of postgraduate work, the gestation period would be even longer. Perhaps this was too pessimistic a view, but the advantages in time of expanding an existing institution were marked. It was argued, further, that the lavish provision of fine buildings and equipment did not necessarily make a great institution; reputation was a slow growth. The exemplary overseas institutions had not sprung full-panoplied from nothing; they were the results of gradual development and achievement. Again, it was argued that applied sciences at their highest levels require close contact with the pure sciences also at the highest level; if applied sciences were to be developed quickly, then surely to build alongside existing strong departments of pure science would bring more rapid results.

If criticism of the narrowness of technological courses in universities was valid, then surely the more effective and rapid solution was not to remove them from the wider sphere of studies found in a university and to develop the technologies in more narrowly based institutes; the remedy was rather to integrate technology more closely with other fields of study, particularly with social studies, than to dissociate them further. Finally, it was urged that medicine, dentistry, veterinary science and agriculture had all been brought successfully within appropriate universities. These subjects represented the application of science to the practical problems of healing sickness and producing food. Was there any inherent reason why other forms of the application of science should not both benefit by inclusion in the universities and in turn bring benefit to the universities ?

These, then, were the main arguments current in 1952 when, on June 10, the Chancellor of the Exchequer asked the University Grants Committee how best rapidly to build up at least one institution of university rank devoted predominantly to the teaching and study of the various forms of technology. The Government, not without previous consultations in various quarters, hinted that, in its view, the best way of achieving this might well be to concentrate this effort on the Imperial College of Science and Technology.

The University Grants Committee had been considering the various issues for the past two years, and had been discussing various possibilities with those who were most likely to be affected by any Government decision. It was therefore in a position to tender its advice to the Chancellor on July 25. It shared the Government's view that the best course was to concentrate on the development of the College and in doing so came down on the side of those who had argued first, that it was preferable to build on existing foundations and, secondly, that, at this particular stage in the development of technology, it was preferable to bring about the expansion in an institution where a wide range of subjects, including arts, social studies, pure science and various other applied sciences were already taught. Imperial College in the wider context of the University of London met these two principles.

Advice on other important issues was also included in the Committee's report to the Chancellor, all of which stemmed from close consultation with the University and the College. First, it was decided to carry out the expansion on the existing site; the possibility of transplanting or of developing the College on more than one site was dismissed. Among the reasons lying behind this decision was the fruitful co-operation which was developing with the London School of Economics, a development which was expected to broaden the education of technologists. Moreover, the College itself attached great importance to its integration with the University of London. It had urged that full emphasis should be placed on the position of the College as a corporate member of the University with which its future was categorically linked; the test of any plan for the further development of the College might well be the nature of the contribution it could make to the stability and welfare of the University as a whole.

Secondly, the College said that it could contemplate, given the necessary recurrent grant, an expansion from the existing level of 1,637 students to 2,100 by 1957. A further major expansion up to 3,000 in the quinquennium 1962–67 and even thereafter to 4,000, was not impossible provided the necessary financial resources were put at the disposal of the College. The expansion would be relatively greater in engineering than in the pure sciences, and there would be a shift in emphasis from undergraduate to postgraduate work, large numbers of postgraduate students being recruited from graduates of other universities.

By an immense effort, the College had prepared, by the end of 1953, its academic plan outlining the means by which it proposed to meet the tasks which had been set for it. This plan was approved by the University of London in December and by the University Grants Committee, with minor reservations, in the same month. Its immediate implications in terms of rebuilding and building were worked out almost immediately. After the resolution of the Collcutt Tower controversy in 1957, this plan became known as the Jubilee Scheme.

In retrospect, the controversy in the late 'forties and early 'fifties, while it undoubtedly caused delay, probably led to a greater and more rapid development of the applied sciences than might otherwise have occurred.

Hard on the heels of the Government decision on Imperial College came, in July 1953, a further invitation to the University Grants Committee to work out plans for higher technological education outside London—an invitation which led to major developments in Glasgow (at both the University and the Royal College of Science and Technology), Manchester (again at both the University and the newly incorporated College of Science and Technology), Leeds and Birmingham, and to considerable developments at Bristol, Cambridge, Sheffield and elsewhere.

The present situation, and the estimates of student numbers for the new quinquennium, bear out the contention of those who argued that expansion should not be confined to one institution but be dispersed, and that the rapid increases in the numbers of American and Russian technologists were the result of widespread expansion and not the development of a single institution. It may well be that the attainment of the highest levels of research and teaching may call for a certain amount of concentration but not attainment of numbers. The universities had some 10,000 full-time students in technology in 1952–53; the expected numbers for this academic year lie between 18,000 and 19,000; the estimates for 1966–67, if the universities' hopes can be realized, suggest a total of more than 27,000. By 1966–67 there may well be at least three university institutions with 2,500–3,000 full-time students in techology and six institutions where the numbers of technologists exceed the numbers in pure science. In general, the universities have not feared an undue 'unbalance' through the expansion of the applied sciences; they have in fact welcomed this development.

In parallel with this development in the universities has been the welcome development of work of university standard in some of the technical colleges. The origins of this growth are found in the reports of the Percy Committee in 1945 and the Barlow Committee in 1946. Again in the Government's White Paper on Higher Technological Education in September 1951, the proposals for expansion in the universities were accompanied by proposals for the development of advanced courses at suitable technical colleges. In 1955 the National Council for Technological Awards was set up, and the first 34 holders of the diploma in technology emerged in 1958. By 1961–62 there were some 111 such courses distributed in 28 colleges, which are now recognized as leading to the diploma in technology, covering 6,200 students. Finally, the establishment of ten colleges of advanced technology with a target set for their next stage of development of 21,000 full-time and sandwich course places, most of which will be in technology, is a further significant indication of the importance of technology in our national life.

I am presuming now to make a rapid survey of what has been achieved in respect of the expectations underlying, and aroused by, the Government's decision ten years ago. First, I would take the tangible results and of these the site of the Imperial College of Science and Technology might take pride of place. The College can now envisage a precinct of some twenty-five acres against the previous nine acres, and the bulk of this is now in its possession. Many doubted whether sites within the rectangle could be made available so quickly, though it must also be clear that the rate of clearance has not completely overtaken the College's academic development plans. On the other hand, the unexpected but immensely welcome availability of the Prince's Gardens site in 1956 must have been a relief to the College as well as a merited recognition of its progress and needs. But much credit must be given to the Government departments, such as the Ministry of Works and the Ministry of Education, which readily co-operated in speeding up plans for the rehousing of their particular responsibilities, such as the Imperial Institute. Controversies also inevitably arose over the planning of the site and the demolition of quasi-historic buildings. Here again credit should be given to the present Home Secretary for the part he played in finding a solution to the "Collcutt problem". It is probably a fair estimate that these difficulties have added about two to two and a half years to the period originally envisaged for the building programme. But ten new buildings have been completed or almost completed, three more have been started and five are planned—a very significant part of the massive building programme, amounting to more than £17,500,000 and aiming at giving the College a total of some 2,320,000 sq. ft. of floor space in what is almost the heart of London. Problems still remain; but it is hoped that it may not be too long before the College enjoys the resources which are necessary for the fulfilment of its academic plans.

The next most tangible evidence of achievement is the growth in student numbers. The Government's hope was that the College would attempt to double its numbers from its existing level of 1,650 students in ten years and the College said that it could contemplate, given the necessary resources, a student population of 3,000 full-time students by 1962. This target has practically been reached at the beginning of this academic year in spite of the delay of two to two and a half years in the provision of accommodation; what is more, the College, in its plans for the present quinquennium, envisages a total of about 3,750 students by 1967.

The physical upheaval has not interrupted the continuous flow of graduates. The output of first-degree students has risen almost continuously from about 280 in 1951–52 to about 500 last year, while the output of students with postgraduate degrees and diplomas has also risen simultaneously from some 260 to more than 500. Surely these figures are a vindication of those who maintained that the building up of an existing institution would give earlier dividends than starting *de novo*. This policy, in the case of the Imperial College alone, has resulted in a bonus of more than 2,000 graduates during the ten years in which the total output of a new institution would have been negligible. It is worthy of note, too, that while the postgraduate output of pure scientists has risen by 43 per cent, the postgraduate output of technologists shows the very remarkable and exceptional expansion of 133 per cent. Nor does there appear to have been a drop in standards; the student wastage-rate has fallen in recent years, and the proportion of students who have completed their course in the minimum time has risen significantly.

So far as academic staff is concerned, its increase has not kept pace with the increase in student numbers, but the proportion of higher posts has been markedly increased; and to-day the College academic staff includes two Nobel prize-winners and twenty-four Fellows of the Royal Society.

There is further evidence of the reputation of the College in its power to attract postgraduate students. This has exceeded even the hopes entertained by the College's advocates in 1952. To-day, some 75 per cent of its graduate students come from other institutions. Some 520 postgraduate students, about 50 per cent of the postgraduate school, come from seventy-one oversea countries—twenty-five Commonwealth and forty-six foreign—a development that entitles the College to be regarded as international.

This is not the occasion or the place to give a detailed account of the academic developments which are the basis of this remarkable achievement nor of the non-academic developments, such as the growth of the corporate life of the College, over the past ten years. It may not be too indiscreet to mention that one of the few reservations which the University Grants Committee raised on the College's academic plan in 1952 was what it considered to be the inadequate attention to the need for student amenities. The development of the Union, the interest in student affairs as evidenced by the College surveys on student views and problems, the acquisition of the Prince's Gardens site which has enabled the College to provide six student residences and three refectories have therefore given the Committee particular satisfaction.

There is at least one academic development by the College which has brought particular satisfaction to the University Grants Committee. In 1950 the Committee, impressed by the need for the expansion of postgraduate instruction, especially for those who had had some professional experience, gave special grants to selected universities to stimulate this provision. The College has been in the forefront in this movement. In 1951–52, it offered some 36 postgraduate instructional courses, two-thirds of which lasted more than six months and one-third were shorter; by 1961–62 the number had increased to 87 of which about one-half were the shorter courses particularly required by industry. Again, evidence of the growing status of the College in the life of the nation is seen in the benefactions which it has received in recent years from sources other than the Government—foundations, individuals and industry.

In the face of all such evidence, it must be agreed that this decade has seen the realization of much that the Government expected. The student target has practically been reached; the proportion of graduate work has risen; the emphasis on technology has grown; standards have risen; the corporate life of the College has grown in vitality and strength.

There still remains a fundamental question. Are the great resources in manpower and materials which are being put at the disposal of the universities and colleges being, or are they likely to be, utilized to the fullest advantage? In other words, is the education provided for these scientists and technologists designed to give the undergraduate or postgraduate those foundations which will be of the greatest value to him and to society in the economic and social circumstances in which he is going to have to live and work? Can one be certain that what has been so admirable in the past will be equally suitable in the next thirty or forty years which constitute the working life of a generation of undergraduates?

Unfavourable comparison between the British university product and his Continental or American counterpart, at least in technology, has already been cited. Now suggestions are being made that the Russian product is better trained and, possibly, better educated. The problem is, of course, closely tied up with the amount of time made available to give this educational background. Prof. Wood, of the University of New South Wales, has given at least a broad picture of the great differences in the length of what he defines as first-degree courses in mechanical engineering. At one extreme is the 'fast' honours course at Cambridge, which requires about 1,100 hours of class attendance spread over two normal academic years, plus part of the long vacation; at the other, Turin, which requires almost 5,000 hours of attendance (the normal age of entry of the students being much the same in each case). It is clear from his investigation that the English universities are given relatively little time in which to do their best for their undergraduates, even if some allowance is made for the greater specialized preparation which the English entrant has received at school—a specialized preparation which only serves to emphasize the need for breadth at a later stage.

Looking first at the content of the academic courses, it is clear that Imperial College in any event has noted the trend in the applied sciences, as in the pure sciences, towards increasing emphasis on firm foundations in the basic sciences and the deferment of more specialist education to the later years of the undergraduate course or even, for a proportion of the students, to a postgraduate instructional year. This broadening of the base of the undergraduate course should commend itself to industry but in spite of this, the corollary—the response of industry to the provision of postgraduate courses—must have been as disappointing to the College as it has been to engineering schools elsewhere in the country. It is generally agreed that the benefits of such specialized postgraduate courses are enhanced, both for the departments concerned and for the students, if these are taken after a period of industrial experience, but it is doubtful whether the response has equalled the effort put into the provision of these courses.

But breadth in education is attained not only in the classroom and the laboratory. This has been fully recognized by the College, as has already been indicated, in its Jubilee scheme. Most important, in my opinion, has been the expansion and improvement in the provision of student amenities in the College. Education of students by students is one of the most important parts of university life, and it becomes impossible if the necessary facilities and amenities are lacking. The Students' Union has doubled in size, refectories and common rooms have been rebuilt and student activities—athletic, cultural and social—have been stimulated. The provision of student rooms in the College precinct has increased from 100 to more than 600. All these are closely interrelated factors in the remarkable growth in the corporate life of the College. The College has wisely recognized the dangers of fission as academic institutions grow in size and has consciously striven to counter them.

Then there is the programme of general studies which the College has undertaken; some 20 per cent of the students regularly, and some 45 per cent frequently, attend the lunch-time meetings. The provision of social and management studies further recognizes the need for the broadening of technological education and for an understanding of human relations as an essential feature of leadership. Links with the London School of Economics have made this possible. Third-year engineering students and some postgraduates attend lectures on economics and business organization at the School, and nearly two-thirds of these voluntarily now submit themselves to an examination, which counts towards the finals.

It may be questioned, however, whether this link with social studies has been developed as strongly as it should be in the engineering schools of Great Britain. The growing importance of social studies is recognized in the universities; most of them have established or are planning separate faculties in order to facilitate their development and expansion. The social studies, in the opinion of many, form the most natural bridge between the sciences and the arts, and their development to meet the specific needs of the scientist and technologist will undoubtedly be encouraged if the newly emerging faculties are given an indication of how they could help. Recent discussions in most universities have disclosed an affinity of interest between those in the social studies and those in the technologies. Students, too, as is shown by the College's own experience, recognize this natural bridge.

Previous Special Visitors to the Imperial College have emphasized in their addresses the need for the combination of quality of mind with quality of character. Lord Bridges stressed the importance of combining technical knowledge with understanding of and sympathy with human problems. Lord Weeks, from his wide experience, suggested that professional competence must be accompanied with a natural sympathy with the aspirations and outlook of people. Lord Hailsham laid the challenge that the universities, if they were to fulfil their role, should be the source of a unity of purpose and should furnish their students with an integrated understanding of man and his environment. It is surely clear from the thoughts of these men of experience and wisdom that technological proficiency is not enough. Can the foundations of these other qualities be more surely laid during the undergraduate years ? Many people believe that they can, but some also think that, since these years are already so short, more could be done if, in addition to a change in emphasis within the undergraduate course, the five years devoted to professional training were re-allocated. There appears to be some justification for these thoughts and they merit at least examination. It is not easy to assess the present trends and to hazard a guess as to circumstances some twenty to thirty years ahead; but it is necessary to attempt to do this if present generations of undergraduates are to be properly equipped to solve the problems which will face them during that period of their lives when they face greatest responsibility and exert greatest influence.

There is one further thought which might also be considered by engineering schools, though not necessarily by Imperial College, which is already fully occupied in the fulfilment of its accepted policy. The Scientific Manpower Committee of the Advisory Council on Scientific Policy in its last report indicated that the possibility must be envisaged that a balance between the supply and demand for certain types of scientists and technologists might be equated sooner than some people thought. This, it rightly insisted, was not a situation to be feared but possibly to be welcomed since it would then make possible a rational use of the scientific disciplines. "It should mean that at long last we shall have a supply of qualified manpower with a scientific training for management, administration and the professions generally, in addition to those who up to the present have been drawn inevitably into vocational employment. We do not doubt that scientific education will adjust itself to this new prospect."

The University Grants Committee, in its most recent annual report, forecast that the prospective increase in the number of graduates will lead not only to their employment in more posts of the kind already filled by graduates, but also in posts not previously occupied by graduates or in new kinds of posts. Why should these new careers for graduates not be met in part by graduates who have been educated through technological disciplines ? Many people believe that many of the basic disciplines embodied in engineering courses in the universities are sound disciplines and, indeed, have some advantages for many students in that they can also include the application of knowledge. Is it not possible then to devise a course or courses which embody some of the engineering disciplines but which would be directed not so much to the gaining of professional qualifications as engineers as to giving undergraduates who may be entering other professions the approach to problems, the methods of analysis and the intellectual disciplines which have stood the engineer in good stead ?

PROGRESS OF CANCER RESEARCH

THE hundreds of investigations in cancer research referred to in the 1961 (thirty-ninth) report of the British Empire Cancer Campaign[*] can be divided into three main groups dealing with: (1) carcinogenesis and the general biology of tumours; (2) radiobiology and radiotherapeutics; (3) clinicopathology.

(1) Carcinogenesis and the General Biology of Tumours

Epstein at the Middlesex Hospital has been studying the electron microscopy of HeLa tumour cells, originally derived from human cervix carcinoma, which show some unusual features: "Rows of open spaced parallel smooth cisternæ were common in the cells, as were membranous elements composed of closely applied pairs of narrow cisternæ arranged either in rings or extending through the cytoplasm in a haphazard manner. In addition, vacuoles containing crystals and areas of material thought to be glycogen were also found".

Ambrose *et al.* find that when erythrocytes are examined by microelectrophoresis the cell surface is observed not to contain exposed phospholipid but is almost completely covered with highly charged groups of neuraminic acid derived from the mucopolysaccharide blood group substance; tumour cells, on the other hand, "contain exposed phospholipid on the membrane; the amount of exposed phospholipid increases progressively as the cells acquire an increased negative surface charge, and show an increasing tendency to form metastases. About 40 per cent of the surface charge on the tumour cell can be removed with neuraminidase. It is therefore suggested that the surface of the tumour cell consists of a complex mosaic of phospholipids, mucopolysaccharides and mucoproteins, in which both phospholipid and mucopolysaccharide contribute to the surface charge".

Mercer and Birbeck, in work on the tumour cell surface, intercellular adhesion and tissue construction, find that the characteristic lesion of many tumour cells is their defective power of tissue construction, a defect probably associated with the failure to secrete adequate amounts of intercellular adhesives: "Replicas of islands (or clusters) of HeLa and hamster kidney cells have been obtained, which reveal that, although these cells seem at first glance to be capable of forming epithelial-like associations, their intercellular gaps are large and common and areas where the surfaces are closely applied and parallel are rare. Cross-sections of such cell clusters reveal the same features". These workers also report that the Walker tumour contains virus-like particles.

The long list of carcinogens continues to grow; the latest additions to the catalogue are: penicillic acid, which induces malignant tumours in rats; complexes of iron and of aluminium with dextran ('Imferron' gave 52 subcutaneous tumours in 160 mice and 38 tumours in 90 rats); the cytotoxic compound ethyl methane sulphonate on injection into mice increased the incidence of lung and kidney tumours from 20 per cent (controls) to 89 per cent, but the more cytotoxic bifunctional alkylating agents myleran and chlorambucil did not increase the incidence of these tumours; cholesterol has been shown to be a potent carcinogen when tested on a sensitive strain of mice (Buffalo strain), 40 per cent of the mice developing subcutaneous sarcoma at the site of injection; cobalt, cadmium and nickel powders have been found to induce fibrosarcoma and rhabdomyosarcoma in rats; arsenic trioxide in the drinking water induced a small percentage of papillomas in mice so treated.

One of us (I. H.) might point out that while the conventional carcinogens have not been detected in the body, neither benzpyrene, nor dimethylbenzanthracene, nor methylcholanthrene, nor stilbene or azo-dyes, let alone plastic film, or cobalt, cadmium or nickel powder—the body *does* contain large amounts of cholesterol.

Glucksmann and Cherry have induced carcinoma in mice and sarcoma in rats by the application of the most potent of carcinogens (dimethylbenzanthracene) to the genital tract in rats and mice; in mice 58 carcinomas and one sarcoma of the vagina and 50 carcinomas of the vulva developed in 80 mice treated; in 352 rats 17 sarcomas and one carcinoma of the vagina, 27 carcinomas and one sarcoma of the vulva, in 24 rats 16 sarcomas of subcutaneous tissue and in 42 rats 35 sarcomas of muscle.

Orr has contined his studies of mammary carcinoma induction in mice by the application of methylcholanthrene; he finds that neoplasia is facilitated by hormonal conditions such as castration of males and subsequent implantation of ovarian grafts, virgin females grafted with mammotropic pituitary tumour, non-susceptible females mated with vasectomized males and breeding without suckling.

From the South African Institute for Medical Research comes the report that in the Bantu Negroes, who are known to have a liver cancer incidence 50 times as high as Whites in the same region . . . "The incidence of cancer of the œsophagus among the Bantu on the Witwatersrand is rising steeply, and this cancer now exceeds cancer of the liver as a cause of death in males and females. Mortality figures for Whites and Asians show no evidence of a rise while mortality for coloured males reveals an increase though not to the same extent as that obtained among the Bantu.

"From figures obtained from the hospital services of the mining industry it is evident that œsophagus cancer among miners also demonstrates a steep increase, and significantly many of these miners were recruited from the Transkei.

"An investigation of cancer of lip in histological preparations in Whites and Bantu in the Transvaal over the period 1949–55 reveals greatly increased susceptibility among Whites and in particular among White males. Among the Bantu the albino also show a high susceptibility. Deaths from cancer of the lip among Whites show a much greater preponderance of males than obtains in cancer of the skin among Whites; therefore, the effect of sunlight would seem inadequate to explain all of this difference, and it seems probable that other factors, such as smoking, contribute largely to the White male susceptibility."

In the field of cancer chemotherapy, Ross and Davis have examined the possibilities of exploiting the

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[*] British Empire Cancer Campaign. Thirty-ninth Annual Report covering the year 1961. Part 1. The Chairman's Statement and the Accounts of the Central Organization. Pp. xxxviii. Part 2: The Scientific Report of the Researches undertaken by the Central Organization and its Autonomous Councils in the United Kingdom, and by some of its Affiliated Organizations Overseas. Pp. xxxix–lxviii + 664. (London: British Empire Cancer Campaign, 1962.)

pH differences between normal and neoplastic cells: "It can be calculated (Ross, 1961) that if the pH of plasma is 7·4, of normal cells is 7·0, and of cancer cells is 6·0, then a base of pK_a greater than 8 can achieve ten times the concentration in cancer cells as in normal cells. Since glucose administration selectively lowers the pH of cancer cells this should tend to concentrate basic drugs in these cells with enhanced cytotoxic activity."

There has been a rapid increase in the application of tissue culture technique to cancer research in the last few years: Easty and Ambrose have been investigating selective cytotoxic effects on tissue cultures of hamster kidney tumour cells and normal kidney cells; they have used royal jelly acid, decanoic acid, carrageenin (a polysaccharide extracted from Irish moss), and snake venoms (from *Naja haje* and *Bitis arietans*) which act via phospholipases on the cell membrane, and at dilutions of less than 1 in a million.

At the Chester Beatty Institute, a group of workers has been investigating the action of the cancer chemotherapeutic drugs on tissue cultures of human tumour biopsy material, such as glioma, seminoma, hypernephroma, melanoma, rectal carcinoma and meningioma: "Observations show that the various nitrogen mustard derivatives differ in their toxicity towards human tumour cells, and in their toxicity towards cells from different *in vivo* sites. So far, it appears that melphalan and chlorambucil are more cytotoxic in most cases than thioTEPA. In experiments where the effects of melphalan, melphalan ester and orthomerphalan were studied on the same cells it was found that melphalan ester was in each case more cytotoxic than the other two compounds".

The furore about the relation of DNA and RNA to neoplasia has declined somewhat; Kirby reports that RNA prepared from rat and mouse tumours by his new method have a similar base composition to RNA from normal rat liver (G: A: C: U; 32·8: 19·2: 31·0: 17·0).

Elson and Whitcutts's studies of the protein characteristics of rat hepatomas induced by azo-dyes and of the normal liver are based on the work of the Millers, Cohen, Sorof and others, who found that during liver carcinogenesis the basic proteins are deleted along with several glycolytic enzymes; starch-gel electrophoresis suggested that hepatomas exhibit a characteristic protein pattern of the cathode bands.

At the Westminster Hospital, chromatography of cell-sap proteins and further examination by immuno-electrophoresis have shown that the general tendency is for basic proteins to decrease and acidic groups of protein to increase in tumours and surrounding tissues. "The results as a whole show that the glycoproteins of tumour tissue are similar to serum glycoproteins in hexose content, kind of sugar present, electrophoretic mobility, chromatographic behaviour and in certain instances immunological specificity. They tend towards high levels in tumours and surrounding tissues. On the other hand, a group of basic proteins, which are absent from serum, are diminished in tumours as opposed to normal tissue."

At Birmingham, Stacey *et al.* have been working on proteins in lung carcinoma, using electrophoresis, diffusion in agar gel against specific sera and immuno-electrophoresis against similar sera; the analysis of eight tumour samples and six normal tissue samples revealed consistently that whereas normal tissues contained albumin, α_1-, α_2- and β-globulin, and very little γ-globulin, tumour tissues contained much more albumin and an abundance of γ-globulin.

Miller, at the Chester Beatty Institute, has been investigating the relation of immune tolerance and the induction of lymphoma by the Gross virus, to thymectomy; mice which have had the thymus surgically removed when one day old are unable to produce antibodies later on in life and 60–70 per cent die at about 4 months from progressive wasting. The capacity for producing antibodies can be restored by grafting thymus from another mouse; moreover, the thymus is essential for the induction of lymphoma by Gross virus. While 2-month-old mice are not susceptible to the virus, thymectomized mice of the same age bearing day-old subcutaneous syngenic thymus grafts are fully susceptible. By the use of a marker chromosome Miller has shown that the dividing cells of the thymic lymphosarcoma were host cells, not graft cells, suggesting that the "host cell lymphocytes in virus infected thymectomized mice can be transformed to leukæmic cells after populating the thymus graft . . . (suggesting) that the thymic influence responsible for the leukæmic change is a non-cellular indirect one, and is in part genetically determined".

Salaman has been studying one of the latest additions to the mouse tumour viruses—the Moloney virus—which induces leukæmia in mice and in new-born rats. He finds that foster-nursing will transmit the virus in the milk and that foster-nursing by untreated females of progeny of virus-inoculated parents prevents leukæmia developing in the young, suggesting that Moloney virus is transmitted via the milk and not via the embryo, which "indicates an important difference between Moloney virus and the agent responsible for the 'spontaneous' disease in AK (mice). Foster-nursing newborn AKs, or even AK embryos removed by Cæsarean section, by females of low-leukæmic strains only slightly reduced the incidence of leukæmia".

Still another virus (*PLDH*-elevating, or Riley virus) has been detected in mouse sarcomas Nos. 37 and 180 and in other serially transplanted tumours and leukæmias but not in most spontaneous tumours or virus-induced tumours and leukæmias; this virus, on injection in mice, can give a 50-fold elevation in the plasma lactic dehydrogenase (*PLDH*).

Investigations of the antigenic properties of cells in tissue culture at the Strangeways Laboratory in Cambridge have opened up some interesting possibilities. Since the classical work of Earle at the U.S. National Cancer Institute, transformation of the characteristics of cells on continued subculturing *in vitro* has been found to give rise to: (*a*) neoplastic changes; (*b*) a loss of Forssman antigens. Daniel has produced evidence that loss of antigens is due to derivation of the clone culture from an antigen-negative cell and not to transformation *in vitro*. This finding might suggest that the cells used in a primary culture of normal cells are a heterogeneous collection some of which are already poised, as it were, for neoplastic transformation. Green's deletion–immunological theory of carcinogenesis postulates that the identity-antigens of normal cells which regulate the proliferative and invasive potentialities in relation to other cells are lost in carcinogenesis, with the result that cells can no longer recognize the monitoring of normal growth.

Baldwin at Birmingham has confirmed that liver cell sap from rats undergoing hepatic carcinogenesis by azo-dye loses two normal antigens as detected by immunodiffusion technique; by using immuno-electrophoresis he finds that at least sixteen antigenic components can be detected in normal rat liver cell sap and six or seven in tumour fractions; these results might be evidence in support of the deletion theory of carcinogenesis except for the unexpected finding that new antigens are formed in tumours at the same time as normal antigens are lost . . . "these differences were not due to dietary effects during carcinogen feeding and thus it is concluded that there is a loss of two major antigenic components of normal liver microsomes during induction of liver tumour with DMAB . . . immuno-electrophoretic analyses are now in progress and results so far obtained confirm that three of the major antigenic components of tumour cell sap are not detectable in normal liver. These findings suggest that abnormal antigens are formed during tumour induction with DMAB".

Koller has examined the chromosomal pattern in murine leukæmias using mice of the *AK* and $C_{58}B1$ strains, and concludes that no gross chromosomal abnormalities can be detected which might differentiate normal cells of thymus, spleen, bone marrow or lymph node from malignant cells. But cytological analysis of leukæmia cells in a C_3H mouse which had been injected at birth with Gross leukæmia virus shows that the thymus, spleen and bone marrow contained many cells with 41 chromosomes (normal number = 40). The frequency of abnormal cells in the three tissues was 88, 27 and 17 per cent.

Clark at Oxford finds that in carcinoma *in situ* of the uterine cervix the chromosomal picture is one of instability and chromosomal aberrations precede the onset of invasion; tetraploidy was frequent (96 chromosomes) and other abnormalities were visible such as cells with 46 chromosomes; in one case 53 was the modal number of chromosomes. Work at the Christie Hospital has shown that abnormal chromosomes characterize tumours in different species of mammals (*Microtus*, rat, man); earlier work in this field referred to: "Peripheral blood and bone-marrow from leukæmic patients have also been studied for chromosome abnormalities, but apart from the already established abnormality of chromosome 21 in chronic myeloid leukæmia (Nowell and Hungerford, 1960, Baikie *et al.*, 1960) the data cannot yet be evaluated. Children with gliomas, reticuloblastomas, neuroblastomas, and epitheliomas, all of embryonic ectodermal origin, have an ABO-blood group distribution which can be explained by trisomy for this locus or the chromosome carrying it".

(2) Radiobiology and Radiotherapeutics

More than one-third of the report deals with projects that come under this heading, and the increased emphasis on radiobiology is particularly noticeable. There are continual improvements in the therapeutic application of radioisotopes, such as the incorporation of radioactive phosphorus into plastic contact lenses for treating tumours of the eye. The use by J. S. Mitchell of drugs which are very highly radioactive for killing those cells in which they concentrate, represents an entirely new approach to the clinical use of radioisotopes though the outcome of an initial clinical trial with forty-eight patients has been rather disappointing. Much research effort has to be devoted to the very high-voltage equipment which is now much more widely available and which requires careful investigation to determine optimum conditions and to develop reliable dosimetry. It is becoming clear, however, that the contribution which further advances in physical and engineering techniques can make in the improvement of radiotherapy is limited and the hope for the future lies in radiobiological approaches such as changing the radiation response of cells in a controlled manner or irradiating cells at their most sensitive stage. Our ignorance about most of the key reactions in the long sequence of events between exposure of tissues to X-rays and the death of cells is still complete, and emphasis is therefore rightly given to basic investigations and, in particular, to the primary radiochemical reactions. Free radicals formed in the surrounding water contribute to these, and their reactions under physiological conditions were thought to be complete within a millionth part of a second. Groups at the Christie Hospital in Manchester and at Mount Vernon Hospital have both used the powerful new techniques of flash radiolysis to study the reaction of free radicals in water and find that they are rather slower (that is, milli- as opposed to micro-seconds) than expected and this indicates that even these very early events are far from understood. Many investigations are directed to determine the changes produced on irradiation in biologically important materials, and the detailed data made available by J. Weiss *et al.* on nucleoproteins represent a highlight in this field, though their relevance to biological problems remains to be determined. The observation by Wormall and Willis that peroxides formed from unsaturated fats are powerful enzyme poisons may provide an explanation for a number of puzzling observations for biological damage which occurs away from the site of irradiation.

Of more direct concern to radiotherapy is the increasing emphasis that is now being given to methods whereby the radiosensitivity of cells can be varied by pre- and post-irradiation treatments. C. Dean, at the Chester Beatty Research Institute, has found that iodoacetamide lowers the dose needed to kill bacteria and that the effect is most dramatic with the most resistant organisms. While iodoacetamide sensitized to X-rays both under aerobic and anaerobic conditions, *N*-ethylmaleimide was observed in one organism studied by D. L. Dewey to sensitize only in the absence of oxygen.

At Mount Vernon a metabolic poison, 6-amino nicotinamide, was tested for sensitization of mammalian cells, at the instigation of L. G. Picciotto, and found to reduce the X-ray dose needed to kill by about 40 per cent, an effect similar in magnitude to that found with iodoacetate in mammalian cells.

W. M. Dale *et al.* have shown that sensitization of anoxic cells with nitric oxide takes place even after all the gas has been removed and that the nitric oxide need not be present during irradiation. They conclude that the sensitizing effect is related to enzyme poisoning and that its sensitizing action is dissimilar from that of oxygen. Interference with the normal metabolism of the cell before irradiation by storage at low temperatures was shown by L. H. Gray to increase the sensitivity of Ehrlich ascites cells as judged by chromosome damage. Quite other considerations led radiotherapists at St. George's and the Royal Marsden Hospitals to examine the possibility that mild hypothermia increased the sensitivity of certain brain tumours, and the clinical results seem to bear out this suggestion.

All these experiments direct attention to the concept proposed more than thirty years ago, but largely neglected in recent years in the enthusiasm for the 'target theory' approach, that the physiological state of the cell has a great influence on the radiation response of cells. In addition to the sensitizing action of metabolic poisons the stage in the cell-cycle at the time of irradiation affects the radiation response, and the time for maximum sensitivity seems to vary for different effects such as cell death or chromosome damage. O. G. Fahmy has found that during spermatogenesis the spermatocytic stages, most probably during a phase of meiosis, are most sensitive to mutagenesis.

Changes in external condition and physiological state apart, how far do mammalian cells differ in their radiosensitivity *per se*? H. B. Hewitt thinks that if oxygenation is controlled, all cells are approximately equally sensitive and he suggests that a quantitative approach to radiotherapy can be based on this premise. In support, he states that five clearly distinguishable murine leukæmias all had closely similar survival curves when irradiated with γ-rays, but P. Alexander and Z. B. Mikulski have found that mutants of varying radiosensitivity arise spontaneously when lymphoma cells are cultured *in vitro*. While the radiation response of the parental strain fell within the range of values found by Hewitt, some mutant strains were twice as sensitive. No difference could be detected between mutants in the DNA content per cell or the chromosome number—both being diploid. *In vivo* studies also emphasize difference in radiosensitivity; R. Barrow has made an extensive investigation of the radiosensitivity of the various lymphocytes and concludes that the primitive stem cells are particularly resistant. A. Glücksmann finds marked differences in the radiation response of cells in the retina between embryonic and new-born rats.

As yet, the practice of radiotherapy has not benefited significantly from radiobiology. Still, there are indications that a scientific basis will replace the enlightened empiricism of the present time

To basic cancer research the carcinogenic action of ionizing radiations is relevant and experiments are reported on the induction of thyroid tumours by radioactive iodine (I. Doniach) and on bone tumours by radioactive metals (Institute of Cancer Research) These investigations seem to have been largely directed to the accumulation of quantitative data and a greater emphasis on mechanism might be more rewarding.

(3) Clinico-pathology

Schade studied numerous early cases of carcinoma of the stomach, and finds them invariably accompanied by severe chronic gastritis. He notes the difficulty of distinguishing epithelial changes due to inflammation and regeneration, and those of early carcinoma by conventional staining methods. On account of these observations an investigation of enzyme patterns was initiated: (1) Intestinal metaplasia of the diseased gastric mucosa shows the same pattern of changes quantitatively as have been found by Wattenberg on the colonic mucosa, suggesting histo-chemical and, therefore, functional as well as morphological identity; (2) the reaction patterns of colonic adenocarcinoma and adenocarcinoma of the stomach showed some similarity; (3) most interesting was the finding that leucine aminopeptidase and alkaline phosphatase gave positive results in gastric carcinoma as well as intestinal metaplasia, while they are negative in normal gastric mucosa; (4) gastric carcinoma showed a variety of reaction patterns.

Although not stated in the report, this sort of investigation is fraught with difficulties and disappointment. It can only be satisfactorily initiated where clinicians and laboratory workers are wholly co-operative, for of necessity quantities of fresh human tumour material are only to be had in busy hospitals, where 'research' to a surgeon who has been in the operating theatre for 6 h is a call of superorogation, and a 5.30 p.m. close to the laboratory workers' day is not sacred. The fairly uniform, repeatable results found in the simple implantation type of experimental carcinogenesis are not to be expected, for two main reasons. First, the stage of its development at which a human tumour is removed is very variable—symptoms or signs may have been present for weeks, months or years—and. secondly, laboratory animals are selectively inbred for the specific purpose of producing uniformity of behaviour, while the behaviour of quite comparable tumours in diversely genotypic man is notoriously variable. Our knowledge of the metabolic activities and histochemical properties of human tumours is minute, but is also uneven for mainly clinical and practical reasons. For example, more is known about thyroid carcinoma in this regard than any other tumour, though it is an infrequent tumour. The reason for this lies in the fact that the thyroid produces a simple aromatic iodine compound, the synthesis and disposal of which is easily followed by accurate radiobiological techniques. Carcinoma of the stomach, on the other hand, becomes available for histochemical study after perhaps 2 h of manipulation and progressive impairment of its blood supply, necessary antecedents to its removal. and presents to the research worker as an ulcerating, fungating, malodorous infected tumour. It is no wonder that the results of histochemical assay vary from tumour to tumour, and even within the same tumour. This last feature may even result from the variety of morphology within an individual tumour, an important fact to be considered where correlation of morphology and histochemistry is attempted.

Galton *et al.* point out that the assessment of the results of therapy in the malignant lymphomas is bedevilled "because the results of investigations in the different basic disciplines are used to create separate nosological entities, instead of being accepted as descriptions of different aspects of each patient's illness" . . . in fact, the fable of the seven blind beggars describing an elephant to each other, each wholly convinced that the part to which he clung was the whole beast. Part of the difficulty lies in the difficult, or debatable, cytology and histology associated with lymphomas (malignant diseases of the reticulo-endothelial system) as a group. This alone makes communication difficult and has our sympathy, but it is inexcusable that there are still scores of classifications of the same disease group in use, up and down the country, and it is not unknown in the same hospital for each member of the staff to have his own system.

In some organs, the incidence of primary tumours is so low that referring cases to a central panel is of value for assistance in the diagnosis of a rare tumour or variants of the usual types found, and for the building up of a body of clinico-pathological knowledge in the country, in time to replace older. less accurate opinions. The activities of the Testicular

Tumour Panel are a case in point. Since the Panel was formed four years ago, 770 specimens have been registered, follow up information is available in the majority, and results of analysis of 457 patients with malignant tumours are presented. The two main types of testicular tumour, seminoma, arising from the tubular epithelium, and teratoma, a mixed tumour of uncertain histogenesis, each accounted for 40 per cent incidence, the former with an 8·6 per cent mortality, the latter with 41·7 per cent. However, in the teratoma groups there is a gradient of malignancy approximately related to the degree of differentiation of the tumour. Sufficient lymphomas have been examined for a gross and histological pattern to be readily recognizable. A report from such a panel is of outstanding assistance to the theory and practice of human medicine.

P. ALEXANDER
I. HIEGER
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OBITUARIES

Dr. A. P. Orr

ANDREW PICKEN ORR was born in Ayrshire on August 6, 1898, and educated at Kilmarnock Academy. He later proceeded to the University of Glasgow and then passed into the army, being wounded and taken prisoner in France in 1918. Returning to the University, he graduated in chemistry and geology and then worked for a time under Prof. Noel Paton in the Department of Physiology. In 1923 he joined, as chemist, the staff of the Millport Laboratory of the Scottish Marine Biological Association. He remained in the service of that Association, which can never have a more devoted and unselfish member of staff, for the rest of his life. He became deputy director and at the time of his death was acting director.

Trained on the broad lines of the old Scottish university curriculum, he found the diversity of disciplines necessary in marine work immediately congenial. Although starting as a chemist, he became gradually more and more preoccupied with biological problems. With his colleague and close collaborator, Sheina Marshall, there soon began that long series of papers first on marine productivity, based on investigations in Loch Striven, and then on the biology of the ubiquitous copepod, *Calanus finmarchicus*, the main food of herring.

These investigations were to make the names of Marshall and Orr familiar to every fishery worker and marine biologist in the world and bring international distinction to the small laboratory at Millport. They are contributions of the highest importance to biological oceanography. The work on *Calanus*—at least until that time, it continued just as vigorously afterwards—was summarized in their joint *Biology of a Marine Copepod* published in 1955.

He was a member of the Great Barrier Reef Expedition during 1928–29. There he was responsible for the hydrographic work, both in the sea within and outside the Barrier, and also in the more intimate conditions on and around reefs where he found a striking diurnal range, especially in oxygen content and in *p*H. His geological interests were responsible for some unusually interesting observations and experiments, carried out again in collaboration with Sheina Marshall, on sedimentation and its effects on corals. For the first time a coral, *Fungia*, was shown to be capable of actively uncovering itself when buried under sediment.

During the Second World War he was engaged on research into the preparation of agar from the red intertidal weed, *Gigartina stellata*. Later, there were visits to Tromsö in Norway and to Woods Hole and elsewhere in the United States; but on both visits work continued to be centred on *Calanus*. He received the degree of D.Sc. from the University of Glasgow in 1934 and was elected a Fellow of the Royal Society of Edinburgh in 1948.

No one who has ever worked at the Millport Laboratory over the past forty years can fail to have known "A. P.". Increasingly, the laboratory came to be associated with his name and personality. He was possessed of an engaging combination of shrewdness and naiveté which can never be forgotten by all who were so fortunate as to become his friends. He was a distinguished man of science whose work will endure, and so, for the life of all of us who knew him, will his endearing personality.

He died on September 19, 1962, leaving his wife, Rachel Orr, a son and a daughter. C. M. YONGE

Prof. D. P. Riabouchinsky

PROF. DIMITRI PAVLOVICH RIABOUCHINSKY died in Paris on August 27, after nearly sixty years of active research in fluid mechanics, both in his native Russia and in France. For his many original contributions to the science, dealing with a great variety of fundamental problems, he may be rated one of the most outstanding scientists Russia ever produced.

Being of a family of considerable wealth, he was enabled to found his own research establishment on the family estate near Moscow: this became the famous Aerodynamic Institute of Kuchino, for some years probably the only large laboratory specially equipped for fundamental research into aeronautics. In his work there he secured the participation of Profs. N. E. Zhukovsky (Joukowski) and V. V. Kuznetsoff, and the *Kuchino Bulletins* included papers contributed by them.

Prof. Riabouchinsky retained personal direction of the Kuchino Institute until 1918 when, to ensure its continuance, he co-operated in its nationalization. Then he left Russia and, after a brief stay in Denmark, settled in Paris. He continued his theoretical and experimental research as associate director of the Fluid Mechanics Institute in the University of Paris and as professor of theoretical mechanics in the Russian Superior Technical School in France. When the Germans overran France in 1940, his laboratories were destroyed, but he was later allowed to resume his research work in a limited way.

The range of his work was unusually wide. He is probably best known for his publications on aerofoils and airscrews, rotating plates and cylinders, autorotation, boundary layers, vorticity, compressible fluid flow, shock waves and laws of dynamic similitude, and for his original designs of wind tunnels, aerodynamic balances, flow visualization methods and aerodynamic–hydrodynamic analogies. He was also keenly interested in recoilless guns and rockets, upper atmosphere research, sun-spot periodicity and astronomy: as early as 1914 he proposed that researches in the direction of interplanetary flight should be included in the programme for the Kuchino Institute.

Riabouchinsky remained active to the end, his most recent paper, reviewing many important results of his researches, appearing in the *Journal of the Royal Aeronautical Society* in August 1962. In 1961, at the invitation of Dr. G. A. Tokaty, head of the Department of Aeronautics and Space Technology in the Northampton College, London, another scientist who found it preferable to come to the West, he visited Great Britain and was welcomed by many distinguished British scientists. Although he made his home for so long in Paris, he never took French nationality and he never lost his pride in the Russian academic and scientific traditions from which he drew inspiration and to which he added so much.

S. BUCHANAN

Prof. A. R. Khan

BY the death on June 13 of Prof. Abdur Rahman Khan, the American Meteor Society lost one of its most eminent and oldest active members. In 1937 he began a correspondence with me and to send copies of his numerous and carefully made observations. His reports continued to arrive up to and including 1954, after which advancing years forced him to stop this exacting type of night work. In our files are found about 11,540 observations of meteors made by him. Most of them were plotted and full details also recorded on the data sheets. His work had special value due to distance of his station from those of other meteor observers. Indeed, during the whole interval mentioned we had only one other active observer in the Indian Peninsula, and him for only about two years. Hence, Prof. Khan's work had exceptional value.

So far, his work has been used here in studies of meteor magnitudes, long-enduring trains, fireball-rates and colours, and especially in *K*3, the *Hourly Rate Catalogue* covering nearly every hour of every night in the year published by the Smithsonian Astrophysical Observatory. They are at present being used in an investigation of fireball radiants, and other uses for his data undoubtedly will be found.

Few people in a great country have the distinction of being the only important contributor to a unique branch of an ancient science such as astronomy, but to my knowledge Prof. Khan attained this in India. In addition to all his valuable work which appeared in the American Meteor Society publications, he contributed many articles on meteors and the history of Indian astronomy to English and local scientific journals. It is not my purpose to review his important work and contributions to education at Osmania University College and elsewhere, as this can more properly be done by those more familiar with this type of his activities. However, one more thing will be mentioned to show the esteem with which he was held in the United States: he was research associate in the Institute of Meteoritics, University of New Mexico. I feel that I personally have sustained a great loss in the death of this able colleague and friend of such long standing.

C. P. OLIVIER

NEWS and VIEWS

Royal Society Awards: Royal Medals

H.M. THE QUEEN has approved recommendations made by the Council of the Royal Society for the award of the two Royal Medals for 1962 as follows: to Prof. S. Chandrasekhar, Morton D. Hull distinguished service professor in the University of Chicago, for his distinguished researches in mathematical physics, particularly those related to the stability of convective motions in fluids with and without magnetic fields; to Sir John Eccles, professor of physiology in the Australian National University, Canberra, for his distinguished investigations of the function of the spinal cord. particularly of the mechanisms of excitation and inhibition.

Experimental Physics at Nottingham : Prof. E. R. Andrew

PROF. E. R. ANDREW, professor of physics in the University College of North Wales, Bangor, has been elected to the chair of experimental physics as from September 1, 1963. In 1964 he will become the Lancashire-Spencer professor and head of the Department of Physics, following the retirement that year of Prof. L. F. Bates. Prof. Andrew was educated at Wellingborough School and Christ's College, Cambridge, where he held an Open Scholarship. After gaining first-class honours in both parts of the Natural Sciences Tripos, he joined the staff of the Radar Research and Development Establishment for three years, but left in 1945 to resume his studies at Cambridge. For his research work he was awarded the degree of Ph.D. in 1948; he was immediately elected to a Commonwealth Fund fellowship, which he held at Harvard for the session 1948–49. On his return he was appointed as lecturer in the Department of Physics at the University of St. Andrews and in 1954 was elected to the chair of physics at the University College of North Wales, Bangor. In addition to a large number of papers in scientific journals he has published an important book on *Nuclear Magnet Resonance*, which is his special field of study.

Zoology at Hong Kong : Prof. J. G. Phillips

DR. PHILLIPS has been appointed, at the age of twenty-nine, to the chair of zoology, University of Hong Kong, and takes up his duties in the New Year. He graduated at the University of Liverpool in 1954 and remained there to join the research group in comparative endocrinology established by Prof. I. Chester Jones. After gaining the degree of Ph.D. he was awarded a Harkness fellowship, which he held at Yale for nearly two years. Since that time he has held a lectureship in zoology in the University of Sheffield. In addition, Dr. Phillips has had the experience of short working visits to research centres in Paris, Basel, Vancouver and Florida. Dr. Phillips has already achieved an international reputation for his work on the nature of adrenocortical secretion in lower vertebrates. He has demonstrated the types of corticosteroids produced by representatives of all the vertebrate classes and has shown the presence of aldosterone in bony fish. More recently, he has investigated the control by corticosterone of the capacity of nasal glands of birds to secrete a sodium chloride solution hypertonic to the body fluids. The move to Hong Kong will make available to him the rich and varied fauna of the East. His research skills and dynamic enthusiasm will ensure that he makes full use of this opportunity.

F.A.O. Fisheries Project in Pakistan :
Mr. E. Nyholm

MR. ERIK NYHOLM, of Grenaa, Denmark, has been appointed by the Food and Agriculture Organization as fisheries economist for a technical-assistance project in Pakistan. Mr. Nyholm, stationed in Dacca, will assist the Pakistani Government in improving the storage, processing, transport and marketing of fish products in East Pakistan. This is Mr. Nyholm's first assignment with the Food and Agriculture Organization. He is familiar, however, with the Organization's work, as he was manager of the fish market in Takoradi, Ghana, from 1959 until June of this year. This market was opened following a pilot scheme by the Food and Agriculture Organization carried out in Ghana. Mr. Nyholm graduated from the Grenaa Handelskole in 1943, and carried out his apprenticeship at the Grenaa Havns Fiskeauktion during 1942–46.

The John Scott Medal Awards for 1962

THE John Scott Awards for 1962 have been given to Mr. J. W. Sparke of the Royal Aircraft Establishment, for his invention of the 'Visual Glide Path Indicator', and to Dr. H. Bickel (University of Marburg), Prof. J. Gerrard (University of Saskatchewan) and Dr. Evelyn Hickmans (formerly, Children's Hospital, Birmingham) for their work on a method of controlling phenylketonuria. Prof. Gerrard and Drs. Bickel and Hickmans carried out their original work at the Children's Hospital, Birmingham, where they devised a diet free from phenylalanine—the substance causing phenylketonuria—which, when started within a few days of birth, prevents mental retardation of children who otherwise would become hopeless inmates of mental institutions. The Award, which consists of a copper medal, scroll and a cash prize of 1,000 dollars for each invention, was established in 1816 by John Scott, a chemist of Edinburgh, who bequeathed 4,000 dollars in trust to the City of Philadelphia. To-day, the funds amount to more than 110,000 dollars. Scott's Will directed that the income "be laid out in premiums to be distributed among ingenious men and women who make useful inventions". The Awards are made by the Board of Directors of City Trusts of Philadelphia.

The Institution of the Rubber Industry

THE Institution of the Rubber Industry has made the following awards for 1962: *The Colwyn Medal*, to Dr. L. C. Bateman, controller of research, Malayan Rubber Fund Board, Kuala Lumpur (see *Nature*, 196, 217; 1962), in recognition of his original work on the chemistry of vulcanization and services to the natural rubber industry; *The Hancock Medal*, to Mr. C. H. Birkitt, director of Hubron Rubber Compounds, Ltd., for his services to the rubber industry and the Institution.

Mrs. L. F. C. Parker has been elected to the fellowship of the Institution and as such is the first woman to gain this honour. Mrs. Parker obtained a science degree at Queen Mary College in 1936, and the Associateship of the Institution by examination in 1938. She is the author of published works on identification and estimation of natural and synthetic rubbers and is engaged in technical and quality control at Pirelli General Cable Works, Ltd., Southampton.

The Imperial College of Science and Technology

IT is very dusty in South Kensington now that the last major phase of the building programme of the College has begun, warned Sir Patrick Linstead, rector of the Imperial College of Science and Technology, University of London, in his report at the annual Commemoration Day ceremony held on October 25 in the Royal Albert Hall, at which Sir Keith Murray, chairman of the University Grants Committee, was the special visitor (see also p. 710 of this issue). Material progress was encouraged by the visit of H.R.H. The Duke of Edinburgh in the summer to inspect the new engineering buildings. Recently, another royal link was confirmed with the discovery in the site of the old City and Guilds building of a casket, containing contemporary coins and newspapers, placed there eighty years ago by King Edward VII, then Prince of Wales. This will be carefully preserved. New buildings for electrical and mechanical engineering have now been finished; one for civil engineering is almost complete, as are the halls of residence in Prince's Gardens. Two other projects have been started, one for the Royal School of Mines and one for the Department of Biochemistry. The next phase is another mammoth job of demolition, including much of the old Imperial Institute. The closing of Imperial Institute Road will eventually produce an attractive College precinct. The number of academic staff has increased by forty-eight, including six new professors. Research has continued at a high level, particularly assisted by the Department of Scientific and Industrial Research and also by many private individuals. Student numbers have now reached 2,900.

Desert Locust Control Organization for Eastern Africa

IN a written answer in the House of Commons on October 25, the Secretary for Technical Co-operation, Mr. D. Vosper, stated that an important advance towards the establishment of a system of effective international organizations for the control of locusts had been made when a Convention for the establishment of a Desert Control Organization for Eastern Africa was signed on August 20 by Ethiopia, Somalia, Kenya, Uganda and Tanganyika. A further step was the holding of a meeting in Teheran on October 1 to initiate the formation of a regional locust control organization for Western Asia. A similar regional organization for West Africa based on Dakar had been operating for some years. The international basis for the organization controlling the migratory locust in its outbreak area in Mali had been broadened under a new Convention subscribed to by sixteen African States, and the analogous organization for control of the red locust in Northern Rhodesia was seeking increased African support. Most of the scientific impetus for new thinking on locust control had come from the Anti-Locust Research Centre in London and the pioneering work of its founder, Sir Boris Uvarov. Mr. Vosper was very glad of the opportunity to pay tribute to the achievements of the Centre which was wholly supported by his Department, which was also ready to supply further technical assistance in locust control and had accepted some responsibility for equipping and financing the new Eastern African Organization in its early years.

Medical Research in the United States

As a pre-requisite in nearly every aspect of medical and biological study, basic research has always received emphasis in the several programmes of the National Institutes of Health. In recent years, however, the importance of fundamental research has grown tremendously. This has arisen following the definition of new disease conditions, the development of new drugs and other new forms of therapy and new medical and biological discoveries on a broad scale which in turn have revealed previously unexplored areas which scientists must now study. The research carried out and supported by the National Institutes of Health is oriented toward specific disease conditions, such as cancer, arthritis and heart ailments. By contrast, the Division of General Medical Sciences supports fundamental research which is not necessarily directed toward specific diseases, but toward a better understanding of the body's structure and function as revealed by research in the sciences basic to medicine and biology. The Division also supports research in certain pre-clinical and clinical fields and in the effects of man's environment on his health. Such research is crucial in nearly all medical investigations and, therefore, plays an important part in determining the ultimate success of applied medicine. The greater part of the basic research supported by the Division may be classified under the headings of developmental biology and the chemistry of life processes. The other main areas supported by the Division are environmental factors in health and disease; public health, medical care and nursing; clinical sciences; and methods and tools of science. The work in each of these sections, as well as that in the Centre for Ageing Research, is described in a booklet, *Division of General Medical Science*, obtainable from the U.S. Government Printing Office, Washington, D.C. (Public Health Service Publication, No. 757).

Science Progress

A RECENT issue of *Science Progress* (vol. 50, No. 200; October 1962. 15*s*.) includes a paper by Dr. A. E. A. Werner on scientific methods used in the examination and conservation of antiques—a subject of increasing importance in museums. Other papers include "On Inertial Reference Frames", by Dr. H. Dingle, and "Plant Nematodes", by Mr. F. G. W. Jones. Recent advances in astronomy, physics, general and physical chemistry, geology, biochemistry and plant physiology are dealt with by various authorities, and the issue concludes with a large number of book reviews.

Japanese Journal of Applied Physics

THE first issue—July 1962—of the *Japanese Journal of Applied Physics*, to be published monthly with the co-operation of the Physical Society of Japan and the Japan Society of Applied Physics, includes eight original papers and four short notes (*Japanese Journal of Applied Physics*, Vol. 1, No. 1; July 1962. Pp. 1–70. Subscription rate: annual, 10 dollars; single copy, 1 dollar. Bunkyo-ku, Tokyo: Faculty of Science, University of Tokyo, 1962). The papers deal with vibrational spectra of ruby and hæmatite in the infra-red region; ammonia maser standard I; piezoelectric properties of triglycine sulphate; epitaxial vapour growth of single crystal Ga; dynatron-type negative resistance observed in the collector voltage-saturation region of the friction transistor; induced radioactivity in iron by high-energy protons; study of field emission cathode by field ion microscopy; and a new apparatus for measuring dynamic viscoelastic properties of polymers.

Bibliography of Electronics

A RECENTLY published guide provides, under 67 main headings, more than 3,300 references to the more important text-books, papers and other publications in the field of electronics (*Electronics: a Bibliographical Guide*. By C. K. Moore and K. J. Spencer. Pp. xvii+411. London: Macdonald and Co. (Publishers), Ltd., 1961. 65*s*. net). For the purposes of their work the authors have interpreted electronics as the "science and technology based on the use of electron tubes and semiconductors", including both infra-red and ultra-sonic techniques. The first and longest section, divided into 13 sub-sections, provides 274 references to other bibliographies, periodicals and publications of a general nature, while succeeding sections deal with specific subjects ranging from electron microscopy to radio astronomy. Approximately half the number of references in each subject are to important papers and reviews, and half to text-books, symposia proceedings and bibliographies.

The main period covered is 1945–59, but several earlier references to pioneer work in each field are included, and an addendum covers significant advances during 1960. Author and subject indexes are provided. The authors are to be congratulated on this valuable and comprehensive volume, and we may hope that they will find it possible to provide revisions or further addenda in future years.

British Inter-Regional Library Coverage Scheme

THE *Library Association Record* for September 1962 (64. No. 9) includes the annual report for 1961 of the National Committee on Regional Library Co-operation. The Inter-Regional Coverage Scheme, which came into operation on January 1, 1959, is working out satisfactorily, and requests for British books published since 1959 now go direct from region to region and not through the National Central Library. Improvement of the coverage of periodicals is now in the hands of the regions, and, with the publication of the *British Technology Index*, it is hoped that in 1963 all regions will be able to accommodate requests for the 400 titles covered. All regions in England were connected by telex by the end of the year, and its installation is also being considered by the Scottish Central Library. It is hoped to bring fully into operation by April 1963 a book-store at Woolwich Arsenal capable of holding 500,000 volumes, thus enabling the National Central Library to accept considerably more donations from the regions and ease their space problems.

Macmillan and Co., Ltd.: Cleaver-Hume Press, Ltd.

SOME time ago, Cleaver-Hume Press, Ltd., found that it would soon become difficult to deal, in the present premises, with its expanding business. This led to discussions with Macmillan and Co., Ltd., because it was felt that their new warehouse, to be built at Basingstoke, might help resolve this problem. As a result, co-operation between the two companies has gone much farther.

Macmillan and Co., Ltd., London, have acquired from Cleaver-Hume, Ltd. (a public company with interests in bookselling, paper converting, postal tuition and other fields as well as in publishing), all the shares in its well-known scientific and technical publishing subsidiary, Cleaver-Hume Press, Ltd. This should enable Cleaver-Hume Press to exploit more effectively, as part of a larger economic unit, its valuable special knowledge and contacts, and would also bring to Macmillans a useful addition to their lists in important fields of science and technology. Since the imprint of Cleaver-Hume Press, Ltd. (and its colophon by the late Allan Wyon) are highly esteemed in scientific and technical circles, it is proposed to retain them and to alter the existing organization only so far as may be appropriate for working economies. Publishing policy, too, will remain unchanged.

The long-standing and cordial relationships with the great Philips concern at Eindhoven, for whose Centrex publishing house Cleaver-Hume Press distributes, in the United Kingdom, both books and scientific journals, and with Infosearch, Ltd., London, publishers of scientific books translated from the Russian, will remain undisturbed. Cleaver-Hume Press also announces that it is at present preparing to distribute works, mainly on computers and other aspects of applied physics, issued by the Spartan Publishing Co., Washington, D.C. Mr. P. J. Edmonds will remain managing director of Cleaver-Hume Press and Mr. Daniel Macmillan, chairman of Macmillan and Co., Ltd., will take over the chairmanship. The continuing interest of Cleaver-Hume, Ltd., in publishing has been ensured by the formation of a new Company, to be called Macmillan and Cleaver, Ltd., which will concentrate mainly on books suitable for use with educational courses of the Cleaver-Hume Group, but which, it is hoped, will command a wider market as well. The first book under the new imprint will be *Careers Encyclopaedia* (Chaffe and Edmonds—third edition), due to be published in February. This title will at first be handled by Macmillan and Co., Ltd., to whom all orders and inquiries should be addressed. Just as Macmillan and Cleaver, Ltd., hope to distribute, in wider fields, books primarily designed for the educational courses, so Cleaver-Hume, Ltd., will still, of course, require for these courses a wide variety of books from other publishers.

Films on Nuclear Energy

ONE of the tasks of the informal Nuclear Public Relations Contact Group, constituted in Rome on January 20, 1961, was to collect together in one publication brief details of films on nuclear energy which are available in different countries. The first edition was published in September 1961, and a second list, compiled by B. M. Adkins, which is a more accurate and complete edition, has recently been issued (O.E.C.D.: European Nuclear Energy Agency. Nuclear Public Relations: Contact Group. *List of Films on Nuclear Energy*. Second Edition. Pp. x+63. Paris: O.E.C.D., European Nuclear Energy Agency, 1962). Films from some twenty-three countries are listed. Films on the military applications of atomic energy, or on defence against such applications, are not included. The films are grouped under their countries of origin, in reversed chronological order, and the title, sponsor, duration, size, whether black-and-white or coloured, and language of each film are given. A short description of the film's contents is also given in many cases.

The Nitrogen Problem

A SYMPOSIUM on the "Nitrogen Problem" was held during a session of the National Academy of Sciences, India, in 1960 at the University of Gorakhpur. About fifty papers were presented; they were mainly from research centres in India but included contributions from a few European countries, and they have been published together in two parts of the *Proceedings of the National Academy of Sciences, India* (31, Section A. *Symposium on Nitrogen Problem*, Part 1. Pp. 1–164. Rs. 7.50. Part 2. Pp. 165–320. Rs. 7.50. Allahabad: National Academy of Sciences, India, 1961). Various aspects of the nitrogen problem in food production are dealt with, such as the industrial fixation of nitrogen and the manufacture of ammonium compounds and urea, the influence of various factors on biological fixation and on the mobilization of inorganic nitrogen and on fixation under sterile conditions, the composting of waste material, the production of fertilizers from coal, the relative responses in crops to different forms of nitrogen, the loss of nitrogen from the soil and the nitrogen balance of arable land. The urgency of increasing agricultural production in India, as well as in other under-developed countries with large populations, and the valuable part that nitrogen can play in raising crop yields are fully stressed. Some statements are of a controversial character, but the large number of statistics and experimental results submitted provide ample testimony to the scope and importance of a serious situation. At the present time the amount of fertilizers used per unit area of cultivated land is no less than 200 times greater in Japan and the Netherlands than in India. Even allowing for differences in climate, soil and agricultural methods, the possibilities of substantially improving food production, were economic difficulties overcome, must be apparent to the most casual observer.

Animal Disease and Human Health

A SERIES of basic studies supporting the 'Freedom from Hunger Campaign' is being published by the Food and Agriculture Organization and other organizations of the United Nations. Sixteen such studies are contemplated in the series. The subjects cover a wide range and include, for example, the possibilities of increasing world food production, the part marketing can play in increasing productivity, education and training in nutrition, animal diseases and human health, economic development through food, population and food supplies, education in relation to agriculture and economic development, hunger and social policy, malnutrition and disease, weather and food. The latest volume, *Animal Disease and Human Health*, issued by the Food and Agriculture Organization, has been prepared by Dr. James H. Steele, chief of the Veterinary Public Health Communicable Disease Center, U.S. Public Health Service, and shows that animal health is necessary for the resolution of many human health problems, including freedom from hunger, as well as for the development of sound economies and commerce (Freedom from Hunger Campaign—Basic Study No. 3. Pp. vii+50. Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1962. 2*s*. 6*d*.; 50 cents). Many problems of animal health are amenable to control and eradication.

A Platinum Blood Flowmeter

The discovery of a new technique is often a matter of chance. Prof. M. Mochizuki, of the Research Institute of Applied Electricity, Hokkaido University, Japan, is well known for his investigations on the determination of oxygen pressure in living tissues such as the lungs. During recent years, Prof. Mochizuki has been interested in measuring the oxygen tension in blood. He utilized the so-called 'Oxigraph', an apparatus which he devised using the principle of polarography. The results of the measurements varied because of pulsation of blood flow and he had to introduce a technique to lessen the errors arising from this. Mochizuki was also aware that there was a strong demand among physicians and surgeons for a new and accurate means of measuring the rate of flow of blood. He realized that his method could be used to measure the rate of flow rather than the oxygen content, and, in order to test his hypothesis, a series of preliminary experiments has been carried out at the Institute. It was found that the signals faithfully represented the trends in the rate of flow and that Mochizuki's method was useful in studying the state of the blood circulation even in a human heart. A monograph under the editorship of Prof. K. Higasi contains four articles by Prof. Mochizuki and his co-workers based on the latest data obtained since 1961 (*Res. Inst. App. Elec., Hokkaido University.* Monograph Series, No. 10: *Platinum Blood Flowmeter.* Pp. v+78. 1962).

The White Rhinoceros

The northern form of the square-lipped African rhinoceros is now found in only three countries, the southern Sudan, where there are between one and two thousand, the Congo, where there are perhaps rather more than a thousand, and Uganda, where there are only about a hundred, and the population is decreasing rapidly. Numbers as small as this are highly dangerous to a slow-breeding animal like the rhinoceros, which is exposed to poaching. The World Wildlife Fund has therefore made it its first objective to save the last remnant of the Uganda white rhinos, and has made a substantial grant to the East African Wild Life Society to preserve, in co-operation with the Uganda Game Department, the white rhinos in districts close to the Upper Nile (*World Wildlife News*, No. 4). The white rhinoceros is not really white, but a pale greyish colour, and is the nearest living relative of the prehistoric woolly rhinoceros. The southern white rhino is now virtually confined to the national parks of Natal, where it is well guarded and thriving.

Research Fellowships in Forest Resources

Research fellowships in forest resources are awarded annually by Harvard University from the Charles Bullard Fund. The fellowships carry stipends up to 15,000 dollars, the amount of each award depending on the professional status and needs of the recipient. The purpose of this fellowship programme is to support advanced research and study by men who show promise of making an important contribution, either as scholars or administrators, to forestry and forest management, these subjects to be defined in their broadest aspects—scientific, economic, political, administrative and legal. The fellowships are open to all men in public service, in academic careers, and in private forestry, not only those who have doctoral degrees or are seeking advanced degrees, but also those without advanced degrees who do not wish them. Bullard Forest Research Fellows can apply for admission to candidacy for advance degrees including Master of forest science, Doctor of philosophy in biology or in economics, Master in public administration and Doctor of public administration, etc. The fellowships are for one year and normally are not renewable. Details of methods of application and further information can be obtained from the Committee on the Charles Bullard Fund for Forest Research, Littauer Center 123, Harvard University, Cambridge 38, Mass. Applications must be received by April 1.

Announcements

Sir Donald Gibson, who has been director-general of works at the War Office since 1958, has been appointed director-general of research and development in the Ministry of Public Building and Works. Sir Donald's responsibilities will include the encouragement of new and rapid methods of construction; standardization of use and production of building components; and dissemination of the best modern practices.

The Institute of Petroleum has taken over the secretarial work of the Gas Chromatography Discussion Group, and all subscriptions and inquiries should now be addressed to the Secretary of the Group, at 61 New Cavendish Street, London, W.1. From the beginning of 1963, the Institute of Petroleum will also be responsible for the Group's publications, including *Gas Chromatography Abstracts*.

The Association for the Study of Animal Behaviour will be holding a symposium on "Gregarious Behaviour" at Birkbeck College, Malet St., London, W.C.1, during December 12–13. Further information can be obtained from J. B. Free, Rothamsted Experimental Station, Harpenden, Herts.

The sixth International Embryological Conference sponsored by the Editorial Board of the *Journal of Embryology and Experimental Morphology* will be held during July 22–25, 1963, in Helsinki. Further information can be obtained, after February 1, 1963, from either Prof. S. Toivonen, Department of Physiological Zoology, the University, Arkadiankatu 7, Helsinki, or Dr. L. Brent, National Institute for Medical Research, the Ridgeway, Mill Hill, London, N.W.7.

The sixty-ninth annual meeting of the American Mathematical Society will be held at the University of California, Berkeley, in conjunction with the annual meetings of the Mathematical Association of America and the Association for Symbolic Logic. The Society will meet during January 24–27, the Mathematical Association of America during January 26–28 and the Association for Symbolic Logic on January 26. The thirty-sixth Josiah Willard Gibbs Lecture will be delivered by Prof. C. E. Shannon, of the Massachusetts Institute of Technology, on January 24. Further information can be obtained from the American Mathematical Society, 190 Hope Street, Providence 6, Rhode Island.

Erratum. In the communication by Dr. D. N. Mullick and V. N. Murty entitled "Effects of Stilbœstrol and Thiouracil on the Electrocardiograms of the Sheep", which appeared on p. 1285 of the June 30 issue of *Nature*, Fig. 1 is printed as it appeared in the original manuscript. However, Dr. Mullick informs the Editor that the block should be turned through 180°.

BRITAIN AND THE EUROPEAN COMMON MARKET

BROADSHEET No. 465, *The Negotiations of Political Union**, part of a major study of Britain and the European Common Market which the Political and Economic Planning Trust began in 1958 with the help of a grant from the Ford Foundation, is the latest in a series of publications issuing from that study. It follows a broadsheet entitled *Cartel Policy and the Common Market* (No. 464)†, issued in August, and an Anglo-French symposium entitled "Aspects of European Integration", produced jointly by P.E.P. and the Institut de Science Economique Appliquée, which was published in September. Broadsheet No. 461, *Trade Unions and the Common Market*, was issued in May, and Broadsheet No. 454, *Economic Planning in France*, was issued in August 1961. Occasional Paper No. 12 was re-issued in March 1961 as broadsheet No. 451, *Four Approaches to the European Problem*, but some eleven occasional papers arising out of the study had then already been published, including one on *Agricultural Policy in the European Economic Community* and a preliminary appraisal of the European Free Trade Association.

The present broadsheet, by M. Palmer, describes and analyses the various drafts that have provided a basis for discussion among the member States of the European Economic Community on closer political union. It first describes briefly the Community's method of operation to show how this process differs from the procedures envisaged by the French Government for the political field and then describes the French proposals and the reactions to them of the other countries. While the French Government and the Governments of the five other parties in the European Economic Community are agreed on the need for a political *relance* which will add foreign policy, defence matters and cultural and scientific co-operation to those fields in which the Six already act together, no clear agreement has yet been reached on the precise arrangements which should be made, nor would agreement on the form and competence of the proposed political union of itself guarantee that common policies can be reached on individual issues in international affairs. The success of the Six in achieving common policies over matters covered by the Treaties of Paris and of Rome is attributed in large measure to their basic community of economic interest

Broadsheet No. 464, written by M. Forsyth, after considering first the significance of the first regulation on restrictive trade practices in the Common Market which came into force on March 13, 1962, considers in succession national legislation in the European Economic Community and the United Kingdom, the example of the European Iron and Steel Community, and the Rome Treaty and the First Regulation. While there are considerable differences between the six countries of the Common Market and the United Kingdom in their policies towards restrictive practices, each policy has to be seen in terms not only of its written form but also of the method and spirit in which it is applied. Italy and Luxembourg still have no specific legislation on the subject, and while in the United Kingdom, the Netherlands and Belgium the criterion is the 'public interest', in Germany and France the criterion is that of 'restricting competition'. Germany and the United Kingdom have perhaps the most developed restrictive legislation in Europe and both are now encountering the same problems, essentially those relating to resale price maintenance and the process of concentration.

The Common Market's present provisions for restrictive trade practices are based on the prohibition *a priori* of restrictive agreements; dominant concerns are also forbidden *a priori* from misusing their power; there are no provisions against mergers, but the Commission has fairly wide powers to investigate sectors of the market if necessary. Appeal is possible to the European Court of Justice. The effectiveness of the rules will depend on how far the various policies and rules of the Community are developed in harmony with them, including how far this policy is balanced by sound common monetary and business cycle policy, in active regional policy and social measures.

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* *Planning*, **28**, No. 465; October 1962 · *The Negotiations on Political Union*. Prepared by Michael Palmer. Pp. 295–326. (London: Political and Economic Planning, 1962.) 3*s*. 6*d*.

† *Planning*, **28**, No. 464, 27 August, 1962 · *Cartel Policy and the Common Market*. Pp. 199–298. (London Political and Economic Planning, 1962.) 7*s*. 6*d*

DOMESTIC FOOD CONSUMPTION AND EXPENDITURE IN BRITAIN

SINCE 1940 yearly surveys have been made of food consumption and expenditure on food in private households in the United Kingdom. The reports of these, printed annually since 1950, contain detailed analyses of the findings. Since the investigations are made in various regions and cover representative rural and urban households of all sizes, belonging to different social and economic classes, they bring to light interesting facts about the country's eating habits not shown by national average food supply statistics.

The 1960 survey is the fifth since 1956, a point at which the conditions created by war and rationing finally ceased to influence the freedom of supply and consumer choice. Changes in eating habits in a free market have occurred during this period, some caused by fluctuating prices rather than by new trends in taste. The see-sawing counter-balances of butter and margarine sales, for example, are related to the price of butter. There are, however, some significant trends in eating habits not due to this cause, one being the increasing popularity of 'convenience foods', bought partly or wholly prepared, and another the remarkable increase of sales of poultry, especially broiler chickens. The average expenditure on 'convenience foods' in 1960 was 5*s*. 6*d*. per person per week, or 18·7 per cent of the total expenditure on food, as compared with 4*s*. 8*d*. and 17·1 per cent in 1956. Expenditure on poultry rose from 3½*d*. in 1959 to 4½*d*. in 1960 per person per week.

Some slower changes have taken place over a longer period of years. The consumption of dairy products has steadily risen, and in 1960 was 42 per cent greater than the 1934–38 level. On the other hand, that of potatoes and cereal products, including bread and flour, is decreasing yearly. In 1960 the decrease for potatoes was temporarily halted as they were cheap and plentiful, but over the five-year period decline in purchases can be seen. Purchases of cereal foods are now 14 per cent less than the pre-War level; only 45·5 oz. of bread per person per week was purchased in 1960 compared with 47·3 in 1959 and 51·1 oz. in 1956. A change of taste is shown in the rapidly growing demand for wrapped bread. Less flour is being bought and more cakes and biscuits.

With respect to 'convenience foods', most of the money is spent on cakes and biscuits, canned or cooked meat and fish, but 21 per cent is spent on fruit and vegetables and the greatest expansion of demand has been for frozen legumes, mainly peas. In 1956, 0·2 oz. of frozen legumes per person per week were purchased, rising to 0·63 oz. in 1960. The popularity of frozen foods is indicated by the fact that they are grouped separately in the 1960 report and not included in other groups. Although an average of 5*s*. 6*d*. per head per week was spent on 'convenience foods', this was by no means uniform. The section on diet and social classes shows that 6*s*. 3*d*. a week was spent on them by the highest earning income group, but only 4*s*. 10*d*. by the lowest, while old-age pensioners spent 3*s*. 11*d*. The section on family composition shows that young childless couples spent 8*s*. 1*d*. per head per week on 'convenience foods' compared with 3*s*. 11*d*. spent by families with four or more children. Geographical comparisons indicate that the highest proportion of the total expenditure on foods devoted to 'convenience foods' was in the north of England and Scotland and the lowest in London and the rural areas.

When the housewife goes out to work she has less time but more money to spend on food, and, as a result, she devotes more money to prepared foods. This trend is likely to increase as mass production and competition reduce the price of these foods and make available a greater variety. Enthusiastic lovers of home-cooking evoke little response from harassed housewives who do not want to be 'slogging away over a hot stove' in a machine-minded age, and can afford to buy foods which reduce the slogging.

Although the average value for food consumed (including 'free supplies' from gardens, etc.) was 30*s*. 6*d*. per person per week in 1960, the highest income group was obtaining food worth 40*s*. 3*d*. and the lowest 27*s*. 4*d*. An increase in the basic pension-rate in 1958 was reflected in a welcome increase of expenditure on food by old-age pensioners from 24*s*. 9*d*. in 1956 to 27*s*. 10*d*. in 1960 (the total value being 28*s*. 6*d*. including 'free foods'); part of this is, however, accounted for by the rise in food prices since 1956. The expenditure of families of different sizes shows a wide range. Families of two adults under 55 spent 40*s*. 3*d*. per head per week (41*s*. 2*d*. with 'free foods'), while families with four or more children spent only 19*s*. 9*d*. (20*s*. 3*d*. with 'free foods').

The survey showed that the population as a whole had a diet adequate in all respects. The higher expenditure of wealthier families means more expensive foods and a greater variety, but little nutritional advantage except for more vitamin C resulting from a higher consumption of fruit and vegetables. The blot in this happy picture remains the consumption of families with several children. Households with three children obtained only 92 per cent and 94 per cent of estimated requirements for protein and calcium respectively. Those with four or more children obtained 99 per cent of the energy value needed, but only 85 per cent of the total protein and 85 per cent of the calcium. Growing children should not go short of protein and calcium. The over-all picture of well-being and rising standards of living, based on encouraging average figures, may conceal the position of some less-fortunate minorities.

JOYCE DOUGHTY

FOOD ADDITIVES AND MEAT HYGIENE

TWO booklets containing reports of Joint Food and Agriculture Organization/World Health Organization Expert Committees have recently been published. Problems in connexion with the satisfactory control of food additives are discussed in No. 31 of the Food and Agriculture Organization Nutrition Meetings Report Series, which is entitled *Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants*[*]. Some 86 pages of the booklet contain a series of monographs relating to a selected number of preservatives and antioxidants. The substances chosen for evaluation by the Joint Committee were those which are widely used in many countries and because they play an important part in reducing food wastage by improving storage efficiency and distribution. Specifications for identity and purity of additives were discussed in previous reports of the Committee. The monographs of this, the sixth report of the Joint Committee on Food Additives, therefore, concentrate largely on the properties that are of the greatest interest to the toxicologist. The biological data are presented under the following headings: acute toxicity, short-term studies, long-term studies and biochemical aspects.

Each monograph records the comments of the Committee on such data, but the report mentions that in most cases they would have liked more evidence from which to draw. For the evaluation of the individual preservatives and antioxidants, the Committee have (wherever possible) estimated: (*a*) the dosage-level causing no significant toxicological effect (mainly from long-term studies in rats); (*b*) the acceptable daily intake zones for man (unconditional and conditional). The latter figures are also usefully given in tabular form on page 12. The Committee recommends that the Food and Agriculture Organization and the World Health Organization should use all means available to promote further research and that further committees should be convened to evaluate the toxic hazards involved in the use of other groups of additives, such as emulsifiers, stabilizers, other preservatives, such as antibiotics, and food colours. It is interesting to note, however, that this report states that "... the needs for food additives and their possible uses and applications vary so widely from country to country that a single pattern of legislation is not feasible on a world-wide basis".

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[*] Food and Agriculture Organization of the United Nations. FAO Nutrition Meetings Report Series. No. 31. *Evaluation of the Toxicity of a Number of Antimicrobials and Antioxidants*. Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives. Pp. 104. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1962.) 6*s*. 8*d*.; 1.25 dollars.

A somewhat different aspect of food quality is discussed in No. 241 of the World Health Organization Technical Report Series†. This publication stresses that the world scarcity of protein makes it necessary to conserve and utilize meat supplies to the fullest possible extent. This requires the application of a meat hygiene system which includes supervision of the meat production chain from the farm, through transport to a well-run abattoir, to distribution to the consumer. This booklet covers each of these stages separately, for example, diseases, slaughter, inspection, refrigeration, transport, marketing, manufacture, sanitation of retail shops and restaurant kitchens, hygiene of handlers, education, newer developments and laboratory methods. A separate section on poultry hygiene is also included. The Committee recommends that the Food and Agriculture Organization and the World Health Organization should encourage further investigation into various problems related to meat hygiene, including those associated with chemical residues, meat-borne diseases, rapid methods for diagnosing organisms and the presence of radionuclides.

D. PEARSON

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† World Health Organization. WHO Technical Report Series, No. 241, and/or FAO Agricultural Studies, No. 58: Joint FAO/WHO Expert Committee on Meat Hygiene—Second Report. Pp. 88. (Geneva: World Health Organization, London: H.M. Stationery Office, 1962.) 3 Swiss francs; 5s.; 1 dollar.

FISHERIES OF NIGERIA

IN an excellent report*, Mr. A. R. Longhurst sums up the fisheries situation in Nigeria and its future requirements. He estimates that at present only some 58,000 out of Nigeria's fish supplies of 203,000 tons are produced locally, the rest being imported, and that, even so, the supply of fish per head of the population is only 11·36 lb. per annum. He doubts whether the indigenous sea fisheries, even if improved by some mechanization and other possible developments, will go far to fill the gap; and the small fleet of foreign-owned inshore trawlers has only limited prospects of expansion. Mr. Longhurst thinks that the best prospects lie in the exploitation of distant-water fisheries, both pelagic (especially the tunas) and demersal. But for this much capital and expertise will be needed, and there is especially the need of a deep-water fishing harbour with all the ancillary facilities without which foreign fishing enterprises are unlikely to be able to work from Nigeria.

There is an interesting account of the freshwater fisheries, indicating scope for expansion in the potentially rich fisheries of Lake Chad. Mr. Longhurst summarizes the research and development work that has already been done in Nigeria and West Africa in general, but the picture was not one of sustained effort. There is a very useful bibliography.

In his summary of the hydrographic situation, Mr. Longhurst shows that Nigeria lies in the area between the relatively rich cold water of the Benguela and Canary Currents, and is subject to the warm nutrient-poor Guinea Current. But the big rivers, and especially the Niger, bring down in their annual floods important amounts of organic matter. The great influence of the thermocline in the distribution of the fish is restated.

Based on this summary, Mr. Longhurst describes the research work begun by the Federal Fisheries Service, which has its headquarters at Lagos but can undertake research anywhere in Nigeria, at the request of the regional authorities. There is an establishment of a director and five research officers, a small band for so large a territory with such diverse problems. A 70-ft. research vessel has been recently acquired, and surveys are being made of the fish and crustacean resources, as well as hydrographic sections and plankton work. Research on fish includes a study of the life-histories of the most important species; in conjunction with studies of mesh-selection and of the fishing effort, it is planned to get the information needed for a rational management of the inshore trawl fisheries.

Those who know how difficult it is to start and carry on such a programme will wish the Federal Fisheries Department well in their important work.

C. F. HICKLING

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* Report on the Fisheries of Nigeria 1961. By A. R. Longhurst. Pp. 58. (Lagos: Federal Fisheries Service, Ministry of Economic Development, 1962.)

AMATEUR RADIO ASTRONOMY

RADIO astronomy has attracted to itself a band of amateurs no less zealous than those who are devoted to optical astronomy. The techniques required for the simplest observations are more complex than those required to build a small optical telescope and most of the work done so far has necessarily been by groups of amateurs who have combined resources and experience to build aerials and receivers and have worked together on the assessment of observations. The Radio Electronics Section of the British Astronomical Association has co-ordinated this work and has recently published a memoir on "Radio Astronomy and Radio Telescopes" (40, No. 1; 1962 February) edited by Dr. J. Heywood.

This memoir sets out to provide a useful introduction for a beginner to the techniques of radio astronomy. A helpful description is given of theoretical and practical fundamentals of the aerial and receiver design of the basic installation necessary for amateur observations.

An encouraging aspect of amateur interest in radio astronomy in Great Britain is its youth. Six of the contributors to the memoir were under twenty-one when they wrote their articles. Two of these were sixth-form schoolboys who wrote their contribution on the physical principles of radio wave propagation and reception. A recent publication in the *Journal of the British Astronomical Association* (72, 19; 1962) describes work by another sixth-form student, J. K. Sabbagh, on "An Analysis of Extraterrestrial Records at 27 Mc/s".

Many types of observation are possible with simple receivers. These include observations of the strong radio sources with interferometers and the effects of the ionosphere on the incoming signals; the Sun and Jupiter can be studied for their variability. The professional radio astronomer heartily encourages this field of amateur activity.

R. D. DAVIES

EFFECTS OF PARTIAL AND MICROBEAM IRRADIATION OF CELLS

A SYMPOSIUM on partial and microbeam irradiation of cells was held in the University Department of Radiotherapeutics, Cambridge, on August 2 and 3, organized by a committee consisting of M. C. Bessis (Paris), R. H. Haynes (Chicago), J. S. Mitchell (Cambridge), C. L. Smith (chairman, Cambridge) and R. B. Uretz (Chicago). The aim of the symposium was to have an informal and intimate discussion on partial and microbeam irradiation of cells and the techniques ancillary to such experiments. All participants were persons who are actively interested in or working in this rapidly growing field. Forty-five people attended, representing most of the European countries, India and the United States. Each morning and afternoon was divided into two sessions in which a subject was introduced briefly by an invited speaker in the hope of provoking a frank, full and spontaneous discussion afterwards. In this it was extremely successful.

The symposium was opened by Prof. J. S. Mitchell, who reminded the delegates that it was just fifty years since the first papers were published in the field of cell micro-puncture (as it was called then) by Chahotin in 1912.

The first morning was reserved for discussions about apparatus and techniques for producing microbeams of ultra-violet and ionizing radiations. In the afternoon, techniques ancillary to partial cell irradiation were discussed; and the next day seven prepared papers, some with films, were presented.

The first morning session was opened by M. C. Bessis with a ciné film showing the effects of localized monochromatic (2460 Å) ultra-violet radiation on human leucocytes with a microbeam 1μ in diameter. Out of this a discussion arose on the difficulties involved in measuring the energy flux over such a small area. R. B. Uretz described his technique of defocusing the beam, allowing it to fall on a 'Willemite' screen, and measuring the phospholuminescence with a photocell. The difficulty of obtaining an absolute quantitative determination of the flux was stressed. Prof. J. S. Mitchell and R. H. Haynes pointed out that the important factor is the absorbed dose, and compared the measurement of energy flux and absorbed dose with the units röntgen and rad. M. C. Bessis proposed using a biological unit, and R. C. von Borstel (Oak Ridge) suggested that phage inactivation might prove a useful method for energy flux measurement as the phage are much smaller than the microspot and the inactivation produced could easily be related to macro experiments for which the dose-inactivation curves are already well known or could easily be measured. Mitchell felt that ultra-violet photomicroscopy of the spot followed by microdensitometer measurements over the spot image should not be too difficult.

I. Nomarski (Paris) explained how it was possible to obtain an ultra-violet microbeam of 0·2μ diameter using the new Zeiss 'Ultrafluar' transmission achromat which has a numerical aperture of 1·25 and is corrected from 2400 Å to 7000 Å. Uretz pointed out that scattering by subcellular constituents in most biological specimens made it impossible to produce an effective spot diameter less than about 1 or 2μ.

Bessis and Nomarski described a very recently built system for producing an optical microbeam of wave-length 6930 Å using a ruby laser and then showed a ciné film of some preliminary irradiations that have been performed with this device on human erythrocytes; one flash produced complete hæmolysis instantaneously.

Uretz then described some experiments on chromosome 'paling' using a polarized microbeam of ultra-violet. No observable difference could be seen between the effects produced by two beams polarized in planes at right angles to each other.

The second morning session was opened by L. D. England (Texas Nuclear Corporation), who described a technique for single proton irradiation of selected areas of single living cells. The protons of 16 MeV energy are produced by the nuclear reaction ^{3}He (d.p.) ^{4}He. The discussion then centred around means of producing microbeams of ionizing radiation. D. Harder (Würzburg) talked about an α-particle microbeam which he had constructed, and Smith described how 5μ holes in platinum could be squeezed and, by plastic deformation, reduced to a diameter of 2μ. Haynes then discussed a method of producing holes down to 1·5μ in diameter by controlled punching of a thin metal foil with a hard-steel needle and stressed that the diameter of the beam must be measured by nuclear plate techniques and not just taken as the diameter of the hole measured optically. He followed this by describing the localization and calibration techniques associated with his own α-particle microbeam.

The afternoon session of the first day, on ancillary techniques, was opened by T. R. Munro (Cambridge) describing his tissue culture technique whereby single S3 HeLa cells could be followed for 7–8 days until they formed clones containing up to 100 or more cells. By this means he hopes to observe long-term effects of partial cell irradiation. Both Uretz and H. Glubrecht (Hanover) then commented on methods of partial cell irradiation using selective radioactive precursors containing hydrogen-3, sulphur-35 and phosphorus-32. Glubrecht stressed the importance of the effect of transmutations, particularly of incorporated phosphorus-32.

J. G. Cruickshank (Cambridge) opened a discussion on the uses of the fluorescent antibody technique and described a proposed experiment involving HeLa cells infected either with Newcastle disease virus or influenza virus. The effect of ultra-violet 'enucleolation' on synthesis of RNA could then be observed either with radioactive precursors (for example, tritiated uridine) or acridine orange. Uretz discussed experiments using a 'phosphoroscope' in which an induced fluorescence with a lifetime $> 10^{-4}$ sec could be observed.

L. Trapp (Zeiss, Oberkochen) described the new Zeiss Caspersson type (ultra-violet and visible) scanning microspectrophotometer with automatic recording.

On the second day, the following papers were presented: P. S. Amenta (Philadelphia), "Effects of Ultra-violet Microbeam Irradiation on the Eosinophils of *Triturus viridescens*"; J. G. Carlson (Knoxville), M. E. Gaulden and J. Jagger (Oak Ridge), "Mitotic Effect of Monochromatic Irradiation of the Nucleolus"; R. Rhines (Chicago), "Some Effects of Irradiating Small Portions of Neurites in Cultures of Nervous Tissue with the Uretz Ultra-violet Microbeam"; J. Jagger and D. M. Prescott (Oak Ridge), "Ultra-violet Microbeam Irradiation of Nucleus and Cytoplasm of *Amoeba proteus*"; H. Ulrich (Zurich), "Partial Cell X-ray Experiments on *Drosophila melanogaster* Eggs: Sensitivity to Killing and Production of Mutations"; M. I. Davis Clegg (Oxford), "Some Preliminary Results of Electron Microscope Studies of Cells Irradiated with a Microbeam of α-Particles"; A. Gibor and S. Granick (New York), "Cytoplasmic Inheritance of Plastids".

The meeting was concluded with an expression of thanks to Euratom for financial support. There was a general feeling among the participants that small meetings of specialists in a given field can stimulate discussion and research probably better than large conferences. At the invitation of Prof. M. C. Bessis it was agreed to hold another informal meeting in Paris in two years time.

The participants then made a tour of the new laboratory, during which they saw the various pieces of microbeam equipment in the laboratory and discussed the work in progress. C. L. SMITH

PRODUCTION OF HYPERSENSITIVE CONDENSATION NUCLEI

By DR. G. R. EVANS and A. A. WATSON

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DURING the course of experiments originally undertaken to ascertain the nature and cause of background fog in a Wilson cloud chamber some results have been obtained which may be of interest to workers in other fields. This fog, which spoils track photography, can be avoided by interposing a number of slow expansions between the fast expansions used for photography: the more dense the fog background, the greater is the number of slow expansions necessary to clean the chamber. The fact that these fog nuclei form drops during slow expansions when the degree of supersaturation is low indicates that they are efficient condensation nuclei.

In normal operation of a cloud chamber, an electric field is maintained across the chamber for the purpose of sweeping away ions formed between expansions. It was discovered that when this field is maintained continuously it becomes more difficult to clean the chamber of fog nuclei, suggesting that an electric field is somehow involved in their formation. Further investigation showed how important it is to remove all traces of fluff from the electrodes if the chamber is to be cleared effectively with the electric field continuously applied.

In order to examine this problem in more detail, a thin strand of cotton was attached to the end of a thick copper electrode and placed at the centre of a cloud chamber, of diameter 20 cm and axial length 20 cm, containing nitrogen at a pressure of 30 atmospheres and saturated with water vapour at 20° C. The electrode was connected to a d.c. voltage which was variable from 0 to 1·2 kV. Adiabatic expansions were made using a range of expansion ratios of 1·01–1·07, the corresponding fall in temperature being from 1° to 8° C. The chamber was first cleaned by slow expansions (in which state no drops are observed even with an expansion ratio of 1·12, provided that in the meantime no electric field has been applied). Thereafter the electric field was applied either during or after an expansion. In such expansions, several jets of drops were seen to issue forth from the tip and side of the cotton strand.

At first, the jet is hollow and shaped like an ear-trumpet, the drops moving away from the region of the cotton with a speed of approximately 2 cm/sec (Fig. 1). Eventually, the drops are dispersed throughout the chamber, the fog becoming too dense to determine for how long the process continues. It is not necessary that the field should be applied during

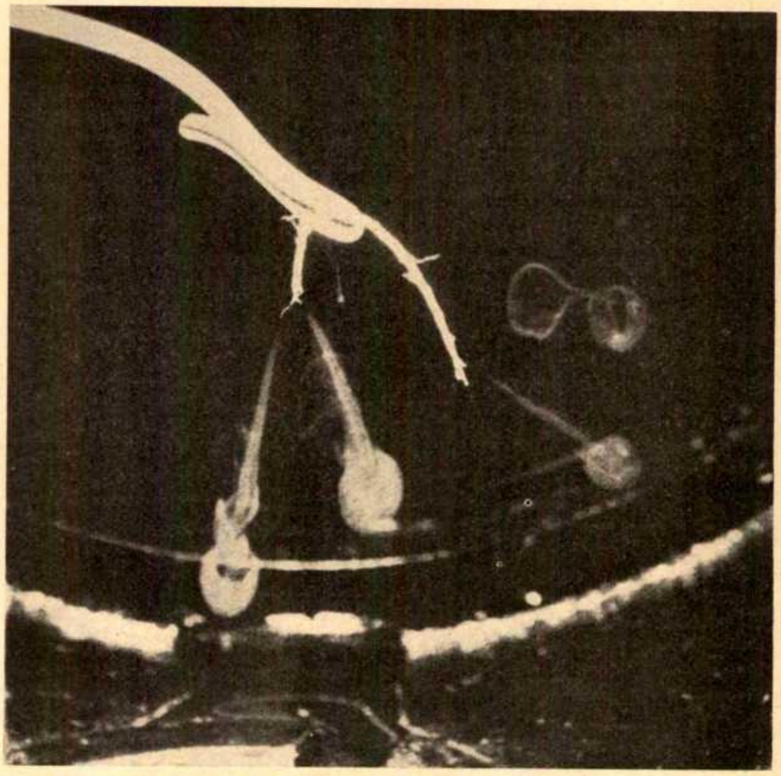

Fig. 1. Jets produced in nitrogen at 30 atms., saturated with water vapour. Photograph taken 2 sec after an expansion of 1·02. Potential on electrode –400 V

the expansion. The effect has been observed when the field was applied 90 sec after an expansion of 1·07.

Other observations made during these experiments can be summarized as follows: (1) The phenomenon occurs after any fast expansion, provided that the expansion ratio is greater than 1·01 and the electrode potential is greater than 400 V, positive or negative. (2) The first slow expansion made after a fast expansion produces fog, even if the time elapsing between the fast and slow expansions is as long as 3 h, provided that there is no electric field across the chamber during this time (if the field is maintained, the chamber clears without the necessity for slow expansions in a period of a few minutes). (3) For fast expansion ratios less than 1·01 no drops are observed, but invariably some fog appears in the first slow expansion made afterwards. (4) Apart from differences in detail, the effects in nitrogen are independent of the gas pressure over a pressure range of 0·8–85 atmospheres. (5) From observations made when the field is established before an expansion, it can be deduced that fog nuclei are formed only when the cotton is damp. (6) Similar effects are observed when: (i) the nitrogen is replaced by argon, except that a delay of approximately 1 sec occurs between the application of the field and the appearance of the first drops; (ii) ethyl alcohol is used in place of water; (iii) the cotton is replaced by a brass needle.

The observations clearly indicate that whenever sufficient voltage is applied to a damp electrode, stable charged nuclei are formed from which drops readily grow from vapour which is only a few per cent supersaturated. If it is assumed that a 'damp' electrode is covered by a thin film of water, it is probable that the action of the field is to produce, by electrolysis in this film, ions of H^+, OH^- and O^-. These ions then combine either with gaseous molecules in solution in the water, or with other ions which are collected by the field from the gas, producing new molecules some of which have a strong affinity for water. The escape of these molecules from the film may be assisted by evaporation and, if they are suitably charged, by the electric field. With nitrogen in the chamber, it is most likely that the most important of these are molecules of nitric acid. Crane and Halpern[1] reported that dense fog can be obtained by introducing a small quantity of nitric acid into a cloud chamber during a fast expansion. They noted also that it was almost impossible to rid the chamber of such fog nuclei by making slow expansions. If the foregoing is the correct explanation, it is at first sight surprising that similar effects have been observed in argon with which no acid molecules can be produced. However, it is impossible to obtain absolutely clean conditions in a cloud chamber and the slow appearance of the effect in argon suggests that some chemical contamination is responsible: sulphur (from the rubber diaphragm), oxygen and carbon are obvious possibilities. In this case, sulphuric and carbonic acids may be formed and behave in a similar way to nitric acid. An alternative explanation might be that a molecule such as hydrogen peroxide is produced[2], a process which would depend on the condensant rather than on the gas used in the chamber.

Unfortunately, it is impossible to calculate the magnitude of the electric field at the surface of the cotton strands, though it appears that there is a critical value of the field below which the phenomenon does not occur. Different metal electrodes with smooth and rough surfaces, for which it will be possible to calculate the magnitude of the electric field, are being prepared for a new series of experiments.

The experimental conditions here described as necessary for the production of hypersensitive condensation nuclei must occur frequently in Nature, particularly in heavily afforested areas. When a leaf is attached to the copper electrode in place of the cotton, effects similar in all respects to those obtained with the strand of cotton have been observed, indicating that the processes involved here should be considered in any theory of cloud formation in the atmosphere. It is well known that rain always contains a minute quantity of nitric acid.

The fogs formed in the rather unusual natural conditions described by Vonnegut[3] probably owe their formation to essentially the same processes as those described here.

We thank Prof. N. Feather for the interest which he has taken in this work. We also thank Mr. J. Paton and Dr. H. C. Sutton, both of this Department, for many helpful discussions, and Mr. J. Scott for his assistance in the operation of the chamber.

[1] Crane, H. R., and Halpern, J., *Phys. Rev.*, **56**, 233 (1939).
[2] Wilson, C. T. R., *Phil. Trans. Roy. Soc.*, A, **192**, 403 (1899).
[3] Vonnegut, B., *J. Atmos. Sci.*, **19**, 194 (1962).

POSSIBILITY OF A 26- OR 27-MONTH PERIODICITY IN THE EQUATORIAL GEOMAGNETIC FIELD AND ITS CORRELATION WITH STRATOSPHERIC WINDS

By F. D. STACEY and P. WESTCOTT

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SEVERAL authors[1-4] have analysed data on stratospheric winds obtained by equatorial meteorological stations in recent years. They found striking evidence of a periodic recurrence of westerly winds encircling the equator. The main features are: (1) the fluctuation is confined to the stratosphere and does not penetrate the tropopause; (2) it is virtually confined to equatorial and near equatorial regions; (3) the phase of the fluctuation advances with increasing height of observation; (4) the average period of recurrence is 26 months. Berlage[5] has reviewed the numerous meteorological periodicities postulated at various times and concluded that apart from the obvious astronomical periods the only one meriting serious consideration was 2¼ years (the southern oscillation). However, none of his evidence was nearly as clear as that now obtained from stratospheric wind data.

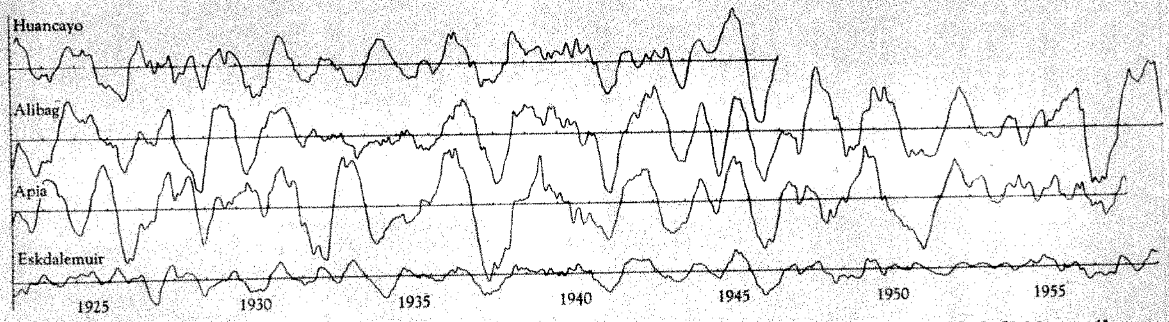

Fig. 1. Running means of *Hq* (12-monthly less 24-monthly) at Huancayo, Alibag, Apia and Eskdalemuir. Each division on the vertical scale represents 1 gamma

The mechanism is unknown. The obvious possibility is a simple momentum transfer of air from ionospheric heights above the equator (at which its conductivity would compel it to rotate more or less with the geomagnetic field), but the required vertical wind speed in the ionosphere would be impossibly high, and Reed *et al.*[3] have pointed out that the ozone deficiency of the equatorial stratosphere constitutes a further serious objection to this possibility. However, the winds occur at the highest level of observation and it appears reasonable to postulate that the fluctuation extends into the ionosphere. If this is so then we may expect the fluctuation to appear also in ionospheric and geomagnetic data. We have examined this possibility and found some evidence for a fluctuation in geomagnetic field with a period of 26 to 27 months, but no evidence for a comparable periodicity in the other measured quantities.

The magnetic values used were monthly means of the horizontal component of the field on magnetically quiet days, *Hq*, at Huancayo (12° S.), Alibag (19° N.) and Apia (14° S.), these stations being approximately equally spaced around the equator, and for comparison Eskdalemuir (55° N.). *Hq* is either tabulated directly in the year-books of the Observatories or is readily calculated from year-book data for the periods represented in Fig. 1, except for Alibag from 1946 onwards, for which unpublished data have been communicated to us by the Director of the Observatory. When the *Hq* values were plotted as 12-monthly running means to eliminate the annual variation, an irregular fluctuation with a period of the order 2 years and amplitude several gammas (1 gamma = 10^{-5} oersted) was immediately apparent. The fluctuation is shown in Fig. 1 after removal of the longer term secular changes by taking the differences of 12 and 24 monthly running means. It is very much stronger at the three equatorial stations (where it is comparable in magnitude with the annual variation) than at Eskdalemuir, but as Fig. 1 shows, the correlation between the equatorial stations is not consistently good.

To determine more objectively whether the *Hq* fluctuation has a periodic tendency rather than being just random, we have carried out spectral analyses of the monthly values by the Tukey method[6] using a digital computer. Results of these analyses are shown in Fig. 2. At Alibag the broad spectral peak in the range of interest is centred at 26 months. At Apia it appears at 27 months for each of two separate periods of observation, although when the whole of the data was analysed together the peak was less strong. We consider this to be evidence that, although the fluctuation tends to be periodic, it is randomly excited. Two analyses for Huancayo, one of which is shown in Fig. 2, gave weak and ill-defined peaks at a slightly longer period. While the spectra appear to constitute good evidence of the extension of the stratospheric fluctuation to ionospheric heights, it must be pointed out that when the spectral peaks are subjected to strict statistical tests, they are found to be only marginally significant. The strong increase in spectral density at periods longer than about 3 years evidently represents the high-frequency end of the secular variation spectrum, which certainly masks any longer-term ionospheric effects.

Ionospheric data from Singapore, Ibadan and Slough have also been analysed. We have used the critical frequencies and virtual heights of the *E* layer at midday and the F_2 layer at midnight for the period 1949–61 (1954–61 at Ibadan), but have found no evidence for a periodicity comparable with that observed in *Hq*. Another ionospheric parameter, which may be more directly relevant, is ionospheric wind. There are virtually no published data for equatorial stations. For middle latitudes we have plotted 12-monthly running means of data given by

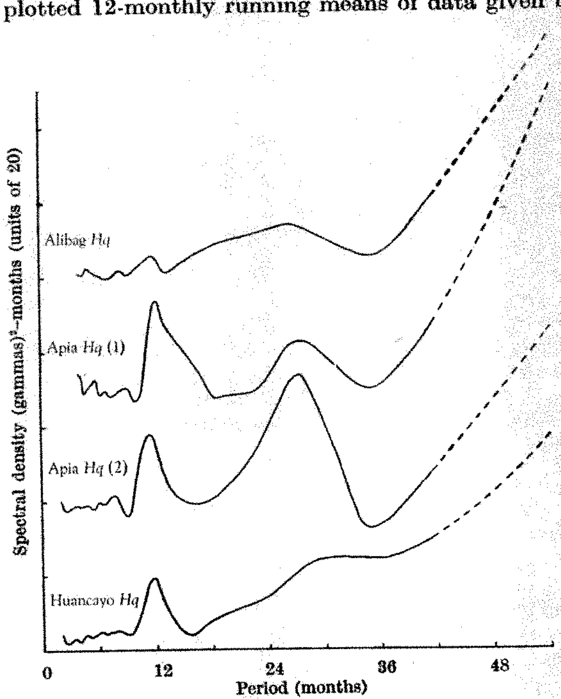

Fig. 2. Power spectra of *Hq* at equatorial observatories. Alibag, Apia (2) and Huancayo for the years 1922–44, Apia (1) for 1939–58. The data were analysed in the normal way in terms of frequencies (months⁻¹) but have been replotted against periods

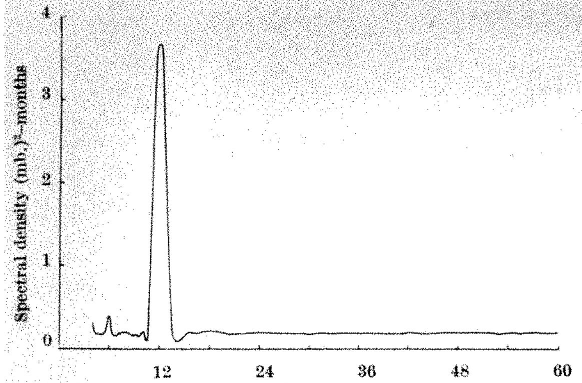

Fig. 3. Power spectrum of surface pressure variations at Apia, 1939–58

Briggs and Spencer[7] for 1949–52 and Greenhow and Neufeld[8] for 1953–58. The latter may indicate a 2-year periodicity, but the record is too short for this to be regarded as significant. Greenhow and Neufeld[9] reported an hour-by-hour correlation between ionospheric wind and surface pressure. A comparison of 12-monthly running means of the Cambridge[7] and Jodrell Bank[8] ionospheric wind data with surface pressure at Kew and Eskdalemuir suggests that the correlation may extend to longer periods. We therefore examined the barometric pressure data for Apia, but found a complete absence of spectral peaks longer than 12 months, as is shown in Fig. 3, although Fairbridge and Krebs[10] suggest that both sea-level and barometric pressure follow the southern oscillation on a world-wide basis.

The apparent downward propagation of the stratospheric wind cycle is very suggestive of an excitation external to the Earth but an analysis of Zurich sunspot numbers[11] gave no evidence of any solar periodicity in the range of interest.

We conclude that the striking 26- or 27-month periodicity in the equatorial stratosphere has a slight and erratic but noticeable extension to ionospheric heights. The magnetic evidence is consistent with the random excitation of an oscillatory mechanism in the upper atmosphere, there being no evidence of a comparable periodicity in solar activity. The extension of the oscillation into the troposphere is apparently slight, contrary to Berlage's[5] suggestion that the meteorological periodicity of 2¼ years arises from an atmosphere–ocean interaction. Since the ionosphere only participates marginally in the stratospheric fluctuation, it appears that the oscillatory mechanism must be a property of the stratosphere itself.

This article is submitted by permission of the Director-General of the Meteorological Office. The computer programme which was used had been prepared by Dr. H. M. Iyer and Mr. V. A. Punton.

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OBSERVATION OF FOSSIL PARTICLE TRACKS IN NATURAL MICAS

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PREVIOUS electron microscope studies have shown that fission fragments from uranium-235 produce tracks in mica[1-3] and other minerals[4,5]. In those experiments the fission fragments were produced by irradiating a uranium source with thermal neutrons from a nuclear reactor. In this article we report observations of tracks in samples of natural minerals which had not been deliberately irradiated. The tracks in these minerals were produced over a long period of time by natural radiation processes and constitute, therefore, a 'fossil' record of the radiation exposure of the minerals.

Experiments on various micas have shown that only very heavy nuclear particles produce visible tracks with any measurable efficiency[5]. There are at least two natural sources of such very heavy particles—fission fragments from the spontaneous fission of uranium impurities and recoil nuclei from cosmic ray induced nuclear disintegrations. We have observed tracks of recoil nuclei from artificially induced nuclear disintegration[6] but have not yet experimentally identified natural fossil tracks arising in this way. The fossil tracks reported here arise from the spontaneous fission of uranium and have been observed in two different types of mica samples—one containing uranium in small inclusions of another crystal, the other from a pegmatite which contained contiguous deposits of a uranium ore (uraninite).

Consider first the sample containing uranium-bearing inclusions. Many natural micas contain small inclusions of other minerals such as apatite or zircon which are surrounded by pleochroic haloes. It has long been known that these haloes arise from radiation damage by α-particles produced from uranium or thorium impurities in the inclusion[7]. Since uranium undergoes spontaneous fission, the region in the immediate vicinity of such an inclusion should show fission fragment tracks, provided such tracks are stable over long periods of time. Fig. 1 shows a transmission electron micrograph of a region of a thin flake of biotite adjacent to a halo-producing inclusion. The inclusion itself has been removed, leaving a hole which shows up in the picture as a large white area. The biotite flake was etched in hydrofluoric acid prior to observation in order to 'develop' and 'fix' the fission fragment tracks by a

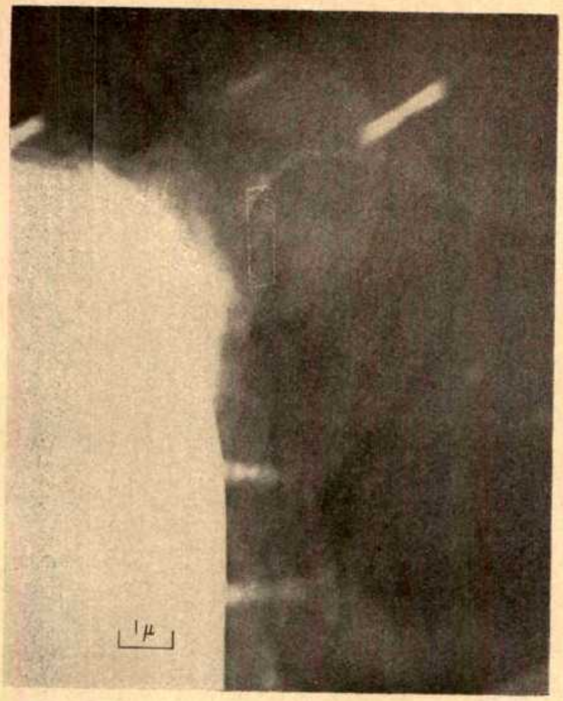

Fig. 1. Transmission electron micrograph of a biotite crystal which originally contained a radioactive inclusion. The channels around the periphery of the inclusion are etched-out regions which indicate the passage of fission fragments

selective etching process which has previously been described[6,8]. Although the details are obscured because of the great thickness of this flake, several channels which have the size and characteristics of etched fission tracks in mica[8] can be seen emanating from the boundary of the inclusion. We attribute these tracks to 'fossil' spontaneous fission events from uranium impurities in the inclusion.

The key question to be answered if tracks are to be used for studies of the radiation history of rocks is whether the tracks, once formed, remain in the samples over long periods of time. In the experiment described here, the amount and distribution of uranium in the inclusion were not determined; hence it was impossible to determine the age of the tracks. In the second experiment, however, the mica was immediately adjacent to a uranium ore, so that the observed number of tracks could be related directly to a track storage time. Fig. 2, in which a number of tracks is visible, shows a portion of an etched mica flake which was removed from the immediate vicinity of the ore. In some samples the track density was as high as $5 \times 10^8/\text{cm}^2$. If all the tracks are attributed to spontaneous fission events, this density corresponds to a storage time or track age of $\sim 10^6$ years for mica in direct contact with the ore. The possibility that some of the tracks were produced by α-particles was ruled out by experiments on the same sample with a polonium α-source.

We regard 10^6 years as a lower limit for track storage time. The uraninite did not occur interleaved with the layer planes of mica but was, instead, always off to one edge. This made it difficult to strip thin samples for electron microscope observation which were definitely within a few microns of the ore and it is likely that our flakes were actually somewhat removed from the ore.

Separate high-temperature annealing experiments suggest that the tracks should be retained almost indefinitely at normal temperatures. When samples of different types of micas containing fission tracks were heated in air and then afterwards examined in the electron microscope, it was found that the tracks suffered no visible change until just below the temperature at which the mica normally decomposed into other crystal phases[3,5] (for example, $\sim 900°$ C in the case of biotite). The tracks thus appear to be relatively permanent features of mica crystals and should remain intact for geologic times.

Unfortunately, this conclusion is weakened by the existence of another effect—the phenomenon of track fading which occurs in natural micas during observation in the electron microscope. It has been shown[5] that this fading phenomenon does not result primarily from a local temperature rise induced by the electron beam. To explain the effect we have advanced the hypothesis that the electron beam produces a particular valence state of a crystal defect which is then mobile near room temperature. However, more work needs to be done on this problem, and until it is completely understood one cannot be sure that a similar fading process does not occur in geologic times under natural exposure conditions. It is thus important to measure directly the maximum storage time for tracks, and it would be of considerable interest to find specimens with still longer storage times than the 10^6 years measured here. Separate reactor irradiation experiments show that storage times of up to 2×10^8 years would give a density of tracks which could still be resolved.

If uranium is distributed throughout the mica as an atomic impurity then the measurement of the track density will give a sensitive measure of the

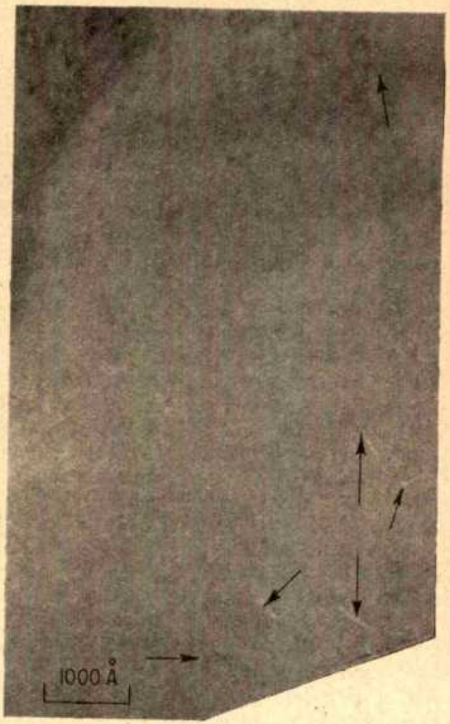

Fig. 2. Etched fission fragment tracks, indicated by arrows, in a portion of a mica crystal which was found adjacent to a uranium ore. Some of the tracks have existed in the crystal for 10^6 years

amount of uranium present. For example, assuming a mica the age of which is 10^8 years, the minimum observable track density ($\sim 10^3/\mathrm{cm}^2$) corresponds to a concentration of only 5×10^{-8} of uranium.

As a corollary, if the uranium content can be independently shown to be less than this amount, then any significant track densities which are observed can be ascribed to other sources such as cosmic rays or the spontaneous fission of other elements. For samples of very low uranium content, which have been well shielded from cosmic rays, it is suggested that the track technique might provide a way of measuring spontaneous fission rates of other heavy elements the life-times of which are too long to detect by conventional techniques.

In this article we have presented evidence for the existence of fossil tracks in samples of natural mica. These tracks were produced by fragments from the spontaneous fission of uranium. From the measured track density we inferred a track storage time of at least 10^8 years. The importance of further storage time measurements was emphasized and it was pointed out that measurements of the type reported here should be possible up to a time of $\sim 2 \times 10^8$ years. The potential applicability of the track technique for measuring low concentrations of uranium impurities was also noted. Finally, we indicated that in samples with very low uranium content it might be possible to measure spontaneous fission lifetimes which are too long to be measured by conventional techniques.

We thank W. G. Johnston for his helpful suggestions and Mrs. Ethel Fontanella for assistance with the experiments.

Note added in proof. We are now able to observe particle tracks in the optical microscope and can work with track densities as low as $10/\mathrm{cm}^2$. Using the optical technique, we have found tracks, arising from spontaneous fission of uranium impurities, which have been stored in mica for $\sim 10^9$ years. The implications of this new technique for geochronology and the measurement of very low uranium concentrations will be reported elsewhere.

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SOME EARLY WORK ON ENERGY METHODS IN THEORY OF STRUCTURES

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CASTIGLIANO[1] is popularly regarded as the originator of energy methods in theory of structures. It is, however, interesting to discover significant contributions in this field by British scientists, namely, H. Moseley and J. H. Cotterill, prior to 1873, the year of Castigliano's thesis to the University of Turin.

In 1843, Moseley, at the time professor of natural philosophy and astronomy in King's College, London, had his celebrated text-book published[2]. It is based on a course of lectures delivered to students of engineering and architecture of King's College, and the influence of the French school, notably Navier and Poncelet, is acknowledged in the preface. There Moseley credits the latter with suggesting a method of calculating the internal work necessary to produce a given deflexion of an elastic beam (*Mécanique Industrielle*, 3me partie, 1831) and its equality to the work done by the load. (Todhunter and Pearson[3] also credit Poncelet with the introduction of the "principle of work", that is, the use of conservation of energy, into practical mechanics.) Articles 368 and 369 of Moseley's book are devoted to the calculation of the internal work or strain energy of beams (having dealt with the simpler problem of an elastic bar in tension in Article 349) and it is interesting to note that the results are correct on the basis of linear elasticity and are presented in the form:

$$U = \frac{P^2}{EI} \times \text{constant } (K) \tag{1}$$

where, in Moseley's notation, U denotes work, P denotes pressure (load), and EI is the flexural rigidity. Then in Article 370 the deflexion of a simply supported linearly elastic beam under a single "deflecting pressure" is calculated in what now seems a very curious manner. In his derivation Moseley notes that if D is deflexion under pressure P:

$$P = \frac{\mathrm{d}U}{\mathrm{d}D} \tag{2}$$

then he writes:

$$P = \frac{\mathrm{d}U}{\mathrm{d}D} = \frac{\mathrm{d}U}{\mathrm{d}P}\,\frac{\mathrm{d}P}{\mathrm{d}D} \tag{3}$$

and noting: $U = K\dfrac{P^2}{EI}$, puts $\dfrac{\mathrm{d}U}{\mathrm{d}P} = 2K\dfrac{P}{EI}$ to give:

$$P = 2K\frac{P}{EI}\,\frac{\mathrm{d}P}{\mathrm{d}D} \tag{4}$$

or:

$$\frac{\mathrm{d}D}{\mathrm{d}P} = \frac{2K}{EI} \tag{5}$$

finally, he integrates to obtain the form:

$$D = 2K\frac{P}{EI} \tag{6}$$

Having noted the similarity of Moseley's notation to that used nowadays, the next ground for comment is the somewhat tortuous procedure adopted when the required result could have been obtained simply by direct application of conservation of energy (of which Moseley was clearly aware in view of his comments on Poncelet's work) thus:

$$\tfrac{1}{2}PD = U = K\frac{P^2}{EI} \qquad (7)$$

whence:

$$D = 2K\frac{P}{EI} \qquad \text{(equ. 6)}$$

for it is not as if Moseley's method, as presented, was capable of generalization.

What is really impressive about this work is Moseley's apparent leaning toward the use of energy derivatives, the more so when it is noted that he proposed what he called the principle of "Least Resistance" in 1833 in connexion with his distinguished work on the mechanics of arches (Articles 333–346 of his book). Reference to this principle is made by Cotterill, whose contribution to energy theory is also highly significant and is probably partly due to his knowledge of Moseley's work. Before considering Cotterill's work, however, it is noteworthy that Moseley's book also presents correctly the differential equation approach for determining deflexions of beams and a correct analysis of continuous beams based on Navier's work[4]. Unfortunately it is impossible to do justice here to Moseley's remarkable book which, it is believed, could still be stimulating to civil and mechanical engineers.

Turning now to Cotterill, he published an article "On an Extension of the Dynamical Principle of Least Action" in 1865 [5] and the essential features of his proposition are contained in the following quotation:

"Now Mr. Moseley has shown that if any number of pressures are in equilibrium, some of which are resistances, then each of these resistances is a minimum, subject to the conditions imposed by the equilibrium of the whole—a principle which he has called the principle of Least Resistance; let us assume this principle, and let us further suppose, for the present, that it is generalized so as to include the case of resisting forces generated as above described [by elastic displacements]; then each of those resisting forces is a minimum, subject to the general conditions stated above; and further, the relative displacements which are the cause of those forces must also be the least possible, and the work done the least possible. Thus in the assumption mentioned, to which I shall return in the sequel, it appears that the work done is a minimum, subject to the law of conservation of energy and the statical conditions of equilibrium; and this principle, analogous to the dynamical principle of Least Action, it is the object of this article to consider and apply.

"If the work done be expressed in terms of the resisting force at all points of the system, or some of them, then, the law of conservation of energy being implicitly satisfied, we have simply to make the work done a minimum, subject to the statical conditions of equilibrium."

Cotterill demonstrates his proposition first by considering a uniform beam loaded by a distributed load of uniform intensity w over its length and subjected to restraining couples M_1 and M_2 at its ends, respectively. If $2c$ is the span, the strain energy of the loaded beam is given as:

$$U = \frac{c}{3EI}\left\{M_1^2 + M_1M_2 + M_2^2 - wc^2(M_1 + M_2) + \frac{2}{5}w^2c^4\right\}$$

Considering the problem of determining the values of M_1 and M_2 to cause the ends of the beam to be fixed horizontally, Cotterill proceeds by asserting that the values of M_1 and M_2, which cannot be found by statics alone, must be such that U is a minimum, that is:

$$2M_1 + M_2 - wc^2 = 0, \quad 2M_2 + M_1 - wc^2 = 0 \qquad (8)$$

whence:

$$M_1 = M_2 = \frac{1}{3}wc^2 \qquad (9)$$

which result, he remarks, "agrees exactly with that given by the ordinary method . . . the reason of which will be seen from what follows. Let i_1, i_2 be the slopes at the extremities of a beam acted on by M_1, M_2 at its extremities, and by the uniform load w then U being, as shown above, an homogeneous quadratic function of M_1, M_2, we have:

$$2U = \frac{dU}{dM_1}M_1 + \frac{dU}{dM_2}M_2 + \frac{dU}{dw}w$$

but by the law of conservation:

$$2U = M_1i_1 + M_2i_2 + wu$$

Where u is the area of deflexion of the beam; and comparing these expressions, we see that:

$$\frac{dU}{dM_1} = i_1, \ \frac{dU}{dM_2} = i_2, \ \frac{dU}{dw} = u$$

but the ordinary method is founded on the consideration that the beam is horizontal at its extremities, in other words, that $i_1 = 0$, $i_2 = 0$; so that the two methods lead to the same result by the same equations. And this will be the case in all questions concerning continuous beams; but the present method enables us to obtain the requisite equations by differentiation of a single function".

Toward the end of his paper, Cotterill proceeds as follows: "Having briefly indicated the mode of applying the principle of Least Action to various problems, I return to its demonstration.

"From the description given of the process by which equilibrium is attained, it is apparent that if the principle of Least Resistance be given, the principle of Least Action follows, and vice versa; and since the principle of Least Resistance is well known, I have assumed it. But inasmuch as that principle has perhaps never been satisfactorily proved, at least in its general form, it will be well to give a direct demonstration of the principle of Least Action in the case where the body is perfectly elastic.

"Let X, Y, Z be the components of one of the forces acting on a free perfectly elastic body, u, v, w the displacements of its point of application parallel to three rectangular axes, U the work done in the body, then:

$$2U = \Sigma(Xu + Yv + Zw)$$

but U may be expressed as a homogeneous quadratic function of the forces; therefore:

$$2U = \Sigma\left\{X\frac{dU}{dX} + Y\frac{dU}{dY} + Z\frac{dU}{dZ}\right\}$$

comparing which expressions for U, we see that:

$$\frac{dU}{dX} = u, \ \frac{dU}{dY} = v, \ \frac{dU}{dZ} = w$$

Now conceive the body, instead of being free, to be immovably attached at certain points to some fixed object, then we shall have for these points:

$$\frac{dU}{dX} = 0, \ \frac{dU}{dY} = 0, \ \frac{dU}{dZ} = 0$$

that is, the variation in U, due to a change in the resisting force at the fixed boundaries of the system, is zero. And the same is true for a change in the resisting force anywhere within the mass; for conceive the body to be divided into two parts by a surface of any form passing through the point, and let U_1, U_2 be the works done in the two portions, then $\frac{dU_1}{dX}$ and $\frac{dU_2}{dX}$ are evidently equal and of opposite sign, that is $\frac{dU}{dX} = 0$ as before. Since, then, the change in U, consequent on any possible change in the resisting forces, is zero, U must be a minimum (the other two possible hypotheses being easily seen to be inadmissible), and the principle is proved for a perfectly elastic body or system of bodies."

There are three striking features of Cotterill's paper. First, it is based initially on a plausible, though fallacious argument depending, it seems, on the assumption of natural economy (his method is valid only for what he calls "perfectly elastic" systems, meaning those the elasticity of which is linear and not elastic systems generally); secondly, he uses the homogeneous quadratic form of U for "perfect" (linear) elasticity to deduce:

$$\frac{dU}{dX} = u, \frac{dU}{dY} = v, \frac{dU}{dZ} = w \qquad (10)$$

and finally, he appears to recognize that his minimization of U provides conditions of compatibility of deformations.

Cotterill followed this article with two more articles[6] on applications of his proposed method. The first of these "On the Equilibrium of Arched Ribs of Uniform Section" is remarkable in that it contains the following: "In my former article I endeavoured to show that if X, Y, Z be the components of one of the forces acting on a perfectly elastic body u, v, w the displacements of its point of application produced by the action of the forces on the body, then:

$$\frac{dU}{dX} = u, \frac{dU}{dY} = v, \frac{dU}{dZ} = w$$

But the reasoning is not so conclusive as the following. Since:

$$2U = \Sigma(Xu + Yv + Zw)$$

$$2\delta U = \Sigma(X\delta u + Y\delta v + Z\delta w) + \Sigma(u\delta X + v\delta Y + w\delta Z)$$

but $\Sigma(X\delta u + Y\delta v + Z\delta w)$ is the increment of the energy expended, which by the law of conservation of energy is equal to δU the increment of work done, therefore, we have also:

$$\delta U = \Sigma(u\delta X + v\delta Y + w\delta Z)$$

whence the above equations follow." Here the final expression actually represents *change of complementary energy* which, in spite of Engesser's work[8], has only comparatively recently been widely accepted as the energy approach to compatibility of strains and deformations of elastic systems, linear or otherwise.

Thus, not only did Cotterill anticipate some of the salient features of Castigliano's work but he eventually realized, apparently, that the whole basis of his method lay in the fact that for a "perfectly elastic" system a small change in strain energy could be written in the form $\Sigma(u\delta X + v\delta Y + w\delta Z)$. His reasoning here is perfectly sound, being based, apparently, on what Lamé[9] called Clapeyron's theorem (that is, $2U = \Sigma(Xu + Yv + Zw)$) for linear systems. It is interesting to speculate whether, if Cotterill's work had received the attention which it warranted, some of the confusion which was to envelop energy methods afterwards would have been avoided.

Perhaps the reason why Cotterill's work has been neglected is that it was based, at first, on an incorrect argument, though he afterwards provided alternative demonstrations, in the same article, which are both correct and illuminating. It is interesting to note that he appears to have been a scholar of St. John's College, Cambridge, in 1865.

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ICE NUCLEATION BY α-PHENAZINE

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ALTHOUGH recent research work[1-3] has shown that many crystalline organic preparations will nucleate ice, relatively few are known in which the majority of particles contain active sites. In many preparations most of the smaller particles are inactive although particles larger than 30μ may operate at higher temperatures.

It was found recently that a number of diazines showed unusually high nucleative power, the greatest activity being exhibited by α-phenazine. Tests in a cloud-chamber showed that more than 10^9 nuclei/g, active at −6° C, could be obtained from a powder of finely crushed phenazine crystals, or greater than 10^{11} nuclei/g active at −12° C from a smoke of sublimed crystals. Since it is estimated that about 10^{12} nuclei/g of silver iodide are effective at −12° C in present cloud seeding operations, α-phenazine can compete with silver iodide as an ice nucleator.

The onset temperature found by cold-stage and cloud-chamber tests was −3·5° C for crushed crystals grown from alcohol or the melt. Cold-stage investigations indicated that all particles larger than 50μ were active at −5° C, while those larger than 10–20μ in size nucleated before −8° C was reached. Ice was seen to grow from invisible nuclei at −12° C.

Density measurements proved that α-phenazine was invariably formed in all the physical preparations, although the β-polymorph has evidently been obtained occasionally by crystallization from solvents.

Cloud-chamber tests were made in the apparatus developed by Edwards and Evans[4], in which provision for counting was made by allowing one in a thousand of the falling ice crystals to nucleate cooled sugar solution on a moving tape. Samples were introduced either by blowing in a known weight of the fines from crushed crystals, or by volatilizing a 5–10 μg sample of phenazine in a flask and injecting an aliquot part with a syringe. Since the larger powder particles did not remain in suspension and because much of the volatilized smoke was deposited on the walls of the flask and syringe used for injection, the counts of nuclei/g quoted here should be regarded as lower limits.

A summary of the tests is given in Fig. 1, where curve *A* is a plot of the activity of crushed powder preparations. It is seen that the majority of particles operate at temperatures above −6° since there is no increase of count above 10^9 nuclei/g at lower temperatures. Due to the small size of the smoke particles produced by flash evaporation, there is less activity at higher temperatures, the number of nuclei/g exceeding those in crushed powders only below −8° C. At −12° C, 10^{11} nuclei/g were obtained, as shown in curve *B*.

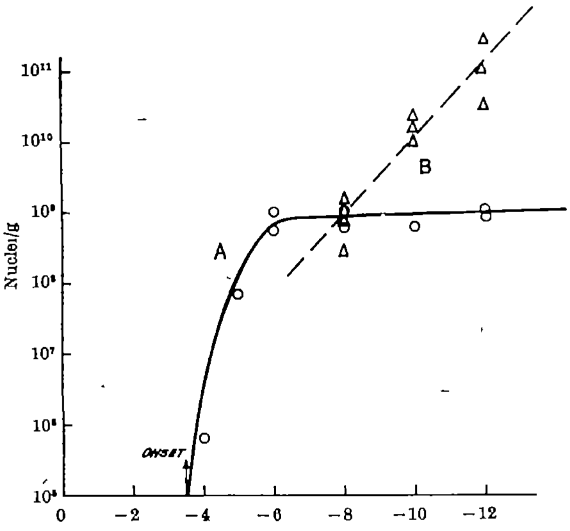
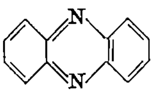
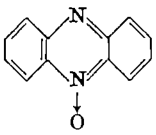
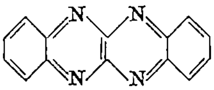
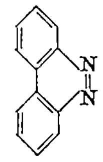
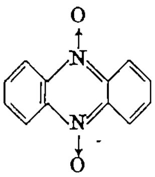


Fig. 1

The activities of a number of other diazines are shown in Table 1. *N*-oxy-phenazine and 5,6,11,12-tetra-aza-naphthacene were almost as active as α-phenazine, although preparations of 3,4-benzocinnoline, an isomer of phenazine, appeared to contain only a small proportion of active particles. α-Phenazine and *N*-oxy-phenazine can both be sublimed to form smokes. At −12° C there is little difference in the nuclei count obtained although the latter compound is much less active than α-phenazine at higher temperatures. It is of interest that the molecular arrangement and lattice parameters of these compounds are almost identical. Hydrogen bonding to the ice structure has been shown to be necessary[3], and in the case of α-phenazine must take place via the nitrogen atoms by virtue of their lone pairs of electrons; in *N*-oxy-phenazine additional bonds may be formed between the heavily polarized oxygen atoms and the ice.

Table 1

Compound	Molecular structure	Activity of powdered crystals at −12° C in cloud chamber (nuclei/g)
α-Phenazine		10^9
N-oxy-Phenazine		5×10^9 *D*
5,6,11,12-Tetra-aza-naphthacene		5×10^9
3,4-Benzo-cinnoline		2×10^7
Phenazine-di-*N*-oxide		10^9 *D*

(*D* refers to delay in nucleation)

The crystal structure of α-phenazine has been determined by Herbstein and Schmidt[5], and that of *N*-oxy-phenazine by Curti *et al.*[6]. The face development of α-phenazine crystals is derived simply from the molecular packing, the most densely populated planes and well-developed faces being (100), (001), and $(20\bar{1})$. A close fit can be shown to exist between the prism faces of ice and these three planes, although no satisfactory fit can be obtained to the (010) plane which is at right-angles to the longest axis of growth. The calculated disregistries of ice with respect to the substrate are given in Table 2. The unit cell dimensions of ice at 0° C were taken as a = 4·51 Å, and c = 7·35 Å.

The extraordinarily large number of active particles characteristic of α-phenazine may be attributed to the fact that as many as three crystal planes fit the ice structure closely. Since the space group of α-phena-

Table 2

Plane of ice	Plane of phenazine	Percentage disregistry along ice axes a	c	Proportion of α-phenazine molecules bonding to ice
$1\bar{1}00$	001	+2·3	−3·2	1 in 3
$1\bar{2}10$	100	+2·8	+3 7	1 in 3
$1\bar{2}10$	$20\bar{1}$	+2 8	−1·9	1 in 3

zine is centro-symmetrical, the same pattern of nitrogen atoms is presented on planes such as (100) and ($\bar{1}$00), which further increases the number of planes in registry. It is evidently not necessary for the two faces formed by a step to register simultaneously with the ice nucleus although such registry occurs in silver iodide where both ice embryo and nucleus crystallize in the same system. It has been observed that nucleation does not take place on plane faces of organic crystals, but at cracks and steps. In α-phenazine there is a much greater chance that such sites will incorporate at least one plane in registry with ice than in crystals like fluorenone (I)[2], where only one plane is in registry and the number of active sites low.

There is no doubt that the brittleness of α-phenazine crystals contributes to the large number of active particles in crushed powder preparations. The needles grown from alcohol cleave easily down their length, resulting in brittle fractures. During an earlier investigation, however, it was found that the active sites in the more waxy crystals such as the steroids are destroyed by plastic deformation unless cooled in liquid oxygen prior to crushing.

The fact that controlled impurities have been found to increase the number of active sites in fluorenone crystals[3] suggests that the large number of active particles found in α-phenazine preparations might be due to the presence of impurities. There is, however, no experimental confirmation of this. The phenazine used in the experimental work was prepared by a method described by Morley[7], in which pyrocatechol was condensed with *o*-phenylene diamine. After chromatographing and recrystallizing the product, the correct melting-point of 176° C (ref. 8) was obtained. This preparation and a commercial sample of phenazine gave counts of 10^9 nuclei/g before and after zone-refining, although crude brownish material from the mother liquor gave only 10^7 nuclei/g. It thus seems more likely that the high number of nuclei/g is due to a fortuitous combination of lattice parameters rather than to the presence of impurities.

Efficient cloud seeding requires the use of nuclei stable in sunlight. While it was not possible to determine the rate of photolytic decay of α-phenazine smoke, owing to the high speed of coagulation, tests on slides showed that, for powdered preparations, no observable decrease of activity took place after exposure to ultra-violet radiation for an hour. However, only 40 per cent of the very small crystals deposited from smokes appear to nucleate after irradiation for 1 hr at a distance of 8 in. from a 550 watt Hanovia quartz mercury arc lamp.

I wish to thank Dr. I. S. Walker, of the Division of Physical Chemistry, Commonwealth Scientific and Industrial Research Organization, for valuable discussions relating to this work.

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γ-EMITTING NUCLIDES IN MILK SINCE 1960

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SINCE October 1957, γ-spectrometric measurements of fission product concentrations in skimmed dried milk have been made in this department[1,2]. This article continues our results for cæsium-137 concentrations in milk up to the end of August 1962 and also gives data for iodine-131 and barium-140 during the period immediately following fresh weapons testing in the autumn of 1961. Milk was obtained from the Milk Marketing Board's creamery at Felin Fach, Cardiganshire, where the supply from a wide area is pooled and steps are taken to ensure a satisfactory sampling procedure[1]. Measurements were normally made monthly by mixing samples from four successive weeks but, on the resumption of testing, the programme was intensified and samples from Cardiganshire were obtained twice weekly. In addition, in order to obtain information on the intake of fission products by consumers in a district near London, a series of measurements was made on liquid milk purchased locally.

Observations were made by placing about 800 g of milk powder or 1,500 g of liquid immediately around a 3-in. × 3-in. sodium iodide crystal. Conclusions were drawn solely on the basis of γ-spectra without chemical treatment of the samples.

Before September 1961 and after December 1961, the only γ-emitting nuclides identified were cæsium-137 and naturally occurring potassium-40. However, in samples obtained immediately after the commencement of the Russian test series, the spectra showed additional photo-peaks due to the short-lived fission products iodine-131 and the barium-140–lanthanum-140 pair (Fig. 1). All the peaks could be ascribed to the foregoing nuclides with the exception of one small one at about 1·05 MeV. This peak was not present when the spectrum of the sample was re-measured two months later and was probably due to cæsium-136, a fission product with very similar half-life (13 days) to that of barium-140 but of considerably lower fission yield. The presence of cæsium-136 in rainwater and in milk has recently been reported by Morgan *et al.*[3].

Fig. 2 shows the concentration of cæsium-137 in milk from Felin Fach; the values prior to September 1961 were the lowest recorded by us since we commenced measurements in 1957. Fig. 3 sets out the data for the period September–December 1961 in greater detail and includes values for iodine-131 and barium-140. The measurements were made on skimmed dried milk; values are quoted in μμc./l. of whole liquid milk on the assumption that 90 g of powder is equivalent to 1 l. of liquid. This figure is based on data from the creamery. Measurements made on whole liquid milk and on a skimmed milk fraction indicated that a negligible proportion of iodine-131 was associated with the fat component; a similar result has been reported by Squire *et al.*[4].

A rise in the concentrations of cæsium-137 as well as the shorter-lived iodine-131 and barium-140 is noticed after September 10, 1961. The mean value measured for iodine-131, namely, 156 μμc./l. over the 12-week period September 16–December 9 is slightly higher than the mean value of 136 μμc./l. given by the Agricultural Research Council for the same period for a neighbouring site in Carmarthenshire[5].

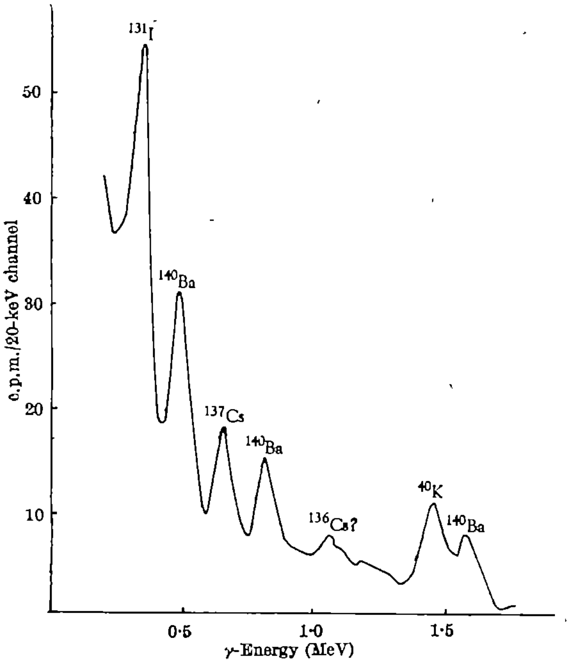

Fig. 1. γ-Spectrum of milk sample from Felin Fach (West Wales) of November 4, 1961 (Typical of period October 1–November 17, 1961)

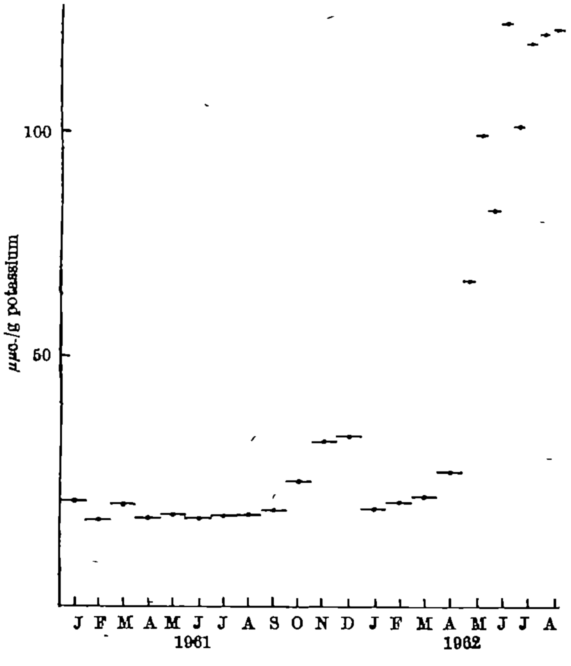

Fig. 2. Monthly average of ^{137}Cs concentration in cows' milk from Felin Fach (West Wales)

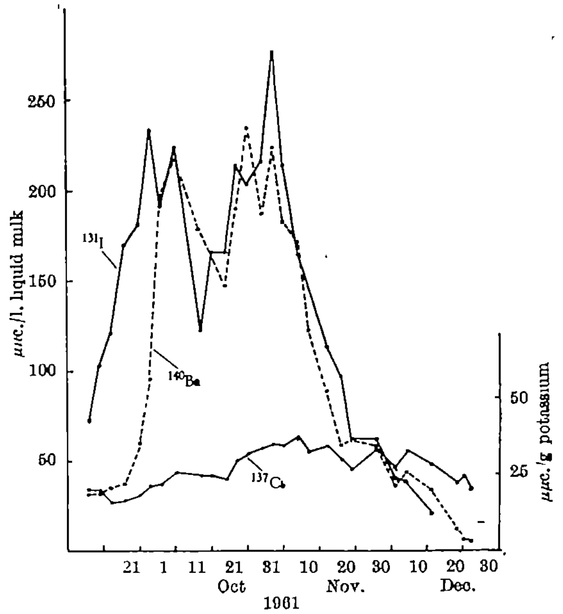

Fig 3 ^{131}I, ^{140}Ba and ^{137}Cs concentrations in cows' milk from Felin Fach (West Wales). Values corrected for decay to date of milking

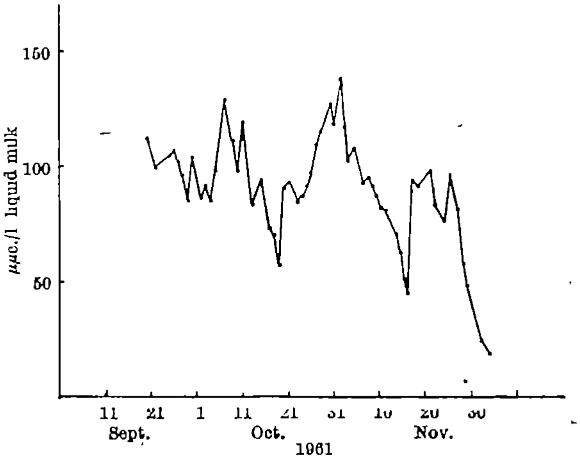

Fig. 4. ^{131}I concentrations in cows' milk delivered at Sutton, Surrey. Values corrected for decay to date of delivery

Fig. 3 shows that there were two main peaks in activity of the shorter-lived nuclides. Similar peaks have been noted in an examination of atmospheric dust and rainwater[6,7], but there is no clear correlation between these and the peaks found in the milk. The fall in the values for milk during November is probably a result of natural decay and of transfer of cattle to stored feeding stuffs. By January 1962 the iodine- and barium-levels were below the limit of detection, and the cæsium-137 values had fallen almost to the pre-1961 test-level. No further significant rise was seen until May (Fig. 2). This latter rise is also a consequence of a change in cattle feeding stuffs, this time back again to summer pastures which will have been subject to high fall-out during the Spring. The cæsium-137 values during June, July and August are 60–80 per cent of the value for May 1959, which was the highest recorded by us in our whole series of measurements[2].

Fig. 4 gives the results of measurements of iodine-131 concentrations in milk as delivered in a district in the London area. A trend very similar to that of the Welsh samples is observed although the absolute values are lower and the relatively high values continue for about a fortnight longer into November. The mean value of 79 μμc./l. for the 12-week period here is similar in magnitude to the nation-wide mean of 91 μμc./l. given in the report of the Agricultural Research Council[5]. Values of barium-140 were not obtained for these samples as the peaks in the γ-spectra were not large enough to allow accurate measurements. The concentration of barium-140 was somewhat less, relative to iodine-131, than in the samples obtained from the creamery in Wales.

It will be seen (Fig. 3) that barium-140 concentrations in milk from Wales during October 1961 were comparable with, and in some cases greater than, the corresponding iodine-131 values. The mean barium-140/iodine-131 ratio for the period September 16–October 5 is 0·33, for the remainder of October, 0·95 and for November, 0·85. It is interesting to compare these figures with the mean ratio for cumulative deposition on grass at Chilton, Berks, given by Fisher *et al.*[6]. For the above periods, the corresponding barium–iodine ratios are 2·6, 4·6 and 5·4 respectively. If similar values are assumed for herbage consumed by cattle in Cardiganshire, it is possible to estimate a discrimination factor between barium and iodine during transfer from grass to milk. From the foregoing figures for milk and for grass there appears to be a factor of roughly 6 to 1 against barium. This is less than the mean discrimination factor of 40 to 1 against barium which may be deduced from the results of Squire *et al.*[4] in an experiment in which cattle were fed with trace quantities of mixed fission products from air filters. This experiment was conducted for six days following a single administration whereas our value results from continuous ingestion for several weeks. Our estimate of 6 to 1 may also be low due to geographical and meteorological differences between Cardiganshire and Berkshire, and it is significant, as mentioned here, that our locally delivered milk showed somewhat lower barium–iodine ratios.

The mean daily iodine-131 concentration (Figs. 3 and 4) averaged over 12 months is 18 μμc./l. for milk delivered near London and 36 μμc./l. for milk produced in West Wales. If no fresh iodine-131 enters the milk before the middle of September 1962, these levels are 14 and 28 per cent respectively of the maximum concentration in milk (130 μμc./l. for one year) allowed by the Medical Research Council for children up to the age of one year[8]. The continuous ingestion for 12 months of 0·7 l. of milk a day containing 36 μμc./l. of iodine-131 would deliver a dose of 280 mrads to the thyroid of a one-year-old child or 8 mrads in the case of an adult. A detailed discussion of the dosimetry along with values of thyroid burdens is given in a recent paper by Eisenbud *et al.*[9].

It is also necessary to consider barium-140 and its daughter lanthanum-140 as a possible health hazard. The mean concentration of barium-140 measured in milk from Wales during the period September 16–December 9, 1961, was 126 μμc./l. This is 29 μμc./l when averaged over a period of 12 months. The maximum permissible level in water for continuous ingestion by the whole population is 3×10^3 μμc./l. (assuming an intake of barium-140 and lanthanum-140 in equilibrium) (ref. 10) which is more than 100 times the value given here.

I thank Prof. W. V. Mayneord for his interest throughout this work and also Dr. W. Anderson for criticism and advice.

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MEASUREMENTS OF IODINE-131 IN HUMAN THYROIDS FOLLOWING NUCLEAR TESTS IN 1961

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DURING September 1961, immediately following the resumption of nuclear testing, when there was widespread public concern that the amount of iodine-131 in human thyroids might reach significant proportions, it was decided to make regular measurements of thyroid radioactivity on a few members of the staff at the Radiological Protection Service. By the end of September, iodine-131 could be detected in the thyroids of these people and thereafter it rose steadily up to a peak in the early part of November. From then on it decreased and was immeasurably small by the middle of January 1962. During the peak period a total of 20 members of the staff were measured and the average amount of iodine-131 in their thyroids was 0·25 mμc. It was estimated that the average radiation dose received by the thyroids of these people as a result of the whole exposure was about 7 mrads. The measured activities were extremely small and the estimated magnitude of the errors of measurement will therefore be of some interest to persons working in this field. It is of even greater interest that the average amount of iodine-131 actually measured in the thyroids of the group could be predicted from a knowledge of the iodine uptake into the thyroid and the known concentration of iodine-131 in milk during this period. This adds weight to the belief, now widely accepted, that fresh milk is the principal vehicle whereby iodine-131 from

Table 1. MEASURED AMOUNT OF IODINE-131 IN HUMAN THYROIDS (mμc.)

Subject	22.9.61	25.9.61	27.9.61	2.10.61	26.10.61	1.11.61	7.11.61	14.11.61	21.11.61	6.12.61	2.1.62	17.1.62
Females												
A		0·33	0·25	0·48	0·8	1·0	0·82	0·98	0·75	0·28		0·02
B	0·15				0·5							
C						0·61	0·67	0·49	0·35	0·16		0
D							0·38	0·49	0·33	0·13	0·03	
Males												
E	0·15				0·07		0·24	0·31	0·25	0·19	0·06	
F		0·22			0·11		0·39	0·45	0·21	0·11	0·04	
G							0·27	0·39	0·35		0·14	
Mean for 20 adults						0·25 ± 0·04						

nuclear test explosions enters humans. Furthermore, it gives added confidence in recommendations which have been made about limiting the supply of milk contaminated with iodine-131 (refs. 1 and 2) since these have been based on the same assumptions regarding thyroid uptake and excretion of iodine-131 which have been used in the present calculations. For these reasons the results of the measurements and a discussion of the calculated values are given in detail here.

Measurements of thyroid radioactivity were made in the manner described in an earlier article[3] reporting the amount of iodine-131 in human thyroids following the Windscale reactor accident of 1957. As reported there, the method used, in which the curved surface of a 2-in. diameter cylindrical sodium iodide crystal scintillation counter was placed in contact with the neck, while having the merit of great sensitivity for the detection of iodine-131, suffers from considerable uncertainty because of the variability in the depth of the thyroid below the neck surface in different people. Some measurements which I made some years ago on more than one hundred people submitted for thyroid investigations using a tracer dose of iodine-131, showed that the mean effective depth of the thyroid below the anterior surface of the neck was about 3 cm with a standard deviation of about 2 cm. The greatest effective depth was 7 cm and the least very close to the surface of the neck. In order to estimate the effect of this on the measurements reported here an experiment was made on a model. The model thyroid consisted of two polyethylene bottles, of dimensions similar to those of the lobes of the adult thyroid, containing a known amount of iodine-131. These were placed 6 cm apart on a water-filled polyethylene model of the human neck. Polyethylene distance pieces of varying thickness were then placed on top of the model and the curved surface of the crystal placed against the top of the whole assembly. It was found that as the depth of the bottles below the surface of the model increased from 0·5 cm to 3 cm, the counting-rate due to scintillations in the crystal decreased by about a factor 2. There was a further reduction factor of about 2 as the depth increased from 3 cm to 7 cm. Since the measured values of iodine-131 in the thyroid which are reported here, and in the earlier article following the Windscale accident, are referred to a calibration in which the model thyroid was 3 cm below the surface of the neck, it will be clear that a measurement on any individual might be in error by about a factor 2 either way. However, for a group of people, the average measured value will approach the true mean value as the size of the group increases. By comparison with the error caused by the depth of the thyroid the other errors of measurement were very small. The iodine-131 in the thyroid of an individual was assessed by comparing the counting-rate within the 0·36-MeV photo-peak of his γ-ray spectrum with the corresponding counting-rate in the spectrum of the model. Each individual was measured for a period of 15 min; the standard error of estimating background in our low background enclosure was equivalent to only 0·05 mμc. iodine-131 in the thyroid. An appropriate correction, which was equivalent to about 0·1 mμc. iodine-131 in the thyroid, was made for the contribution from potassium-40 and cæsium-137 in the body. No correction was made for the contribution from iodine-131 distributed throughout the body, as opposed to that in the thyroid, but an experiment showed that the amount in the thyroid would be overestimated by only 5 per cent on this account.

The values obtained for 7 members of the staff, 3 males and 4 females, who were followed over a period of about 4 months are shown in Table 1. It will be seen from this that the highest values were measured in the early part of November. The pattern of rise and fall of thyroid radioactivity is more clearly demonstrated in the upper section of Fig. 1, which shows the values obtained for a female subject *A* who had the highest measured values. In view of the discussion here, these relatively high values for subject *A* could be due to a truly high thyroid radioactivity but might also reflect the fact that her thyroid was nearer the surface of the neck than that of the average person. All that can be said with certainty is that she did not contain more than 2 mμc. iodine-131 and not less than 0·5 mμc. iodine-131 on November 1. With this possible source of error in mind a larger group of people were measured on November 1 in order to obtain a representative mean value. The results for 10 males and 10 females showed that the mean amount of iodine-131 in their thyroids was 0·25 mμc. and the standard error of this mean value was ± 0·04 mμc. The highest measured value was 1 mμc., subject *A*, and the lowest 0·06 mμc., a value which was barely significant.

It has been suggested that the principal source of iodine-131 at this time was in fresh milk. No correlation was in fact found between the milk consumption of these individuals and the measured amount of iodine-131 in their thyroids. However, in view of the large errors which are possible in the measurement of a single individual this does not necessarily imply that no correlation exists. The mean milk consumption of the group of 20 people was 1 pint a day with a standard error of 0·15 pints a day, and it is perhaps of interest that subject *A* drank an average of 2 pints daily. In fact the shape of the curve shown in Fig. 1 for subject *A* and the magnitude of the mean measured iodine-131 in the thyroids of the group can be estimated by assuming that the sole source of iodine-131 in human beings is fresh milk.

Values for the concentration of iodine-131 in milk at different times during the period September 1961–January 1962 have been reported by the Agricultural Research Council[4]. In order to simplify calculation of the amount of iodine-131 in the thyroid, information

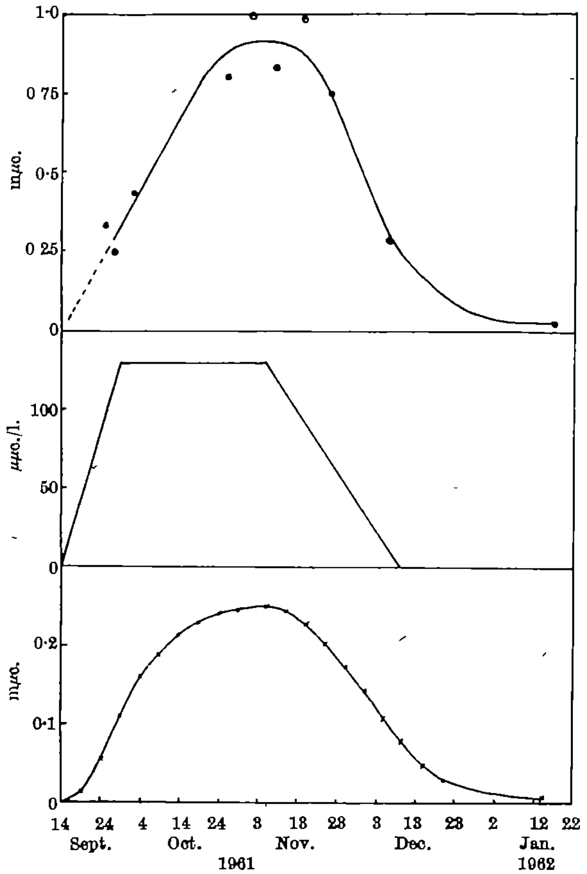

Fig. 1. Top, measured amounts of iodine-131 in the thyroid of subject *A*, middle, concentration of iodine-131 in milk; bottom, calculated amount of iodine-131 in the thyroid of an adult drinking one pint of milk a day

given by the Agricultural Research Council has been greatly simplified as shown in the central graph of Fig. 1. However, all the important details of the information have been preserved. For example, the rise and fall of activity as well as the length of the plateau shown in Fig. 1 are substantially the same as that given in the detailed results reported by the Agricultural Research Council. Furthermore, the total iodine-131 in milk during the whole period, as calculated from the area under the curve in the figure, namely, 8,000 μμc. days/l., is exactly the same as that reported by the Agricultural Research Council for the integrated total iodine-131 in milk during the same period; using a country-wide mean weighted for milk production. Using this information and making certain assumptions about the uptake and elimination of iodine-131 it is possible to calculate how iodine-131 in the thyroid of individuals on a specified daily milk intake varied with time during this period. This has been done by integrating equations of the following general type:

$$\frac{dq}{dt} = f.F(t) - \lambda q \qquad (1)$$

where q is the amount of iodine-131 in the thyroid at any time t; f is the fraction of ingested iodine-131 reaching the thyroid; $F(t)$ is a function which describes the variation of iodine-131 consumption with time; λ is the elimination constant of iodine-131 from the thyroid.

For convenience, the period under investigation was divided into four main parts which were determined by the rise and fall of iodine-131 in milk as shown in the middle curve of Fig. 1. If the intake of iodine-131 from milk for the individuals considered is put equal to P during the period covered by the plateau in the curve, the appropriate values of $F(t)$ for the four periods considered are as follows:

Period I Sept. 14–Sept. 29. $F(t) = Pt/15$ where t varies from 0–15 days (2)

Period II Sept. 29–Nov 5. $F(t) = P$ where t varies from 0–37 days (3)

Period III Nov. 5–Dec. 9. $F(t) = (P - Pt/34)$ where t varies from 0–34 days (4)

Period IV Dec. 9 to infinity $F(t) = 0$ (5)

It was assumed, following a report of the medical Research Council[1], that 30 per cent of ingested iodine-131 would reach the thyroid where it would remain without appreciable biological elimination. The value used for λ, the elimination constant, was therefore 0·087 days^{-1}, the radioactive decay constant of the radio-nuclide. With these assumptions, q, the amount of iodine-131 in the thyroid at any time, was found by integration of equation (1). For the four periods and the values of t specified in equations (2), (3), (4) and (5) here, the corresponding values of q were as follows:

Period I $q = 0{\cdot}23\,P(t - 11{\cdot}5 + 11{\cdot}5e^{-\lambda t})$ (6)

Period II $q = P(3{\cdot}45 - 1{\cdot}95e^{-\lambda t})$ (7)

Period III $q = P(4{\cdot}6 - 1{\cdot}22e^{-\lambda t} - 0{\cdot}10t)$ (8)

Period IV $q = 1{\cdot}07\,P\,e^{-\lambda t}$ (9

These values are shown plotted in the lower curve of the figure for $P = 74$ μμc. iodine-131, which is appropriate for a person consuming 1 pint of milk a day. It will be seen that the shape of the calculated curve is almost identical with that showing the measured values for subject *A*. Furthermore, the calculated value for the maximum amount of iodine-131 in the thyroid of a person drinking 1 pint of milk a day is exactly the same as the mean measured value for the group of 20 adults, namely, 0·25 mμc. This mean value was measured on November 1, which is very near to the time of maximum thyroid radioactivity, and the average milk intake of the group, 1 pint a day, was the same as that used in the calculation. While the agreement between calculation and experiment may be somewhat fortuitous, in view of the fact that average values have been taken for the milk consumption of the group and country-wide averages for the concentration of iodine-131 in milk, it does inspire confidence in the measurements and also in the basic assumptions which have been made about the uptake and elimination of iodine-131 in the thyroid.

In view of this close agreement between the measured and predicted values it is reasonable to calculate the average radiation dose received by the thyroids of the group of 20 adults from the lower curve in Fig. 1. (In fact the shapes of the curves for subject *A* and that calculated for a person drinking

1 pint of milk a day are so nearly identical that an equally good estimate is obtained from the curve for subject A, making an appropriate allowance for the different maxima of thyroid radioactivity in the two cases.) The integrated activity in the thyroid, which is the area under the curve, is found to be 15 mμc. days. Using values given in the Medical Research Council's[1] report for the effective energy per disintegration, 0·23 MeV and the mass of the thyroid, 25 g, the corresponding mean radiation dose is about 7 mrads. It will also be clear, from the way this has been computed, that this is also the mean radiation dose delivered to the thyroid of all those members of the population who were drinking an average of 1 pint of milk a day during the relevant period.

It is important to stress that the results reported here are all for adults. The doses to children on the same average milk intake would be greater because the iodine-131 would be distributed in smaller thyroids. There are, of course, other differences, notably that the thyroid uptake of iodine is greater in young children than in adults. According to the report of the Medical Research Council[1], the percentage uptake of ingested iodine-131 in children is about 50 per cent as against 30 per cent in adults, the thyroid weight below 1 year is about one-tenth of that assumed here for adults, and the average milk intake is 0·7 l., which is 1·2 times the average for my group of adults. Taking into account these factors it may be inferred that the average radiation dose received by the thyroids of young children, less than 1 year old, who were fed solely on fresh cows' milk during the period considered, amounted to about 150 mrads. This is only 15 per cent of that, 1 rad, which is considered by the International Commission on Radiological Protection[5] to be an acceptable upper limit for the annual radiation dose received by the thyroids of members of the population, assuming that they are exposed to this dose-rate continuously.

[1] Report of the Medical Research Council on Maximum Permissible Dietary Contamination after the accidental release of radioactive material from a nuclear reactor. *Brit. Med. J.*, 967 (1959).

[2] *Statement Med. Res. Coun. Radioactive Fall-out in the Light of the Resumption of Nuclear Weapon Testing M.R.C.* 61/1061 (circulated to the Press, October 24, 1961)

[3] Maycock, G., and Vennart, J., *Nature*, 182, 1545 (1958).

[4] *Rep. on Radioactivity in Diet.* Agric. Res. Coun. Radiobiol. Res. Lab. (1961 and 1962, *ARCRL* 6 and 7).

[5] *Recommendations of the International Commission on Radiological Protection. Report of Committee II on Permissible Dose for Internal Radiations* (Pergamon Press, 1959).

STRUCTURE-ACTIVITY CORRELATIONS OF ACTINOMYCINS AND THEIR DERIVATIVES

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ACTINOMYCINS[1] are highly toxic peptide-containing antibiotics. They are produced by strains of *Streptomyces antibioticus* as mixtures of closely related substances, which may be resolved into their components by counter-current distribution and partition chromatography[2].

Previous work has shown that low concentrations of actinomycin may selectively and completely inhibit RNA biosynthesis in mammalian cells in culture (as well as in some bacteria) while permitting normal growth of the RNA viruses Mengo and poliovirus[3-5]. These observations suggested that virtually the entire complement of the RNA of uninfected cells is produced in a DNA-dependent reaction, and experiments conducted with purified mammalian and bacterial extracts capable of catalysing such a reaction have demonstrated that it is exceedingly susceptible to inhibition by actinomycin[6,7]. Moreover, several authors have reported that actinomycin readily complexes to DNA[8-10]. Since the latter acts as the template for RNA synthesis, the binding of actinomycins to DNA provides a concrete physical basis for its inhibition of RNA biosynthesis.

It has been reported that the anti-tumour[11] and antibiotic[2] potency of natural actinomycins and their synthetic derivatives may be altered by structural changes affecting different parts of the molecule. These facts made it appear worth while to seek a correlation between the inhibition which a compound displayed toward the growth of cells in culture and bacteria, its action on DNA-dependent enzymatic RNA synthesis, and its binding to DNA. The results reported in this article demonstrate that these properties of actinomycins are closely correlated.

Growth inhibition of HeLa cells. When actinomycin is added to the growth medium, mammalian cells in culture respond in one of two ways, depending on the concentration of the antibiotic: (I) at a marginal concentration, some mitotic activity persists for approximately 24 h after which the cells begin to detach slowly from the surface of the Petri dish; (II) concentrations of actinomycin 2–5-fold greater than those permitting slight residual mitotic activity inhibit cell division immediately, and some loss of cells from the surface of the culture dish may begin within 4 h. The inhibitory properties of the various actinomycins have been classified according to the concentration required to produce each response, and the results shown in Table 1, column A, I and II. It is seen that the requirements for minimal growth inhibition by the compounds listed extend over more than a thousand-fold range of concentrations. Changes involving the pentapeptide portions of the molecule may affect its ability to inhibit the growth of HeLa cells; for example, actinomycin F_1 is reproducibly less active than actinomycins C_1, C_2 or C_3, confirming expectations based on previously reported observations in animals. Actinomycin X_2 is perceptibly more active than any of its reduction products. As might have been anticipated from previous results[2,11],

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† Operated on behalf of the U.S. Atomic Energy Commission by the University of Chicago.

Table 1

Actinomycin	*A* I µg/ml.	*A* II µg/ml.	*B* µg/ml.	*C* µg/ml.	*C* UT³²P Incorporated (mµmoles)	*C* Inhibition (per cent)	*D* µg/ml.	*D* UT³²P Incorporated (mµmoles)	*D* Inhibition (per cent)	*E*
X_2	0·01 (1)	0·0025 (1)	0 1 (1)	0·5	0·785	59·0 (1)	5	0·114	36·3 (2)	(1)
C_2	0·02 (2)	0·005 (2)	0·25 (2)	0·5	0·93	52·5 (2)	5	0·115	35·7 (2)	0
C_1	0·02 (2)	0·005 (2)	0·25 (2)	0·5	0·98	49·0 (3)	5	0·115	35·7 (2)	(2)
C_3	0·02 (2)	0·005 (2)	0·25 (2)	0·5	0·97	50·5 (3)	5	0·109	39·0 (1)	0
$X_0\delta$	0·02 (2)	0·01 (5)	0·35 (5)	0·5	1·02	46·8 (5)	5	0·132	26·2 (8)	(2)
$X_0\delta$ acetate	0·05 (6)	0·02 (6)	0·5 (6)	0·5	1·11	42·2 (6)	5	0·115	35·7 (2)	(2)
F_1	0·2 (7)	0·10 (7)	1·0 (7)	0·5	1·19	37·9 (8)	5	0·123	31·3 (6)	(2)
$X_0\beta$	0·5 (8)	0 10 (7)	1·5 (9)	0·5	1·16	39·6 (7)	5	0·122	31·4 (6)	(2)
$X_0\beta$ acetate	1·0 (9)	0·10 (7)	2·5 (10)	0·5	1·24	35·4 (9)	5	0·128	28 5 (8)	(8)
N-methyl C_3	1·0 (9)	0·10 (7)	1·0 (7)	1·0	1·39	27·1 (10)	25	0·07	60·9 (10)	(7)
N-β-diethylamino-ethyl C_3	1·0 (9)	0·10 (7)	2·5 (10)	1·0	1·63	15·1 (11)	25	0·124	30·7 (11)	(9)
Desamino C_3	>10 (i)	10 (i)	>10 (i)	10	1·93	— (i)	25	0·181	—	(i)
Chloro C_3	>10 (i)	0·1 (†)	>10 (i)	10	1·89	1·5 (i)	25	0 102	43	(i)
N-β-aminoethyl C_3	>10 (i)	1·0 (†)	>10 (i)	10	1·81	5·7 (i)	25	0·181	—	(i)*
N-*p*-aminophenyl C_3	>10 (i)	10 (i)	>10 (i)	10	1·90	1·1 (i)	25	0·194	—	(i)
N-amino C_3	>10 (i)	1·0 (†)	>10 (i)	10	1·83	4·7 (i)	25	0·085	52·5	(i)
Control					1 92	—		0·180	—	

The numbers in parenthesis represent the ranking, in order of decreasing inhibitory effectiveness, of the compounds tested in each experiment. (i) Considered inactive; >, indicates that the concentration shown was the highest tested and was without effect; 0, compound not tested; (†), observed activity considered due to transformation to active compound—see text.

Column A. Concentration of compound required to inhibit division of HeLa cells (I) without delay, (II) after 24 h incubation. Aliquots of HeLa (strain *S*-3) spinner cell cultures were added to plastic Petri dishes at 2·5 × 10^4 per dish. Cell counts were performed after 8 and 24 h on representative areas delineated on each plate, as previously described (ref. 12). The medium was changed immediately prior to the second cell count, appropriate concentrations of aqueous solutions of the test compounds added and cell counts repeated at 24-h intervals. Two plates were used for determining the activity of each concentration of every compound.

Column B. Concentration of compound required to inhibit completely the growth of *Bacillus subtilis* (strain Marburg, wild-type). Equal aliquots of an exponentially growing culture of the bacterium were inoculated into large test tubes containing warmed nutrient broth and various concentrations of the test compounds. The optical density of the suspension was recorded before and after 1 h incubation at 37° with rapid rotary shaking. The concentrations listed are the minimum levels of each compound required to prevent any increase in optical density (600 mµ) during the 60-min incubation period.

Columns C and D. Effect of actinomycins and derivatives on *E. coli* and HeLa RNA-polymerases, respectively. Experimental conditions as described for Fig. 2.

Column E. Ranking of actinomycins and derivatives with respect to their affinity for binding to DNA, measured as described for Fig. 4.

*, Interactions observed between compound and DNA, but not of type characteristic of biologically active forms. See spectra in Fig. 3.

structural changes involving the nuclear amino-group yield products the activity of which may be much lower than that of the parent compounds.

The action of the acetates of actinomycins $X_0\beta$ and $X_0\delta$ should be noted in relation to that of the parent unacylated compounds. $X_0\delta$ was more active than its acetate in causing immediate and delayed growth inhibition. Likewise, $X_0\beta$ was somewhat more effective in causing immediate growth inhibition than $X_0\beta$ acetate, but after 24-h incubation this difference could no longer be detected. It seems reasonable to suppose that this might be due to slow deacetylation of $X_0\beta$ acetate, yielding the parent compound $X_0\beta$.

Inhibitory effects of actinomycins on growth of B. subtilis. The results of a representative experiment are shown in Table 1, column *B*. The order of inhibitory potency resembles that found for HeLa cells, but is not identical with it. It has already been shown[9,10] that the actinomycin derivatives which we have found to be ineffective in inhibiting the growth of HeLa cells are inactive against *B. subtilis*, and the results in Table 1 confirm this. These results differ somewhat from those reported by Brockmann *et al.*[2,19] for the relative activities of $X_0\beta$, $X_0\delta$ and their acetates. They observed a ten-fold greater activity with $X_0\beta$ than with $X_0\beta$ acetate, and a five-fold greater activity with $X_0\delta$ than with $X_0\beta$. Our findings resemble qualitatively those of Brockmann *et al.*, and the relatively small quantitative discrepancies may be attributable to slight differences in culture conditions, bacterial strain, or experimental design.

Effects of actinomycins on E. coli and HeLa RNA-polymerase. *E. coli* RNA-polymerase is strongly inhibited by actinomycin. The results in Fig. 2*b* demonstrate the inhibition of incorporation of UT³²P as a function of actinomycin concentration. A comparison of the effects of various actinomycins and their derivatives is given in Table 1, column *C*.

In this test the various actinomycins and their derivatives show a graded series of activities which resembles that found for toxicity to HeLa cells and *B. subtilis*. The inhibition of *E. coli* RNA-polymerase by actinomycin is not due to an effect on the enzyme

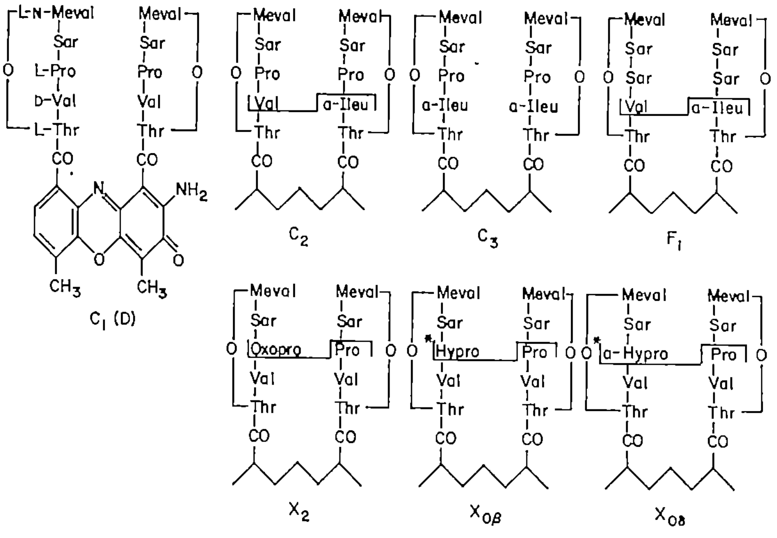


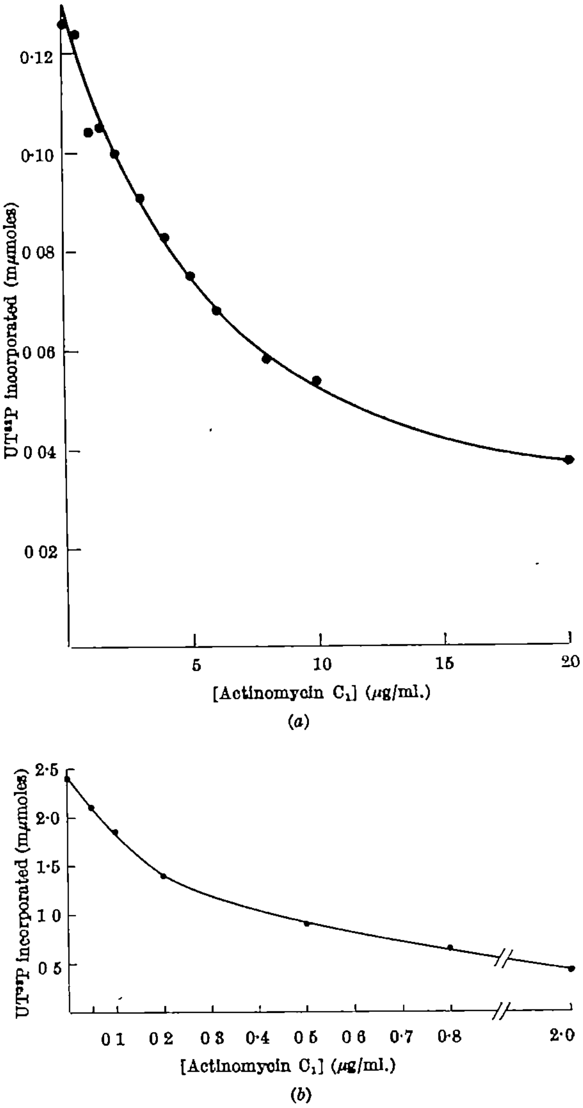
Fig. 1. Structures of some actinomycins according to Brockmann (ref. 2). The asterisks show the hydroxyl groups which are acylated to yield the corresponding acetates. Derivatives which were used in the present study having structural changes involving the aromatic amino-group are as follows: desaminoactinomycin and chloroactinomycin, in which the amino-group is replaced by hydroxyl and chlorine, respectively; *N*-substituted actinomycins, in which one hydrogen of the amino is replaced by β-aminoethyl, *p*-aminophenyl, methyl, β-diethylaminoethyl, and amino-groups

Abbreviations. Meval, *N*-methyl valine; Sar, sarcosine; Pro, proline; Hypro, hydroxyproline; a-Hypro, allohydroxyproline; Val, valine; Thr, threonine directly, but results from the binding of the antibiotic to the DNA primer. This is shown by the fact that the inhibition due to a given concentration of actinomycin is not affected by increasing the concentration of enzyme, but may be diminished to any desired extent by suitable additions of DNA. Similar observations have been recorded[6] for HeLa RNA-polymerase. For those actinomycins the structural differences of which involve only the peptide portions of the molecule, the resulting variations in effectiveness of enzyme inhibition can best be demonstrated when the concentration of DNA in the reaction mixture is not far above the saturating level. This may be seen by contrasting the findings summarized in Table 1, column *D*, with those in column *C*. In this experiment the various compounds were assayed for inhibitory potency using an enzyme preparation, obtained from HeLa cell nuclei, which is not dependent on added DNA for ribopolynucleotide synthesis. The active fraction is very viscous since it contains large amounts of DNA, thereby reducing the precision of the assay, and correspondingly higher concentrations of actinomycins are required to obtain degrees of inhibition comparable with those observed with the bacterial enzyme preparation (Fig. 2).

It is of interest that 3-chloroactinomycin and *N*-amino-actinomycin, which were inert in the reaction catalysed by the bacterial enzyme preparation, show appreciable inhibitory activity with the HeLa enzyme. It should be noted that some of the *N*-substituted actinomycins are somewhat unstable, reverting spontaneously to the parent compounds. Furthermore, chloroactinomycin is notably reactive, and readily yields free or *N*-alkyl actinomycins on treatment with ammonia or alkylamines[2]. Since the HeLa enzyme, in contrast to the bacterial enzyme, is best assayed in the presence of high concentrations of ammonium sulphate, the varying behaviour of these otherwise inactive actinomycin derivatives in the two enzyme systems is probably due to their partial transformation to active forms in the HeLa incubation mixture.

Effect of actinomycins on pseudouridine incorporation into polynucleotides. Pseudouridine triphosphate (ψUTP) has been shown to be incorporated into ribopolynucleotides by RNA polymerase preparations from mammalian[16] and bacterial[17,18] sources. The incorporation pattern of this rare nucleotide differs from that of uridine nucleotide[16,17] in the presence of uridine triphosphate. Nevertheless, the degree of inhibition of incorporation of radioactive ψUTP by the complete series of compounds listed in Table 1 paralleled that found for UTP.

Effect of DNA on spectral properties of actinomycins and derivatives. Kirk[8] found that a biologically active actinomycin complexes with DNA whereas actinomycinic acid, which is formed on hydrolysis of the lactone rings and which is biologically inactive, does not. The changes seen in the presence of DNA resemble those which may occur on alkylation of the amino-group, namely, broadening of the absorption peak and its shift to longer wave-lengths. We have examined the spectral changes of the actinomycins and their derivatives which are observed on interaction with DNA. The results are summarized in Fig. 3, where the difference spectra in the visible region are shown. Under identical conditions and concentrations the spectral changes observed in the presence of DNA were very similar in position and magnitude for actinomycins C_1, C_2, C_3, X_2, $X_0\delta$, $X_0\delta$ acetate, F_1 and $X_0\beta$, but differed quantitatively from those observed for $X_0\beta$ acetate.



(*a*)

(*b*)

Fig. 2. *a*, Inhibition of HeLa RNA-polymerase by actinomycin. The enzyme was prepared and assayed, using ^{32}P-labelled uridine triphosphate (UT^{32}P) to measure incorporation of uridylic acid into RNA (ref. 15). The reaction mixture (final volume 0·5 ml.) contained 125 μg of DNA in the form of the 'aggregate' enzyme (ref. 15)

b, Inhibition by actinomycin of RNA-polymerase from *E. coli* B. The enzyme was prepared and enzymatic reactions performed according to Chamberlin and Berg (ref. 13). The enzyme fraction used was totally dependent for activity on added DNA and nucleoside triphosphates. UT^{32}P, prepared as previously described (ref. 14), was used as tracer for nucleotide incorporation into acid-insoluble form. The reaction mixture, which contained 18·5 μg of enzyme protein, was incubated for 10 min at 37°. The reaction was terminated by the addition of trichloroacetic acid (TCA) to a final concentration of 5 per cent in the presence of carrier yeast RNA and serum albumin. The resulting precipitate was washed three times with TCA, dissolved in ammonium hydroxide, plated, and its radioactivity measured in a thin-window gas flow counter. A single preparation of calf thymus DNA (Sigma Chemical Co., St. Louis, Missouri) was used for all studies with the enzyme from *E. coli*. The concentration of DNA–P was 176 mμmoles/ml., a level which was just above that saturating the enzyme under our conditions

N-Methyl and *N*-β-diethylaminoethyl actinomycins were found to be active in toxicity and enzymatic tests. While these compounds interacted with DNA in solution, and their spectral behaviour qualitatively resembled that of the parent substances, the optical

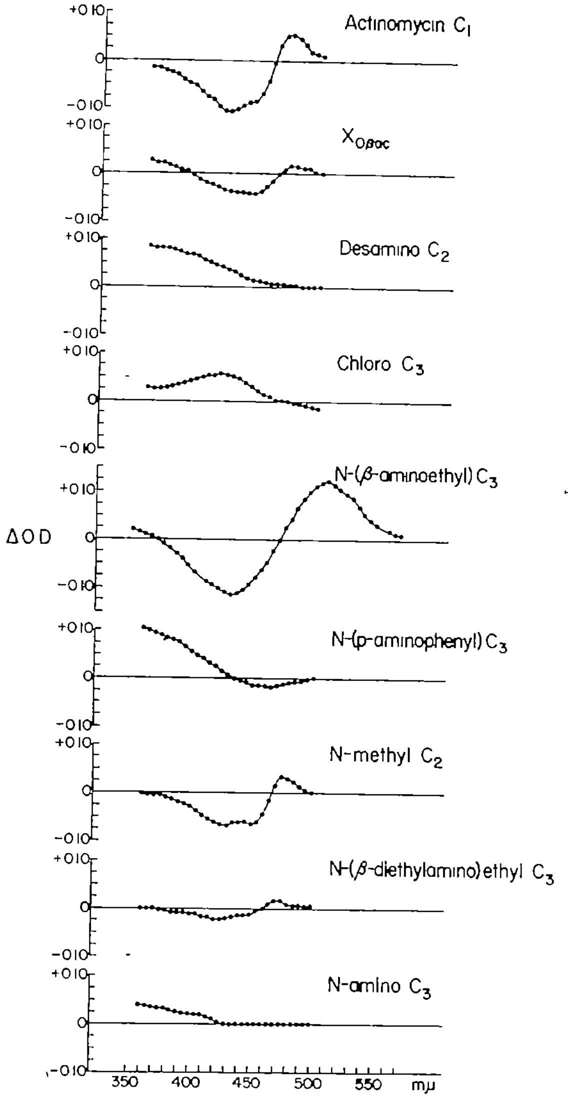


Fig. 3. Difference spectra obtained by recording (Cary spectrophotometer model 11) the spectrum of a solution containing an actinomycin and DNA against a reference solution containing only the same concentration of the actinomycin. All solutions were 0·01 M sodium chloride–0·01 M *tris* buffer (*p*H 7·4). The spectra of actinomycins C_1, C_2, C_3, X_2, $X_0\delta$, $X_0\delta$ acetate, $X_0\beta$, $X_0\beta$ acetate, F_1, N-methyl C_2 were obtained at a concentration of 8 × 10^{-5} M in the presence of 176 mμmoles/ml. of calf thymus DNA-P The spectra of the remaining derivatives were recorded at 10^{-4} M in the presence of 440 mμmoles/ml. of the same calf thymus DNA preparation. Although the changes induced in solutions of actinomycins by DNA affect all portions of the actinomycin spectrum, we have examined only the visible region 350–600 mμ

changes were smaller, on a molar basis, than those found for the parent compounds. The contour of the difference spectrum seen with N-methyl actinomycin is similar to that of the parent substance, but the peaks and cross-over points occur at somewhat different wave-lengths. For this reason it appears unlikely that the spectral changes, and therefore the binding of N-methyl actinomycin to DNA, are the result of a substantial degree of contamination of the preparation with free actinomycin. It would follow that methylation of the amino-group does not destroy the biological activity of the actinomycin. N-Diethylaminoethyl actinomycin possesses measurable, but considerably less, biological activity than its parent compound. If the spectral changes induced by DNA were assumed to be due entirely to the presence of unsubstituted actinomycin, the concentration of the latter would be of the proper order of magnitude to account for the observed activity, provided the N-substituted species were totally inactive. Desaminoactinomycin, 3-chloroactinomycin and N-p-aminophenyl actinomycin were inert, or showed optical changes which were minor in degree and differed qualitatively from those of parent compounds. N-β-Amino-ethyl actinomycin, which did not inhibit the growth of HeLa cells or *B. subtilis*, unexpectedly showed marked spectral changes in the presence of DNA; as is seen in Fig. 3, these differed from those observed for the biologically active compounds. This suggests that while actinomycins and their derivatives may interact with DNA in various ways, probably only one type of interaction is effective in suppressing DNA-dependent RNA synthesis.

The effect of varying concentration of DNA and actinomycin on the spectral changes associated with complex formation deserves comment. For a given level of actinomycin C_1 increasing DNA concentrations induce progressive and approximately proportional changes in spectral behaviour (cf. Fig. 4 and ref. 10), until a level is reached beyond which additional DNA has no further effect. Raising the actinomycin concentration at this level of DNA leads to further complex formation, as shown by the accompanying increased spectral changes. Such dependence of binding on actinomycin concentration might be observed if all available actinomycin were bound and the resultant complex almost entirely undissociated, at DNA concentrations the effect of which is maximal for a given actinomycin level This interpretation is supported by the fact that the absolute value of the spectral changes observed at a fixed level of DNA is exactly proportional, over a wide range of actinomycin concentrations, to the amount of actinomycin present. This would not be observed if, at equilibrium, more than a few per cent of the actinomycin remained uncomplexed to DNA.

The binding of a series of actinomycins has been examined at different DNA-levels. Some of the findings are shown in Fig. 4. A number of the actinomycins tested (C_1, X_2, $X_0\delta$, $X_0\delta$ acetate, $X_0\beta$, $X_0\beta$ acetate, F_1) differ structurally in ways which might be expected to influence the stereochemical properties of their complex with DNA Therefore, it cannot be

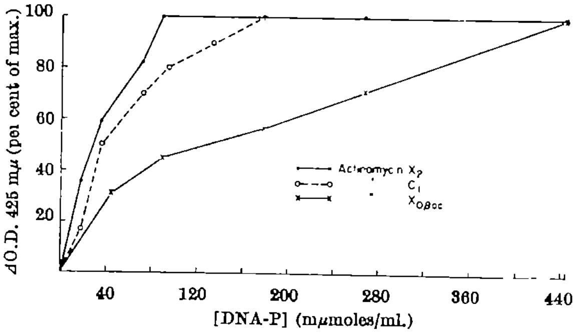


Fig. 4. Binding of actinomycins to DNA was measured by the decrease in absorption at 425 mμ on addition of standard amounts of DNA. All solutions were 0·01 M sodium chloride–0·01 M *tris* buffer (*p*H 7·4). The calf thymus DNA preparation contained 184·5 mμmoles of phosphorus per unit optical density (260 mμ) and a hyperchromic effect of 42·5 per cent (*p*H 7·0, 260 mμ). The concentration of each actinomycin was 8 × 10^{-5} M

assumed that the molar extinction coefficients of the various actinomycin–DNA complexes are identical, and since these have not been established, for each compound, the results have been charted as shown. The maximal optical density change observed, and by inference the maximal binding at a given concentration of the antibiotic, occurs at a lower concentration of DNA for actinomycin X_2 than for C_1 or $X_0\beta$ acetate. Curves of this type have been recorded under identical conditions also for actinomycins F_1, $X_0\delta$, $X_0\delta$ acetate, and although the biological activity of these compounds differs significantly from that of C_1, their spectral behaviour at different DNA concentrations is indistinguishable from the curve shown for C_1. The simplest explanation of these findings is provided by the assumption that the differences in optical behaviour (and therefore presumably in binding) between the various actinomycins reflect the degree of dissociation of the particular actinomycin–DNA complexes. Actinomycin X_2, for example, would be assumed to reach a binding equilibrium, characteristic for any given drug concentration, at a lower concentration of DNA. It is possible that the binding differences may reflect differences in the numbers and/or properties of sites available on DNA for the various actinomycins.

These experiments have been conducted with a single DNA preparation at a single *p*H. Variations of concentration of sodium chloride over the range 0–1·0 M did not affect the spectral changes produced by a given concentration of DNA, and the effects of changing other variables (for example, polyvalent cations or anions, temperature, or different DNA preparations) have not been systematically explored. Heating the DNA preparation (100°, 10 min, 0·01 M sodium chloride, 0·01 M *tris* buffer *p*H 7·4), followed by rapid cooling, reduced its ability to bind a given amount of actinomycin by approximately 15 per cent.

In Table 1 the various compounds used in this study have been ranked in decreasing order of potency for each test system in which they have been examined. Except for the HeLa enzyme assay, the relative activities, with minor deviations, correlated in all tests. The high DNA content and viscosity of HeLa RNA-polymerase probably introduce imprecisions which are of sufficient magnitude to prevent demonstration of the changes in activity resulting from amino-acid substitutions in the polypeptide chains of actinomycins. However, even the HeLa enzyme assay could easily distinguish between the *N*-substituted and parent compounds.

The differences in tumour-inhibitory activity reported for actinomycins *in vivo*[11] closely parallel those recorded here for the compounds used in both studies. Although the various systems available for estimating the potency of actinomycins possess approximately equivalent resolving power for detecting activity differences, it is apparent that the most sensitive measure is provided by the growth response of the HeLa cell line. The precision of this procedure is at least equal, if not superior, to that of the other avialable test systems.

It may be recalled that actinomycin does not affect the growth of several RNA viruses in cultured mammalian cell lines[5], and thus appears not to affect the many complex cellular enzymatic and structural properties which are required to sustain virus growth. Therefore, it is noteworthy that the activities of the large family of compounds examined in this investigation appear, with the exceptions noted here, to correlate strongly at the level of the intact animal, the intact mammalian and bacterial cell, the bacterial enzyme assay, and, to a considerable extent, even with the optical changes produced on complexing with DNA. These facts make it highly probable that the toxicity and biological activity of actinomycin are due exclusively to its binding to DNA and to the consequent interference with DNA metabolism, the molecule being otherwise innocuous to other physiological processes. This is a further illustration of the remarkable specificity of action characteristic of many antibiotics.

No explanation is available for the fact that several actinomycins having lower biological activity appear to bind to DNA as efficiently as C_1. Demonstration of small variations in binding might require ionic conditions other than those used in these experiments. Alternatively, differences in biological activity may reflect not merely the quantitative aspects of binding of actinomycins, but might also be partially determined by the structural features of the different bound forms or of the complex with DNA.

While structural features of actinomycins affect their reaction with DNA, the base composition of the latter influences the extent of binding and the degree of inhibition of enzymatic RNA synthesis. Synthetic deoxyadenylic–thymidylic copolymer[21] (dAT) directs the synthesis by RNA-polymerase, of a corresponding polyriboadenylic–uridylic polymer[22] in a reaction which does not require the presence of guanosine or cytidine triphosphates. This synthesis is totally resistant to very elevated concentrations of actinomycin (150 μg/ml.)[23]. In contrast, when the naturally occurring dAT polymer[24], which is found in crab DNA, is used to prime RNA formation, all four ribonucleoside triphosphates are required for optimal synthesis, the RNA produced contains approximately 2·5 per cent guanine and cytosine nucleotides[23], and the reaction is reproducibly inhibited (25 per cent) by actinomycin (100 μg/ml.). Moreover, crab dAT induces small but definite spectral shifts, resembling those normally produced by DNA in actinomycin solutions, whereas synthetic dAT does not[23]. In tests of a series of DNA preparations from different sources the amount of actinomycin bound and the degree of inhibition of RNA-polymerase reactions were found to be a function of the guanine–cytosine content of the DNA[23]. The results of these and other experiments, which will be described more fully[23], demonstrate that actinomycin binding to DNA and its inhibition of RNA synthesis is mediated through the guanine residues of the primer template. This fact fulfils the prediction of Kersten[25], who found that of the normal constituents of nucleic acids deoxyguanosine could most effectively induce spectral changes of actinomycins similar to those produced by DNA.

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APPEARANCE OF STREPTOMYCIN RESISTANCE FOLLOWING THE UPTAKE OF TRANSFORMING DEOXYRIBONUCLEIC ACID IN *PNEUMOCOCCUS*

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In bacterial transformation, the experimenter is able to examine the physiological action of normal hereditary determinants, introduced into cells in the form of DNA, by examining the events leading to the manifestation of the newly acquired hereditary trait. Thus far, such studies are limited in number[1,2]. In one[1], the appearance of resistance to streptomycin was followed in populations of pneumococci which had been made to fix DNA from a streptomycin-resistant donor strain. The conclusion reached was that the resistant phenotype appears as a discrete change from sensitive to resistant: no stages of partial resistance could be recognized. It was found, furthermore, that the probability of a cell becoming resistant was normally distributed over a time-interval ranging from about 15 min to 90 min following penetration of the streptomycin-resistance gene, even though DNA fixation had been limited to a 5-min period. The most probable moment for a cell to become resistant was about 60 min following uptake of DNA. The interpretation of these results at the time of their publication was rendered difficult owing to the absence of information concerning the mechanism of streptomycin-resistance and the types of syntheses involved in its establishment. Later, a brief description was made of experiments showing that the discrete event, described here, is not in fact the development of typical streptomycin-resistance, but, after all, only an intermediate stage in its development[3]. Experiments documenting this contention are presented here, in conjunction with a hypothesis concerning the mode of action of the streptomycin-resistance gene. The hypothesis is a development of the recently published theory of Spotts and Stanier[4] concerning the mode of action of streptomycin and the nature of streptomycin-resistance. Since it may open some interesting new approaches to the study of gene action, and, in particular, to the question of the relationship between genes and ribosomes, the publication of these experiments, and the accompanying hypothesis on the mode of action of the streptomycin-gene, seems worth while.

(1) *The experimental demonstration of the appearance of resistance.* All investigations of the appearance of antibiotic resistance following uptake of transforming DNA use a single basic procedure. Following a period of DNA fixation which is sharply limited by the destruction of unabsorbed DNA with DNase, the cells are diluted into fresh medium and incubated at 37° C. At intervals, samples are withdrawn and plated in agar containing the antibiotic. The number of cells able to give rise to a colony in the presence of the antibiotic are thus scored. Fig. 1, curve *a*, shows how pneumococci transforming for streptomycin-resistance develop this ability. This curve is typical of those obtained by Fox[1] and Schaeffer[5] as well as by me. The number of cells able to give rise to colonies in streptomycin-agar rises rapidly from about the 15th min following DNA fixation. A shoulder is observed at about 80–90 min, following which the numbers increase again, but at a slower, exponential rate characteristic of the overall population increase of the growing culture. Various experiments[6,7] have shown that: (1) at 90 min, virtually every cell which fixed a transforming molecule is able to form a colony in the presence of streptomycin; (2) that the increase observed after 90 min is due to the formation of genetically transformed daughter cells. In fact, it is established that the transmission of an acquired gene to both daughter cells may begin as early as the second generation after DNA fixation[7], that is, at about 45 min. It may, however, begin only at a third or fourth generation in some cells[7]. The early transmission of an acquired gene is, however, not reflected immediately in the numbers of streptomycin-resistant colony-forming units observed, owing to the tendency of sister cells to remain attached after cell division. Differences in the degree to which a shoulder is observed at 80–90 min are almost certainly due to differences in the extent of chain formation in different media.

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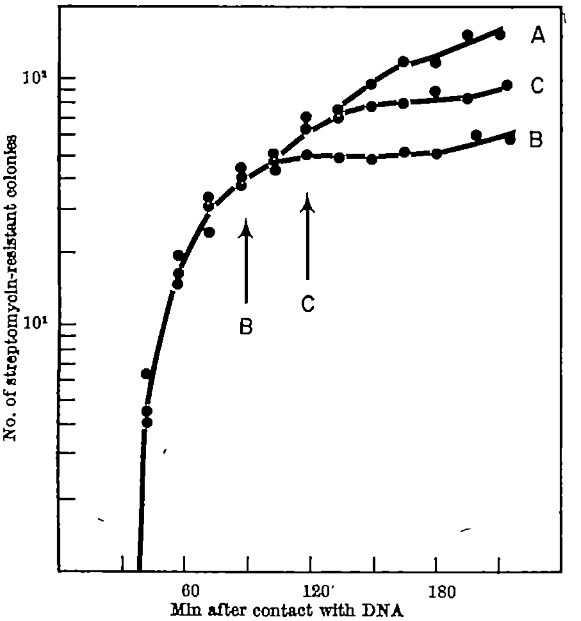

Fig. 1. Evolution of the numbers of transformed cells able to form colonies in streptomycin agar. *A*, Transformation culture growing in absence of streptomycin; *B*, streptomycin has been added 90 min after DNA uptake; *C*, streptomycin has been added 135 min after DNA uptake

If, in the agar medium used to score the resistant cells, the concentration of streptomycin is insufficient to arrest rapid growth of the recipient strain, residual metabolism in the presence of streptomycin will cause some transformants to complete the change from sensitive to resistant on the agar plate[1]. Thus, the transformed phenotype will seem to appear earlier at concentrations of streptomycin below a critical level, and the precocity of the appearance of the phenotype will appear to be a function of streptomycin concentration. Therefore, in order to avoid underestimation of the time required for resistance to develop it is necessary to challenge the cells in agar containing streptomycin at a concentration yielding a maximally selective effect. In the experiments reported here, it was found that the rate of appearance of streptomycin-resistant cells is the same at all concentrations of streptomycin equal to or greater than 200 μg/ml. The majority of experiments were performed at 200 μg/ml., but in some the concentrations were higher.

(2) *Limitations of the procedure for demonstrating resistance.* The foregoing procedure for determining the appearance of resistance, adequate at first sight, in fact, leaves one parameter unexplored. The method reveals only when a transforming cell can form a colony at a maximally selective concentration of streptomycin. It does not tell us whether the transformant does so at once, or whether its growth and division is temporarily suspended by the challenge. The streptomycin-resistant donor strain is completely indifferent to streptomycin at the concentrations used: Is the newly resistant cell, which yields a colony, also really indifferent to streptomycin?

(3) *Two steps in development of resistance.* To test this question, instead of challenging the transformants in streptomycin agar, a small amount of streptomycin was added to the cells in liquid medium, at a time when resistance is generally presumed to be complete. Following the addition of streptomycin, platings in agar were performed in order to determine the evolution of the numbers of resistant cells. Fig. 1 shows the results of one such experiment, in which cells which had fixed DNA for 2 min were diluted 100-fold into fresh medium and the culture divided into three portions: (*a*) no streptomycin is present in the liquid culture and platings are made directly into streptomycin agar; (*b*) 50 μg/ml. of streptomycin were added after 90 min of growth, and platings made into streptomycin agar (at 200 μg/ml.); and (*c*) 50 μg/ml. of streptomycin were added after 135 min of growth, and platings are made in streptomycin agar. If, as is generally believed, all transformants have achieved the synthesis of the streptomycin-resistant phenotype by 90 min, there should be no difference in the numbers of streptomycin-resistant cells present in these three liquid cultures. This is, however, clearly not the case. The increase in the numbers of resistant cells present in the cultures receiving a small amount of streptomycin is almost immediately arrested by the antibiotic. Thus, the immediate replication of the newly formed resistant cells is blocked by as little as 50 μg/ml. of streptomycin. Yet these cells are able to form colonies in agar containing 200 μg/ml. or more. Other experiments showed that, in fact, streptomycin transformants become completely indifferent to streptomycin only after some 150–180 min have elapsed following DNA fixation.

Two explanations of these observations can be offered: (1) that resistance develops in two steps. First, the bacteria are altered so that streptomycin is no longer bacteriocidal, and secondly, they become completely indifferent to streptomycin. If this explanation is to be retained, it must be assumed also that cells can pass from the first state to the second in the presence of streptomycin. (2) That the cells which survive the streptomycin challenge are not genetically transformed. For example, the acquired factor can be supposed to be not yet a part of the linear array of genes of the bacterial chromosome, but transmitted via an extra-chromosomal mechanism. Streptomycin could then be supposed to block the extra-chromosomal mechanism so that the majority of the daughter cells produced in its presence would be streptomycin-sensitive and therefore die. This would be analogous to the situation found in the induction of 'petites' by acriflavine acting on yeast[8]. The eventual formation of a colony in streptomycin-agar would reflect a shift from the extra-chromosomal state to a chromosomal state, achieved through recombination at one of the numerous cell divisions which the mother cell could make.

Results of a number of types of experiments invalidate the second hypothesis. One critical argument against it is the fact that when a cell acquires a DNA particle, recombination does ensue very shortly thereafter[3,9]. Further, it is reported that when a particle of transforming DNA is genetically marked at several points, so that it is able to give rise to several types of different, recognizable recombinants, one observes that a unique recombinant type is formed from a single absorbed particle, most if not all of the time[10]. Were the acquired particle transmitted at the outset by an extra-chromosomal mechanism prior to recombination, this result could not be observed. The first hypothesis is, therefore, to be retained in considering why streptomycin arrests the multiplication of resistant cells newly formed by transformation.

Accordingly, in order to explain the results exemplified by Fig. 1, we can assume that even though from

90 min on, every cell which acquired the streptomycin-resistance gene can form a colony at high concentrations of streptomycin they do so only after a considerable period of arrested growth. In other words, at this stage of phenotypic transformation, streptomycin is a bacteriostatic substance from the effects of which the transforming cell can eventually escape. This characterizes what we shall call stage 1 in the development of resistance, while complete indifference characterizes stage 2, the definitive state.

Another type of experiment confirms this point of view, and, in addition, informs us of further characteristics of stage 1. Following a challenge of 500 μg/ml. of streptomycin for a 30-min period at 37° C, surviving transformed cells are washed on a membrane filter to eliminate unbound streptomycin, transferred to fresh medium by washing them off the membrane, and their growth followed by plating samples at intervals, in two different media: agar with and without streptomycin. In the experiment shown in Fig 2, the resistant transformants were selected 60, 110 and 180 min after DNA fixation as described.

One may note the following features of the curves in Fig. 2. (1) There is a small lag in the onset of replication of the resistant cells selected 180 min after DNA fixation which is not observed when streptomycin is simply added at this time to a transforming culture and left there. The lag observed in Fig. 2 is almost certainly caused by the vigorous aeration of the cells during washing on the membrane filter (*Pneumococci* are microaerophilic). (2) Growth of streptomycin-resistant cells selected 60 and 110 min after DNA fixation is severely retarded, even though the antibiotic has been removed. This means that either the cells have fixed enough streptomycin so that the extracellular concentration of the antibiotic is no longer critical, or that the 30-min treatment has inflicted a finite damage which is not subject to reversal by the removal of streptomycin. The latter seems more likely since the amount of streptomycin bound to bacteria is very small[11]. (3) No sensitive cells survive the selection at 500 μg/ml., and few or no sensitive progeny are formed by stage 1 resistant cells.

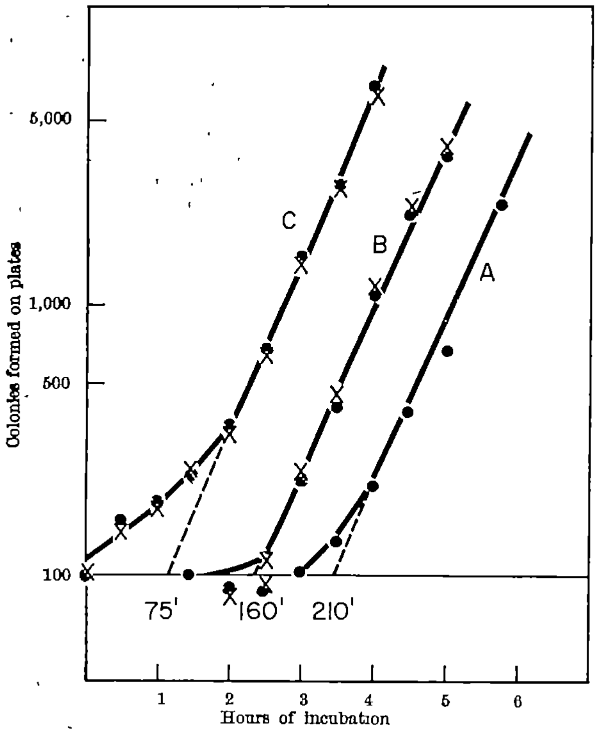

Fig 2. Onset of division of streptomycin-resistant transformants in the absence of extracellular streptomycin. The resistant transformants were selected: *A*, 60 min; *B*, 110 min; *C*, 180 min after DNA uptake by treating the transforming population with 500 μg/ml. of streptomycin for 30 min. Survivors were collected on a 'Millipore' membrane, washed, resuspended in medium, and their growth followed: ×, on plates containing no streptomycin and ● on 200 μg/ml. of streptomycin

By extrapolating the exponential slopes of the curves of Fig. 2, one can calculate from curve *c* the delay caused by aeration, and from curves *a* and *b* the delay caused by the combined factors of aeration and streptomycin-inflicted damage. Correcting for the delay caused by aeration, one finds that the cells in *a* required 135 min of incubation to resume exponential increase, while the cells in *b* required 85 min. The difference between these two times is 50 min, which is the same as the difference in the incubation times of the two cultures prior to the streptomycin challenge. In other words, the time required for definitive resistance to develop is constant, and independent of the moment of application of the streptomycin challenge. Thus, cells which are at stage 1 in the development of resistance, and which may have arrived at this stage at very different moments, are a homogeneous population in so far as their attainment of definitive resistance is concerned. With respect to definitive resistance, primary transformants are apparently no different from their second, third or even fourth generation daughters.

In the experiment of Fig. 1, 50 μg/ml. of streptomycin was added to the liquid culture, while in the experiment of Fig. 2, 500 μg/ml. were added. Both concentrations arrested the multiplication of the resistant transformants. Irrespective of whether the damage to the cells was inflicted by 50 or 500 μg/ml., and of whether the streptomycin was left in contact with the survivors, the moment of onset of increase of the streptomycin-resistant cells was at about 180 min. This again suggests that streptomycin inflicts finite damage on stage 1 transformants, and that their recovery is independent of the external concentration of streptomycin. To show this more clearly, an experiment was performed in which fluctuations in the numbers of streptomycin-resistant colony-forming units was followed in a control and two streptomycin-containing cultures. The latter received 50 and 500 μg/ml. of streptomycin, respectively, 60 min after DNA fixation. Fig. 3 shows the results of such an experiment. It can be seen that the time required for stage 1 resistant transformants to resume division after the addition of streptomycin is approximately the same, irrespective of the external streptomycin concentration. Hence, the conversion of a stage 1 resistant transformant into a definitely resistant cell is essentially independent of streptomycin concentration. Further, the damage inflicted on the stage 1 cells must be finite and independent of streptomycin concentration, within the limits explored

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The most striking feature of the way in which definitive resistance develops in a transforming population is that cells destined to transform, and their immediate progeny, show this resistance at the same time, that is, about 180 min after fixation of transforming DNA. Yet some of these cells are the original transformants and some are their first,

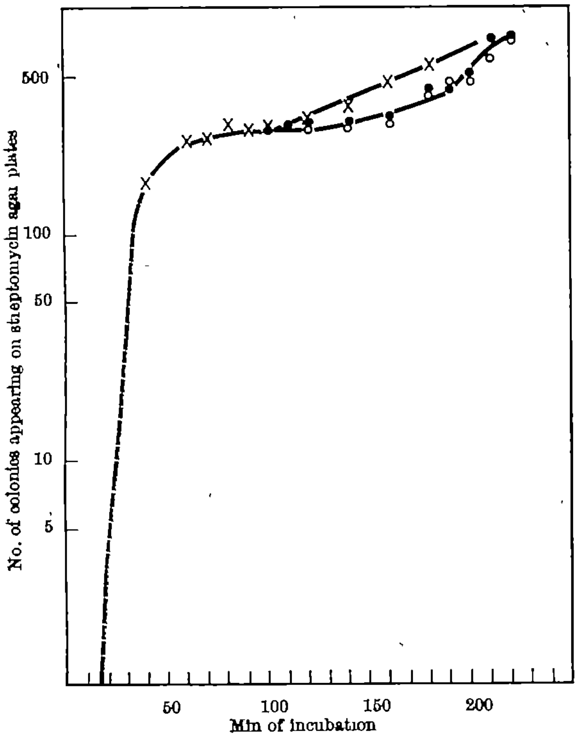

Fig. 3. Evolution of the numbers of streptomycin-resistant cells in a transforming culture × in the absence of streptomycin; in the presence of ● 50 μg/ml.; ○, 500 μg/ml. of streptomycin added 60 min after DNA uptake. Some of the irregularities in the curves are probably due to synchrony of division.

second or later generation progeny. Therefore, as transforming cells grow and divide, they must produce daughters which are similar to themselves not only genotypically but also with respect to the degree to which they have developed definitive phenotypic resistance. Since genetic integration usually occurs at one of the first two or three divisions following DNA fixation, it is difficult to imagine that this phenotypic uniformity of transformants and their progeny is established by the process of genetic integration itself. On the other hand, DNA fixation has occurred during a very short interval. Its penetration into the cell could very well be the event which initiates the process of phenotypic transformation.

If this were the case, the following mechanism of the development of definitive resistance can be advanced. On penetration of the DNA, the streptomycin *r* gene immediately induces the formation of the system which confers resistance. Since the complete phenotype is manifested only some 180 min after DNA fixation, we can suppose that resistance results from the synthesis of a very large number of specific macromolecules. As cell division proceeds, both the generating system initiated by the acquired gene and the specific macromolecules which it determines are distributed more or less equally between sister cells. In those cells where the resistance gene is fixed permanently by genetic recombination, the resistance gene will also be transmitted. It is only in these cells that the generating system will be stable enough for final resistance to be manifested. What. then, is stage 1 resistance? As shown by Fox, it is a discrete change which occurs on an average after about 60 min have elapsed following DNA-uptake, and which shows a normal but fairly wide distribution with respect to the moment it occurs[1]. As shown by the foregoing experiments, it is a change which enables a cell to survive a challenge of maximally selective amounts of streptomycin, and to escape from a strong bacteriostatic effect of the antibiotic. Further, the rate at which a stage 1 resistant escapes is independent of the external streptomycin concentration in the growth medium.

A suitable explanation of stage 1 resistance was not evident so long as theories of the nature of streptomycin resistance were based on supposing the resistant cell impermeable to streptomycin. Even with the publication of a theory[12] to the effect that, in the presence of streptomycin, sensitive bacteria synthesize an abnormal membrane constituent which results in disruption of transport mechanisms, an explanation of stage1 resistance did not seem possible. Supposing that, at the onset, the acquired resistance gene were to confer on the cell the capacity to form normal membrane substance in the presence of streptomycin, at early stages the cell membrane could be at best a mosaic, for the old membrane and membrane-forming system should still be present in the cell. It is hard to see how a mosaic membrane could confer on cells an immunity to the lethal effects of streptomycin.

The recent hypothesis of Spotts and Stanier[4] provides, on the other hand, an explanation of the nature of stage 1 resistance. According to these authors, streptomycin attacks the ribosomes of sensitive cells, causing their disruption. Resistant cells, according to the theory, contain ribosomes which do not combine with streptomycin, and are, therefore, resistant to its action. There is, indeed, some direct evidence in favour of this view[13]. In the light of this hypothesis, stage 1 resistance can be interpreted as resulting from the synthesis of adequate numbers of streptomycin-resistant ribosomes so that at least one copy of each of the different messenger RNA's of the cell which are necessary for the continuation of vital specific functions could be housed in streptomycin-resistant ribosomes. Bacteriostasis would ensue, however, at this stage owing to the destruction of residual streptomycin-sensitive ribosomes, which could still represent the majority of the ribosomes of the cell. Stage 1 resistant cells would recover their ability to divide as soon as the streptomycin-resistant ribosome population were built up to a level compatible with normal growth and division. Recovery-rate would be independent of the amount of streptomycin in the system, for recovery would result from the function of surviving streptomycin-resistant ribosomes.

The ribosome hypothesis is particularly satisfying because it explains why stage 1 resistance appears after an interval which is normally distributed over a fairly wide time-range.

There are presumably many different messenger RNA's determining vital functions which must be housed, and the probability that any one cell possesses one streptomycin-resistant ribosome-messenger RNA particle of each type would be expected to be distributed in this way, assuming random association of the RNA with ribosomes. Further, the ribosome hypothesis of streptomycin action can explain why all transforming cells and their progeny show definitive resistance at about the same time: the existing ribosomes would be shared at each division.

However, it should be mentioned that there is one fact concerning the action of streptomycin which the Spotts and Stanier theory does not explain. This is the observation that if chloramphenicol and streptomycin are added simultaneously to sensitive cells, streptomycin has no lethal action. It appears, thus, that the lethal effects of streptomycin are the consequence of protein synthesis. It has been proposed that streptomycin does not enter cells unless a special permease is synthesized[14], following contact of cells with streptomycin, and this could account for the protective effect of chloramphenicol. Since so many of the biological actions of streptomycin can be explained by the theory of Spotts and Stanier, including the very particular way in which resistance develops following transformation, one is inclined to conclude that only a minor modification of it may be necessary in order to explain why chloramphenicol eliminates the bacteriocidal effect of streptomycin.

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INTRACELLULAR LOCATION OF DNA NUCLEOTIDYLTRANSFERASE

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IT has invariably been observed that DNA nucleotidyltransferase of mammalian cells is more readily obtained from soluble supernatant fiactions after high-speed centrifugation of disrupted cell preparations, than from the sediments which contain the nuclei and other intracellular particles[1-4]. In view of the problems thus arising from the concept of synthesis of a nuclear component, DNA, by an apparently cytoplasmic enzyme, Smellie and Eason[5] investigated the intracellular location of DNA nucleotidyltransferase and found only low activities of the enzyme in variously prepared nuclear fractions, while the corresponding cytoplasmic fractions were highly active. We have continued the work of Smellie and Eason by examining more extensively, and with improved assay techniques, the DNA nucleotidyltransferase activities in nuclei and cytoplasm isolated in non-aqueous solvents by a modification of the Behrens principle[6], in order that complications introduced by possible diffusion of enzymes across the nuclear membrane during manipulations in aqueous media might be eliminated. The tissue used was regenerating rat liver (regeneration time = 29 ∓ 0·5 h), and three fractions were studied—nuclei, cytoplasm and whole tissue which had been exposed to the various treatments with organic solvents. DNA nucleotidyltransferase and deoxyribonuclease I were assayed as described by Keir[7].

The dry tissue powders were homogenized in buffer and centrifuged at 105,000*g* for 60 min, the supernatant extracts being taken for enzyme assay. When homogenization was in 0·01 M potassium phosphate buffer *p*H 7·5, the nuclear extract had a DNA nucleotidyltransferase activity higher than the cytoplasmic and whole tissue extracts; this was particularly marked after the longer incubation periods (Fig. 1). The activities declined gradually in all extracts by about 80 per cent on storage at 0°–2° for 22 days. During this time, however, the deoxyribonuclease I activities of the extracts increased substantially (Table 1), a phenomenon which is probably attributable to the lability on storage of deoxyribonuclease I inhibitor initially present in the extracts (cf. ref. 8). This diminution of the inhibitor effect may contribute to the apparent loss of nucleotidyltransferase activity after storage, by allowing deoxyribonuclease to act unimpeded on newly synthesized polydeoxyribonucleotide during the nucleotidyltransferase assays. Further, since the results shown in Fig. 1 were obtained from freshly prepared extracts, the differences in nucleotidyltransferase activity between the nuclear and cytoplasmic fractions may be a consequence, at least in part, of the disparity in deoxyribonuclease activities in the respective fresh extracts. However, the situation is complex since limited deoxyribonuclease activity can enhance the nucleotidyltransferase reaction by providing 3′-hydroxy-terminal groups on the DNA primer[7]; also, the deoxyribonuclease I inhibitor in mammalian tissues is labile on incubation at 37° as well as on storage in the cold[8,9]. The inhibitor effect might thus decrease during nucleotidyltransferase assays, releasing previously masked deoxyribonuclease activity.

Inclusion of potassium chloride at 0·15 M in the extraction buffer resulted in a nuclear extract with greatly increased levels of nucleotidyltransferase activity, although the activity of the cytoplasmic

Table 1. DEOXYRIBONUCLEASE I ACTIVITIES IN EXTRACTS OF VARIOUS FRACTIONS OF REGENERATING RAT LIVER PREPARED IN NON-AQUEOUS SOLVENTS

Fraction	DNA rendered acid-soluble/mg protein/3 h (extinction units at 260 mμ) 2 day-old extract	22 day-old extract
Nuclei	0 79	9·45
Cytoplasm	4·03	7·47
Whole tissue	3·14	—

These extracts were assayed for DNA nucleotidyltransferase immediately after preparation (see Fig. 1), and were afterwards stored at 0°–2°. The deoxyribonuclease assays were performed under nucleotidyltransferase assay conditions but in the absence of the four deoxyribonucleoside triphosphates (ref. 7).

Table 2. DEOXYRIBONUCLEASE I ACTIVITIES IN NUCLEAR AND CYTOPLASMIC EXTRACTS AND IN FRACTIONS PREPARED FROM THEM AT *p*H 4·8

Fraction	DNA rendered acid-soluble/mg protein/3 h (extinction units at 260 mμ) Nuclear	Cytoplasmic
Soluble extract	2·15	16·85
*p*H 4 8-precipitate	1·82	19·10
*p*H 4·8-soluble fraction	4·60	6·07

The assays were performed 3 days after preparation of the extracts and 2 days after assay of the DNA nucleotidyltransferase in the extracts (Fig. 2).

Table 3. DNA NUCLEOTIDYLTRANSFERASE AND DEOXYRIBONUCLEASE I ACTIVITIES OF FRACTIONS OBTAINED FROM A CYTOPLASMIC EXTRACT

Fraction	Percentage of total activity in all fractions obtained from the initial soluble extract DNA nucleotidyl-transferase	Deoxyribonuclease I
*p*H 5 8-precipitate	7	37
*p*H 5·4-precipitate	6	25
*p*H 4 8-precipitate	10	3
*p*H 4·8-soluble fraction	77	35

The cytoplasmic extract was prepared by homogenizing the dry powder in 0·01 M potassium phosphate buffer *p*H 7·5 and centrifuging at 105,000*g* for 1 h. The extract was then fractionated with acid (no potassium chloride added) to give the four fractions shown.

extract rose only slightly (Fig. 2). Acid fractionation of each extract yielded a '*p*H 4·8-soluble' fraction and a '*p*H 4·8-precipitate'. The latter contained 75 per cent of the nuclear nucleotidyltransferase with a 2·5-fold purification (17 μm.moles of [^{32}P]TMP incorporated/mg protein/4 h), but no purification was obtained in the corresponding cytoplasmic '*p*H 4·8-precipitate' (0·35 μm.moles [^{32}P]TMP incorporated/mg protein/4 h). A probable explanation of this is that the nucleotidyltransferase was masked by the relatively high deoxyribonuclease activity obtained in the cytoplasmic fractions after extraction of the dry powder with buffer containing 0·15 M potassium chloride (Table 2). A cytoplasmic extract made in 0·01 M potassium phosphate buffer *p*H 7·5 was therefore fractionated with acid; under these conditions of low ionic strength (no potassium chloride added) the acid fractionation characteristics were different from those shown in Fig. 2 and Table 2. This variation revealed higher specific activity nucleotidyltransferase (1·6 μm.moles [^{32}P]TMP incorporated/mg protein/3 h), 77 per cent of which was located in the '*p*H 4·8-soluble' fraction along with 35 per cent of the deoxyribonuclease I activity (Table 3). Heterogeneity of the cytoplasmic deoxyribonuclease was indicated by the fact that most of the remainder of the deoxyribonuclease was precipitable at *p*H 5·4 and *p*H 5·8.

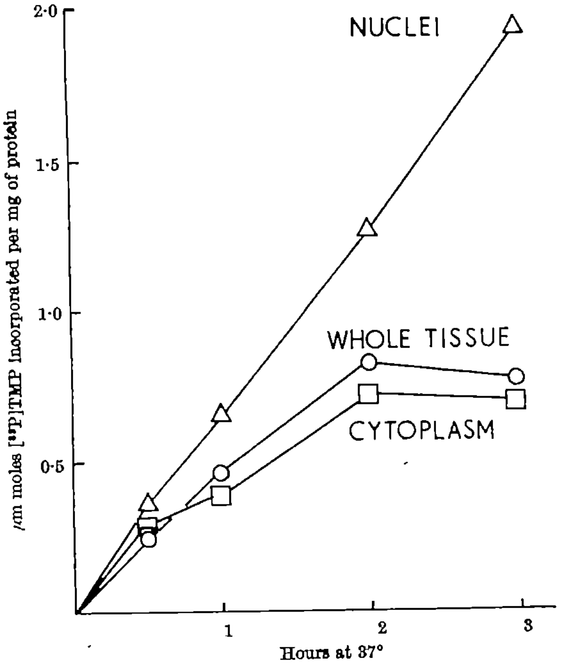

Fig. 1. DNA nucleotidyltransferase activities in extracts of various fractions of regenerating rat liver isolated in non-aqueous solvents. The extracts were made in 0 01 M potassium phosphate buffer *p*H 7·5. The assay volume was 0·25 ml. and contained 10 μmoles *tris*-HCl buffer *p*H 7·5, 1 μmole potassium phosphate buffer *p*H 7 5, 1 μmole MgCl$_2$, 0·08 μmole ethylenediaminetetra-acetate, 9 μmoles KCl, 50 μg thermally denatured DNA (ref. 7), 50 μm.moles each of deoxyadenosine-, deoxycytidine- and deoxyguanosine-5′-triphosphates, 50 μm.moles of thymidine-5′-triphosphate containing phosphorus-32 in the α-phosphate group [^{32}P]TTP (20·7 × 10^6 counts/min/μmole), and 60 μg protein from the tissue extract. Incubation was at 37°. Polydeoxyribonucleotide synthesis was measured by incorporation of ^{32}P-labelled thymidine-5′-monophosphate, [^{32}P]TMP, into an acid-insoluble form

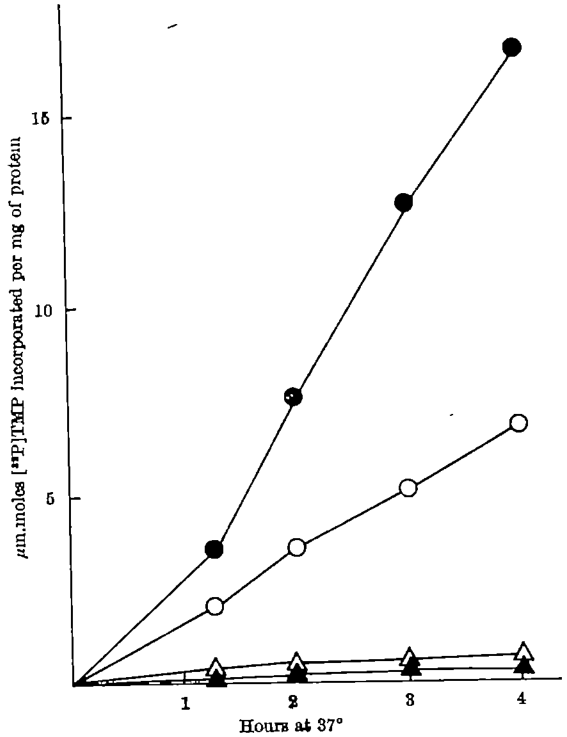

Fig. 2. DNA nucleotidyltransferase in nuclear and cytoplasmic extracts made in 0 15 M KCl–0 01 M potassium phosphate buffer *p*H 7·5, and in '*p*H 4·8-precipitate' fractions derived from these extracts. Incubation conditions were as in Fig. 1. The specific activity of the [^{32}P]TTP was 9·5 × 10^6 counts/min/μmole ○, Nuclear extract–90 μg protein; ●, nuclear '*p*H 4·8-precipitate'–60 μg protein; △, cytoplasmic extract–90 μg protein; ▲, cytoplasmic '*p*H 4 8-precipitate'–90 μg protein

It is improbable that triphosphatase activity interfered seriously with the nucleotidyltransferase reactions in the nuclear and cytoplasmic extracts, for even after prolonged incubations, 40 and 50 per cent of the initial [^{32}P]TTP remained in the nuclear and cytoplasmic reaction mixtures respectively.

The possibility existed that the nuclear extracts contained DNA which could serve as additional primer in the nucleotidyltransferase assays and thereby account for the specific activities superior to those obtained for the transferase in the cytoplasmic extracts. Estimations of the DNA concentrations of nuclear and cytoplasmic extracts were accordingly carried out[10]; they revealed that the extracts contained approximately equal amounts of DNA, and in all cases this was less than 1 μg DNA/ml. Only

0·05–0·1 ml. of extract was used in any nucleotidyltransferase assay, and since the standard assay mixture contained 50 μg of DNA primer it is unlikely that the endogenous DNA made any significant contribution.

These observations are interpreted as an indication of the existence of DNA nucleotidyltransferase activity in both nuclei and cytoplasm. While the limited contamination of nuclei with cytoplasmic material in these preparations cannot be ignored (nuclear ratio DNA/RNA = 2·0 and cytoplasmic ratio DNA/RNA = 0·03), it is very unlikely that the high apparent specific activity of the nucleotidyltransferase in the nuclear extracts can be ascribed exclusively to a non-nuclear enzyme since any cytoplasmic contamination would be expected to result in a lower specific activity in nuclear, as compared with cytoplasmic, extracts. Moreover, highly purified non-aqueous nuclei prepared from rabbit and calf thymus gland (nuclear ratio DNA/RNA > 5·0) have also been shown to contain high specific activity nucleotidyltransferase (Smith and Keir—to be published).

In conclusion, the high DNA nucleotidyltransferase yields and activities in 105,000*g* supernatant fractions from mammalian tissues disrupted in *aqueous media* might be explained by: (1) the location of a proportion of the cellular nucleotidyltransferase in the cytoplasm; (2) leaching out of the enzyme from the nuclei; (3) removal of much of the deoxyribonuclease I in the 105,000*g* sediment; on the other hand, high deoxyribonuclease activities would be expected in extracts of non-aqueous type preparations in which organic solvents had destroyed the integrity of lipid-containing subcellular structures.

We thank Prof. J. N. Davidson for his interest in this work and for providing the facilities with the aid of grants from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research and the Rankin Fund of the University of Glasgow.

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ESTIMATION OF LOW CONCENTRATIONS OF PERCHLORATE IN NATURAL MATERIALS

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BAAS-BECKING *et al.*[1,2] claimed the occurrence of perchlorate in sea-water, natural salts, plant tissues, and body-fluids in concentrations of 10–10,000 p.p.m., with an average value of the order of 100 p.p.m. The main diagnostic reactions used for perchlorate were based on the interactions of perchlorate with methylene blue and other metachromatic dyes, the formation of the insoluble strychnine salt, and the lowering of the char-temperature of cellulose.

Greenhalgh and Riley[3,4] devised a new procedure based on the co-precipitation of perchlorate with tetraphenyl arsonium perrhenate. The perchlorate was estimated by quantitative infra-red spectrophotometry. By this method, Greenhalgh and Riley could find no perchlorate in sea-water down to a level of 0·03 p.p.m. They suggested that the previous results were based on faulty analytical techniques, and resulted from interference by chloride or sulphate.

A procedure has been developed to check the occurrence of perchlorate in the natural environment. Perchlorate ion has a high affinity for quaternary-ammonium ion-exchange resins[5], so it can be separated from other ions by ion-exchange chromatography and estimated in the eluate by a modification of Greenhalgh and Riley's method. The chromatographic step removes other inorganic anions and permits the concentration of the perchlorate content of large samples.

Preliminary separations on a 'Dowex 2-*X*8' ion-exchange resin column showed that mixtures of the common stable inorganic anions could be separated at levels of up to 0·1 μmoles. Iodate, phosphate, sulphate and chloride are separated and eluted by 1 M sodium acetate or 1 M ammonium formate solutions; nitrate and bromide are separated and eluted by 1 M potassium chloride solution; iodide and perchlorate are separated and eluted by 1 M ammonium trichloroacetate solution. These solutions form a sequence of eluting agents of increasing activity, in the order given. All the anions are eluted, and perchlorate is separated from the others, by 1 M ammonium trichloroacetate.

Organic samples were chopped, boiled with distilled water, filtered through muslin, and clarified by treatment with a small excess of lead acetate at *p*H 8·5 (adjusted with ammonium hydroxide), followed by filtration. The filtrates were concentrated in a cyclone evaporator and decolorized by percolation through a charcoal column. Each clarified sample was then passed through a 12·5 cm × 1·2 cm column of 'Dowex 2-*X*8' (200–400 mesh) ion-exchange resin in the trichloroacetate form. After washing the column with water, it was eluted with 1 M ammonium trichloroacetate solution (*p*H 7·3). The first 100 ml. of eluate were discarded; the second 100 ml. were collected for analysis.

5·00 ml. of 0·40 per cent tetraphenyl arsonium chloride solution and 2·50 ml. of 0·120 per cent potassium perrhenate solution were added to the eluate and the mixture was shaken slowly at 0° C for 48 h. The fine precipitate of tetraphenyl arsonium perrhenate, containing co-precipitated perchlorate, was collected on a micro-Hirsch funnel, dried *in vacuo*

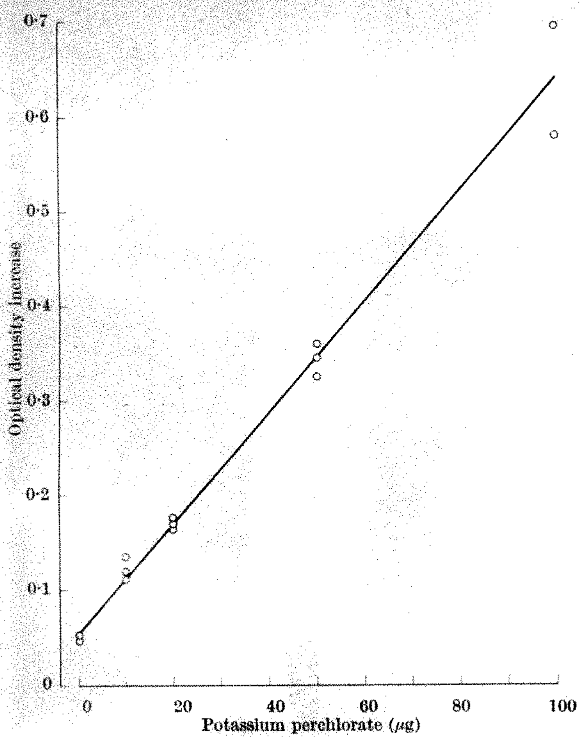

Fig. 1

ground with 200 mg potassium bromide in an agate mortar and pressed into a 13-mm disk in a hydraulic vacuum-die press under 60 tons load for 3 min. The infra-red absorption spectrum of the disk was recorded with a Perkin–Elmer model 21 spectrophotometer with sodium chloride optics. Perchlorate produced an absorption peak or shoulder at 1,100 cm^{-1}. A base-line was drawn on the chart tangential to the absorption minima at 1,050 cm^{-1} and 1,140 cm^{-1} respectively. The height of the perchlorate peak above the base-line at 1,100 cm^{-1}, expressed in optical density units, was a measure of the perchlorate content of the sample. The absolute perchlorate content was obtained by comparison with a calibration curve prepared from standard samples (Fig. 1).

The coefficient of variation of the method is 14 per cent (11 samples) and the limit of detection is approximately 5 μgm potassium perchlorate. The method is thought to be specific for perchlorate; anions less strongly absorbed on 'Dowex 2' than perchlorate are eliminated by the anion-exchange separation, and interference by any remaining substances should be detectable by examination of the infra-red absorption spectrum.

Analyses were carried out on a variety of natural materials. Approximately 2-kg samples were taken for analysis. Inorganic and standard solutions were simply filtered before adsorption on the resin column; the organic samples were prepared as described here. The vegetables were bought at a local greengrocery; the urines were collected over a period of 14 days, stored at 0° C, and combined for analysis. Results are shown in Table 1. The perchlorate contents were expressed as potassium perchlorate. Analysis of urine enriched with 25 μgm potassium perchlorate showed complete recovery of the added perchlorate within the limits of reproducibility of the method.

Table 1. PERCHLORATE CONTENTS OF SAMPLES

	μgm $KClO_4$ per kgm sample
Blank sample (400 gm Whatman cellulose powder)	not detected
Sea-water	not detected
Cabbage	9
Silver beet (sample 1)	12
Silver beet (sample 2)	9
Urine (sample 1)	28
Urine (sample 2)	21
Urine (sample 2 + 25 μgm $KClO_4$)	51

These results confirm that perchlorate does exist in the natural environment. However, the levels are lower than those reported by Baas-Becking *et al.* by a factor of approximately 10^{-4}. Greenhalgh and Riley's negative findings for sea-water are also confirmed.

The mode of formation of perchlorate and its reactions (if any) in the natural environment remain unknown, but its low concentration would not seem to favour any important role in the natural economy.

I thank Dr. E. Spinner and Dr. A. R. Gilby for the provision of facilities for infra-red measurements, and Dr. F. P. Dwyer for a gift of arsonium salts. This work was supported jointly by the Australian Mining Industry Research Association, the Commonwealth Bureau of Mineral Resources, and the Commonwealth Scientific and Industrial Research Organization.

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TETRAPLOID HYBRID SORGHUM

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TETRAPLOID cultivated sorghum hybrids may be of value in under-developed areas where farmers lack both the desire and the resources to purchase fresh seed for each planting, as would be necessary to make proper use of diploid hybrids. In many such areas it is customary for each grower to save his own seed from one season to the next.

If the yield increase from heterosis is related to the degree of heterozygosity, the tetraploid hybrid will lose the yield increment more slowly[1] than the diploid. Seed of tetraploid hybrids could thus be renewed at longer intervals, the grower saving seed from his own crop for the intervening plantings. Segregation would not be a serious problem, since mixtures of varieties are grown in any event, and it would merely be necessary to ensure that a few main characters were present in both parents of the hybrid.

The principal defect of raw autotetraploid cultivated sorghum varieties is their low seed set relative to the diploids. Results have already been obtained[2] showing the increase in seed set of the F_1 and F_2 when two autotetraploid cultivated sorghum lines were crossed together. Comparable material was again grown, and selection in it continued, with the results shown in Table 1.

Table 1. SEED SET OF AN AUTOTETRAPLOID CULTIVATED SORGHUM HYBRID ($A \times B$), AS MEASURED BY THE PERCENTAGE OF SESSILE SPIKELETS PRODUCING SEED, OVER SEVERAL GENERATIONS OF SELECTION

	Percentage seed set		Percentage seed set
Parent *A*	19	$A \times B$ F_6	70·7
Parent *B*	33	F_7 (all)	70·1
$A \times B$ F_1	66	F_7, best family	75·3
F_2	67	F_7 best line	78·9
F_3	56	F_8 (all)	77·3
F_4	71·4	F_8, best family	81·0
F_5	70·5	F_8, best line	86·1

Genetic variance is now low, and the tetraploids stable, whereas in the early generations occasional reversions to diploid were found. Mean diploid seed sets varied between 88 and 95 per cent, and it is still not certain that an average seed set of more than 80 per cent will be obtained from this tetraploid cross.

However, a commercial sample of the natural tetraploid fodder grass *S. almum* showed high seed sets, and this, when crossed with the autotetraploid cultivated sorghum line *A*, gave high seed set in the F_1. The segregation in the F_2 suggested that high seed set is a dominant inherited character. A condensed frequency table is shown in Table 2.

Table 2. DISTRIBUTION OF SEED SET IN A CROSS BETWEEN AUTOTETRAPLOID SORGHUM LINE *A* AND *S. almum*

Percentage set	No. of plants			
Class	Parent *A*	*S. almum*	$A \times S.\ almum$, F_1	$A \times S.\ almum$, F_2
0–9	6			2
10–19	8			4
20–29	10			5
30–39	3			12
40–49				10
50–59		1		30
60–69		2	4	46
70–79		9	6	79
80–89		23	7	67
90–99		28	2	14

The generations in Table 2 were not grown simultaneously, but since neither parent had been selfed or selected for seed set, the data could in any event be used only to give a general indication that high fertility is an inherited character. Inbred lines are now available to repeat the cross for more precise studies.

It has proved easy to transfer the high seed set from *S. almum* on to cultivated autotetraploid types, and many plants from the second back-cross are virtually cultivated sorghums. Several such plants from the F_2 of the second back-cross have seed sets of more than 90 per cent, the highest recorded so far being 96 per cent (Fig. 1). The analysis of the whole F_2 generation has not yet been completed.

The third back-cross is being made to a range of autotetraploid cultivated varieties which will be recurrent parents in subsequent back-crosses. In this way, high seed set will be obtained in all the parents of the eventual tetraploid hybrids.

It is known that *S. almum* arose as a hybrid between a diploid sorghum and the ancient tetraploid *S. halepense*, a grain sorghum being the probable diploid parent[3]. *S. almum* cannot be old, as *S. halepense* was not introduced to the New World until 1830[4].

Presumably *S. halepense* has accumulated mutations giving high seed set, which were inherited by *S. almum*, and selection has tended to favour plants with good seed set in *S. almum*. *S. halepense* is a prohibited weed in East Africa so its fertility has not been checked by me.

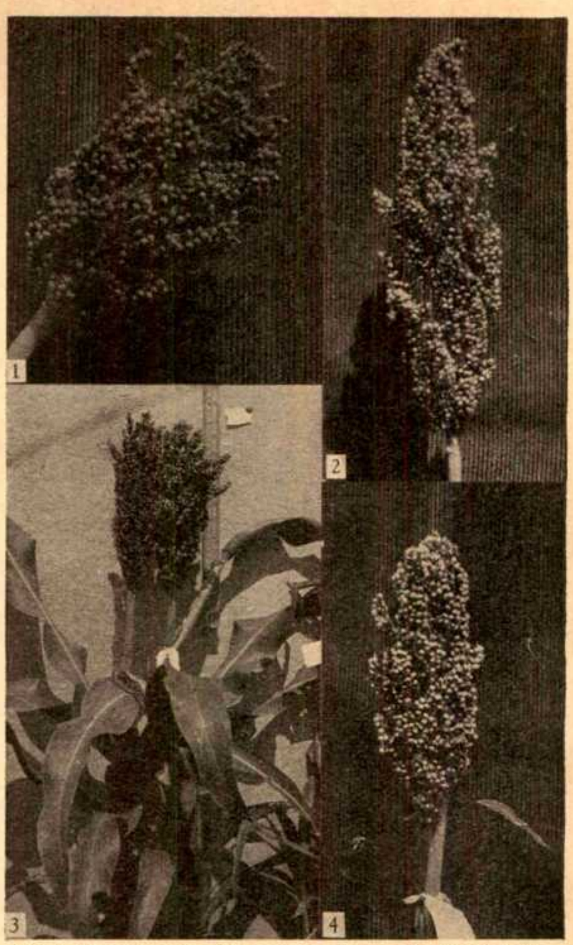

Fig. 1. Segregates from the second back-cross of cultivated autotetraploid sorghum × *S. almum*
1, 91 per cent seed set; 2, 96 per cent seed set; 3, combine type segregates; 4, 88 per cent seed set. [*Photo: K. Woodcock*]

Results from the autotetraploid cultivated sorghums suggest that certain 'neutral' genes present at the diploid level influence the seed set of the autotetraploids. Certainly the behaviour of the hybrid $A \times B$ in Table 1 resembles complementary gene action, and selection has been effective in stabilizing and increasing fertility. There is also evidence that different sorghum varieties show differing autotetraploid seed sets, though no differences between seed sets at the diploid level have been found.

There is no evidence that diploid pollen interferes with tetraploid seed set, nor vice versa. In a trial using single-row plots in three replications, fully randomized, two diploid entries showed 91·9 and 90·4 per cent seed sets while two of the *S. almum* entries gave 90·6 and 90·4 per cent seed sets. These figures are virtually identical.

Fully fertile tetraploid grain sorghum hybrids should thus not prove difficult to develop, and may be of considerable practical interest.

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LETTERS TO THE EDITOR

GEOPHYSICS

A New Method of Determining the Electron Content of the Ionosphere

Recently, the detection of a linearly polarized component in the galactic background radiation at 408 Mc/s was reported both by the Mullard Radio Astronomy Observatory, Cambridge[1,2], and the Leyden Observatory[3,4]. Both Observatories used the correlation between the observed plane of polarization and the ionospheric Faraday rotation, predicted from ionospheric sounding data, to test the existence of a true polarized component.

Although such a correlation exists, both the Leyden results[4], based on the model of a parabolic electron density distribution in the F_2-layer, and the Cambridge results[1], based on a more extensive model, show a spread of 15°–20° in the polarization angle observed for a given value of the predicted Faraday rotation for the highest intensity points. Since the latest measurements obtained at Leyden have a mean error of about 5° in the polarization angle, ionospheric sounding data are not accurate enough to allow a correction for the ionospheric Faraday rotation to be made. Therefore, an attempt was made to determine the Faraday rotation directly from the measurements.

To compute the Faraday rotation from an observation of a given point in the sky, the value of the polarization angle of that point beyond the Earth's ionosphere must first be determined. This was done by comparing the observed polarization angle, measured with respect to the Galactic Pole, θ_{gal}, with the Faraday rotation, expected on the base of ionosonde data, θ_f.

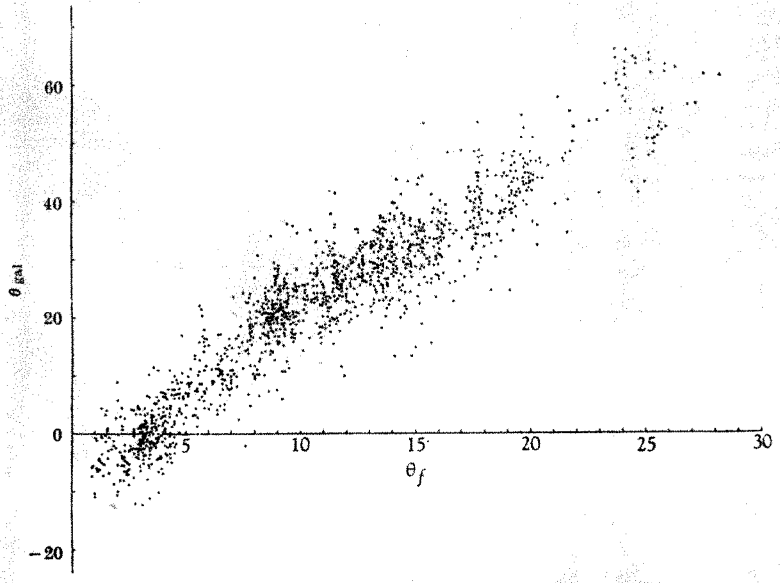

Fig. 1. Comparison of the observed polarization angle (θ_{gal}) and the Faraday rotation expected on the base of a parabolic F_2-layer (θ_f)

θ_f is given[5] by:

$$\theta_f = \frac{1{\cdot}4 \times 10^{-6}}{f^2} \int NH \cos\Phi \sec z \, \mathrm{d}h \text{ degrees,}$$

where N is number of electrons per cm^3, H is magnetic field-strength in oersteds, Φ is angle between magnetic field and direction of propagation, z is zenith distance of the observed point, h is height above the Earth in cm and f is frequency in Mc/s.

Thus, if f is 408 Mc/s:

$$\theta_f = 8{\cdot}1 \times 10^{-12} \, \overline{H \cos \Phi \sec z} \int N \, \mathrm{d}h \text{ degrees.}$$

If we assume a parabolic distribution of the electron density, then:

$$\int N \, \mathrm{d}h = 16{\cdot}5 \times 10^{-9} (f_0F_2)^2 (y_mF_2) \text{ electrons per cm}^2,$$

where f_0F_2 is ordinary-wave critical frequency of the F_2-layer in c/s, and y_mF_2 is half-thickness of parabola at zero electron density.

Using ionospheric sounding data, kindly provided by the Dutch Meteorological Institute, K.M.N.I., De Bilt, θ_f was calculated for each observation.

The results for all the observations made of the point $\alpha = 57°$, $\delta = 64°$, between March 20 and April 30, 1962, are shown in Fig. 1. Filled circles indicate measurements made at night, open circles are measurements made in the daytime, and, therefore, possibly disturbed by solar side-lobe radiation.

Least-squares solutions to fit a straight line through all the points yielded, for this, and a few other points, a mean slope of 2·3, or a ratio of the number of electrons above $h_{\max}F_2$ (n_a) to the number below (n_b) of $n_a : n_b = 3{\cdot}6 : 1$. This value is in agreement with values derived from Moon radar observations[5,6]. Furthermore, the least-squares solutions yielded the values of the polarization angles outside the Earth's ionosphere.

The electron content of the ionosphere could now be calculated from:

$$\int N \, \mathrm{d}h = \frac{(\theta_{gal} - \theta_0) \times 0{\cdot}12 \times 10^{12}}{\overline{H \cos \Phi \sec z}}$$

electrons per cm^2, where θ_{gal} is the observed polarization angle in degrees, and θ_0 the polarization angle outside the Earth's ionosphere in degrees.

The electron content of the ionosphere derived in the foregoing manner is shown in Fig. 2b, as a function of the local mean time, which is the solar mean time at the sub-ionospheric point. The filled circles indicate values obtained from measurements at $\alpha = 57°$, $\delta = 64°$, open circles values obtained from measurements at $\alpha = 217°$, $\delta = 14°$. No day-time measurements of the last point are present, since this point rose and set approximately during sunset and sunrise respectively.

In Fig. 2a the electron content of the ionosphere, as derived from the ionospheric sounding data, is plotted. A parabolic electron density distribution was assumed, and, in addition, the ratio $n_a/n_b = 3\cdot6$ was used. It is obvious that both parts of Fig. 2 are almost identical.

The mean error for the electron content derived from the polarization measurements is $1\cdot8 \times 10^{12}$ electrons per cm² per measurement.

The foregoing results show that even with a feed not especially designed for accurate angle measurements, and at a frequency where the maximum ionospheric Faraday rotation is about 80°, the electron content of the ionosphere can be determined from polarization measurements with an accuracy only slightly less than that from the Moon radar measurements. It seems, therefore, quite probable that in the future polarization measurements made with an especially designed feed, at a lower frequency, will be a superior method used for measuring the electron content of the ionosphere. Such measurements are, furthermore, not restricted to the period during which the Moon or an artificial satellite is above the horizon.

We thank Mr. R. S. Roger, of Jodrell Bank, who kindly provided us with a table of $\overline{H}\cos\Phi\sec z$, Mr. H. J. A. Vesseur, of the Dutch Meteorological Institute, who provided us with the ionospheric sounding data, Prof. C. A. Muller and his staff for building and maintaining the equipment, the Computer Centre of the University of Leyden, and the group at Cambridge for continuous information concerning their results. The measurements were made with the 25-m telescope at Dwingeloo, and financially supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

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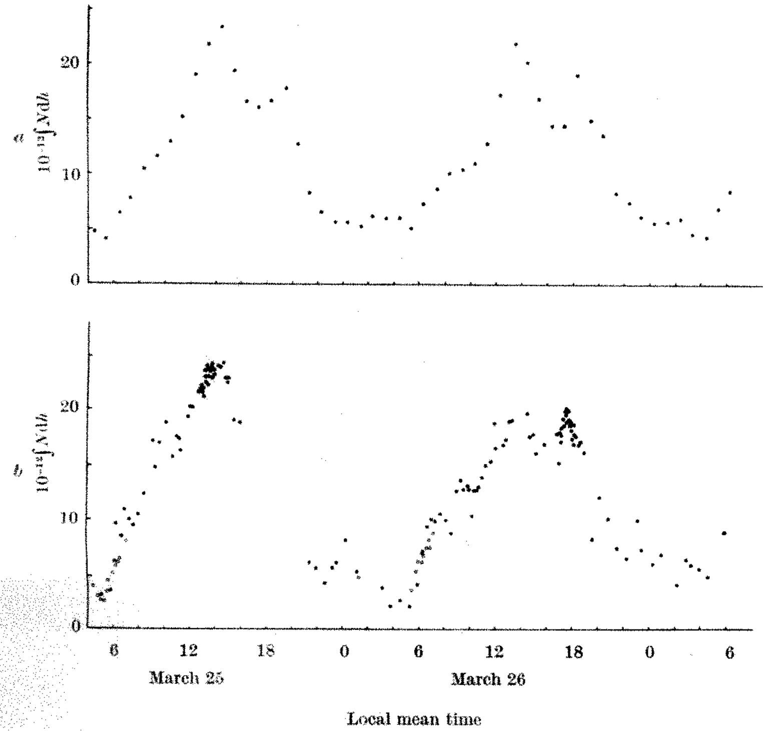

Fig. 2. *a*, Electron content of the ionosphere, derived from ionospheric soundings; *b*, total electron content of the ionosphere as a function of local mean time derived from the polarization measurements

Non-Seasonal Variation in the *E*-Layer Ionization

SOME time ago, Shimazaki[1] studied *foE* as a function of cos χ for stations in many different locations using only noon values of *foE* obtained during the International Geophysical Year. According to Chapman's theory, we should have:

$$(foE)^4 = K\cos\chi$$

but Shimazaki puts:

$$(foE)^4 = K'(\cos\chi)^y$$

K should have a constant value all over the Earth, determined by the level of solar activity. Waldmeier and also Harnischmacher found, in 1944, that *y* is greater than 1·00; this shows that Chapman's theory is not completely valid, and that we must be cautious in preferring the exponent four in the first equation. The calculated values of *K'* for many stations were used by Shimazaki to show that they tended to increase during the months November, December and January. This effect persists even if one corrects for the variations of solar activity and of the distance between the Sun and the Earth. Shimazaki explained his results by a hypothetical additional ionizing agent.

Minnis and Bazzard[2] considered, for a constant level of solar activity, the values of $Ch_E = (foE)^4 \sec\chi$, which should be a world-wide constant according to Chapman's theory. When plotting the median value of Ch_E at eight stations all over the world as a function of the month, they found a semi-annual modulation with two maxima, one in July–August and one in January–February. It seems to us, however, that we should look at the problem on a world-wide scale. In a recent publication[3] I stated that, at sunspot minimum, the *K'* values are constant for stations where the magnetic distortion found by Beynon and Brown[4] is of little importance. It has also been stated in ref. 3 that the higher *K'* values in December appear in only the northern hemisphere at temperate latitude stations; for equatorial stations no variation of *K'* with season is seen. The effect is more accurately described not as a winter increase

Table 1. C DETERMINED FROM MONTHLY MEDIAN DIURNAL VARIATION (SUNSPOT MINIMUM)

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Lwiro	3·57	3·50	3·52	3·52	3·52	3·54	3·61	3·51	3·52	3·61	3·55	3·55
Nha-Trang	3·54	3·50	3·47	3·51	3·50	3·49	3·48	3·50	3·49	3·47	3·49	3·54
Mean	3·56	3·50	3·50	3·52	3·51	3·52	3·54	3·50	3·50	3·54	3·52	3·54
Yearly mean						3·520						
Freiburg	3·50	3·54	3·39	3·38	3·33	3·33	3·33	3·40	3·49	3·50	3·55	3·57
Lindau	3·62	3·43	3·35	3·34	3·29	3·31	3·29	3·33	3·33	3·37	3·46	3·30
Mean	3·56	3·48	3·37	3·36	3·31	3·32	3·31	3·38	3·41	3·44	3·50	3·43
Yearly mean						3·405						

but as a summer decrease at northern temperate latitudes. This is shown in Table 1.

I have now investigated the seasonal variation of K' at ten stations. The minimum seasonal effect was found at Lwiro (2·3° S., 28·3° E.) using data for the period February, 1952–July, 1960; observations made at all times of day, and not only the noon values, were used. All values of log foE were plotted against log cos χ measured between sunrise and sunset and fitted the points to a straight line corresponding to the formula:

$$foE = C \cos^n \chi$$

C was then found by extrapolating to $\chi = 0$. In this way we could take account of all observations so that the basic information was considerably increased. The results thus obtained were first corrected for the variable distance between the Sun and the Earth; they were then compared with the Covington index, Φ, of solar radio-noise intensity at $\lambda = 10$ cm as given in the *F*-Series of the *CRPL Bulletin, Part B*. We also used the intensity of $\lambda = 20$ cm radiation as given by the Heinrich Hertz Institut, Berlin-Adlershof; but the dispersion was larger than for $\lambda = 10$ cm. Fig. 1 is a correlogram between Φ and the fourth power of the monthly median C values for Lwiro. If any residual seasonal effect is present, it is extremely small (0·1 MHz in C) as compared with, for example, 0·3 MHz at Freiburg. Also the phase of the remaining small effect at Lwiro is different from that reported in refs. 1 and 2 in so far as the November–February values are in the middle of the field of points.

Table 2. CORRELATION COEFFICIENT BETWEEN THE SOLAR RADIO RADIATION AT $\lambda = 10$ CM AND MINNIS'S E-INDEX $(foE)^4$ SEC χ FOR SLOUGH (FEBRUARY 1947–DECEMBER 1956 (REF. 2) AND OUR INDEX C^4 AT LWIRO (FEBRUARY 1952–JULY 1960)

Month	Slough	Lwiro
January	0·99	0·982
February	0·95	0·979
March	0·87	0·972
April	0·93	0·974
May	0·95	0·983
June	0·93	0·981
July	0·98	0·986
August	0·91	0·985
September	0·97	0·986
October	0·96	0·988
November	0·97	0·999
December	0·99	0·995
All months	0·90	0·9798
Mean value from January to December	0·950	0·9842

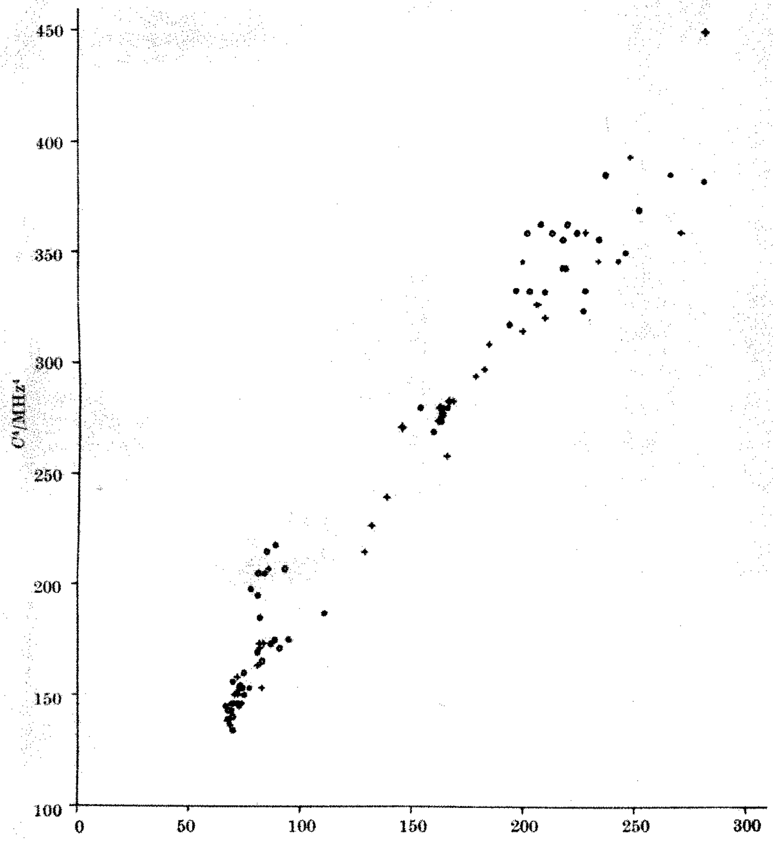

Fig. 1. The parameter C^4 at Lwiro (2·3° S., 28·8° E., February 1952–July 1960) plotted as a function of the Covington index Φ (λ = 10 cm). ○, Summer months; +, winter months; ●, equinoxes

Correlation coefficients between Ch_E and Φ have been determined by Minnis and Bazzard[5] for Slough. As a consequence of the seasonal effect, they found considerably higher coefficients for individual months than for all months taken together. This differs from what was found at Lwiro, where the average monthly coefficient equals that obtained for all months together (Table 2). This shows that Shimazaki's hypothesis of an additional ionizing agency must be rejected.

As an explanation of the differences found between stations, we feel that the magnetic distortion[4] ought to be considered as a perturbation which varies with time. In that case, complications must be present in the E-layer near the poles of the magnetic eddies. Nearly all the stations summarized by Minnis and Shimazaki lie in this zone and one may obtain quite different apparent seasonal variations according to the selection of the stations.

On the other hand, these investigations confirm the difference between northern and southern hemisphere stations found by Shimazaki[1]. An example is shown in Fig. 2, where log foE for December is plotted against log cos χ for a large number of stations in a limited

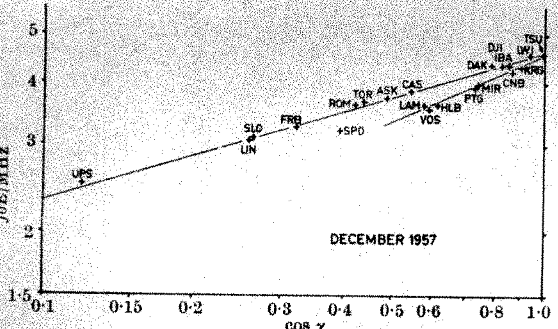

Fig. 2. Dependence of the monthly median critical frequency of the E-layer at noon on the zenith angle χ, December 1957. Upper line, northern hemisphere; lower line, so uthern hemisphere

longitude range. One finds two straight lines instead of only one as would be expected. It is always the winter hemisphere which gives the higher values, and hence the lines are inverted on changing from December to July. The 'scissors' effect disappears at the equinoxes.

It follows that a few stations, such as Lwiro, Chiclayo and Chimbote, are in a much better position for E-layer observations because the perturbing effects that cause seasonal variations, etc., seem to be unimportant at these places.

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RADIOPHYSICS

Faraday Rotation Effects associated with the Radio Source Centaurus A

The remarkable observations of Cooper and Price[1] indicate that the radiation from the radio source Centaurus A is linearly polarized and is subject to Faraday rotation of an amount such that:

$$\int n\, H \cos \alpha \, dr \sim 2{\cdot}5 \times 10^{14} \text{ gauss cm}^{-2} \qquad (1)$$

where n is the number of free electrons per cm^3, H is the magnetic field in gauss and α is the angle between the magnetic field and the line of sight. Cooper and Price assert that "the majority of the rotation must be occurring either within our own Galaxy or in the outer regions of Centaurus A". Nevertheless they find that both of these possibilities are quantitatively somewhat implausible.

For the Galaxy, Cooper and Price take values representative of the halo. I differ from them by assuming $n \sim 10^{-3}$ cm^{-3}, $H \sim 10^{-6}$ gauss (their value of 10^{-5} gauss seems much too large[2]), and $\int \cos \alpha \, dr \sim$ one-fifth of the dimensions of the region (a somewhat arbitrary choice which I make for all the regions mentioned in this communication). Taking the dimensions of the halo as 10 kiloparsecs we have:

$$\int n\, H \cos \alpha \, dr \sim 6 \times 10^{12} \text{ gauss cm}^{-2}$$

which is rather less than expression (1).

As regards the possibility that the rotation occurs in the outer envelope of Centaurus A, Cooper and Price point out that it is remarkable that the effect varies so little from one line of sight to another. This point is somewhat strengthened by the following consideration. If we assume that the dependence of the percentage polarization on frequency is due to Faraday rotation in the emitting regions themselves, one can calculate: (1) the amount of this rotation (which turns out to be about one-tenth of the total rotation observed); (2) the percentage polarization in the absence of this rotation (which is a measure of the uniformity of the magnetic field in the emitting regions). The calculation shows that both these quantities vary from one line of sight to another by more than a factor 2, in contrast to the 15 per cent or so for the total Faraday rotation.

The difficulty is less severe in an alternative explanation which I wish to propose, namely, that most of the rotation takes place in the local cluster of galaxies (or possibly in the cluster containing Centaurus A). According to a model recently developed by me[3], the local cluster contains a magnetic field of about 5×10^{-7} gauss. In this model $n \sim 1/3 \times 10^{-3}$ cm^{-3}, and the dimensions of the region are about one megaparsec[4]. This increase in dimensions as compared with the galactic halo more than compensates for the decrease in n and H. In fact for the local cluster:

$$\int n\, H \cos \alpha \, dr \sim 10^{14} \text{ gauss cm}^{-2}$$

In view of the uncertainties in the parameters of the cluster, this agrees reasonably well with expression (1). The small variation in the rotation from one line of sight to another is now more understandable, since the regions involved lie closer together in space, and their separation is a smaller fraction of the dimensions of the magnetically active medium.

One should also examine the possibility that the Faraday rotation occurs mainly in the intergalactic space between the source and the local cluster. Most cosmologists believe[5] that the average particle density in the universe as a whole is about 10^{-5} cm^{-3}, and it is probable[6-8] that most of this is in the form of ionized hydrogen. Accordingly, $n \sim 10^{-5}$ cm^{-3}. Taking $H \sim 10^{-7}$ gauss[9] and the distance of Centaurus A as 4 megaparsecs[1], we have:

$$\int n\, H \cos \alpha \, dr \sim 2 \times 10^{12} \text{ gauss cm}^{-2}$$

which is again too small.

I conclude that the observations of Cooper and Price may be explained in terms of a magnetic field in the local cluster of galaxies. If other polarized sources can be detected, it might be possible to check this conclusion by studying the dependence of the amount of Faraday rotation on the direction of the source.

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Dr. Sciama has proposed an interesting explanation of the Faraday rotation which is observed to be associated with the radiation from Centaurus A. The agreement between the observed rotation and that which would be produced by magnetic fields, electron densities and path lengths appropriate to his model of the local cluster of galaxies[1] is indeed very good. Experimental evidence for or against this model may soon be obtained from observations of other polarized sources at various galactic latitudes and longitudes, and we are able to report that Drs. Gardner and Whiteoak of this laboratory have now detected Faraday rotation in some 16 additional sources. Their results, which are soon to be published, indicate that there is a substantial galactic contribution to the rotation; in general, sources at high galactic latitudes show less rotation than those close to the plane.

In considering a possible intergalactic source of the rotation it should be pointed out that Prof. F. Hoyle[2] now sees no objection to a field as high as 10^{-6} gauss in inter-galactic space. With such a field, and an acceptable value of 2×10^{-5} cm^{-3} for the inter-galactic electron density, the rotation may be produced in a path length of 3 megaparsecs.

On reflexion, we are prepared to admit that the magnetic field of $\sim 10^{-5}$ gauss which we postulated for the galactic halo is higher than most current estimates. However, it is still possible that such fields do exist in the first 1–2 kiloparsecs of the path between the Earth and Centaurus A; this passes close to the Sagittarius arm of the Galaxy. Reducing the effective path-length to ~ 1 kpc the electron density then requires to be raised to $\sim 10^{-2}$ cm^{-3}, an acceptable value in this restricted region.

In summary, we hope that the experimental data will soon be numerous enough to arbitrate between the various models which have been proposed.

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PHYSICS

Neutron–Proton Interaction

Our attention has been directed to a note by Y. P. Varshni on neutron–proton interaction in the deuteron (ref. 1, hereafter referred to as I). The object of this communication is to point out that the magnetic dipole interaction suggested by Varshni does not lead to a bound state of the deuteron and his method has many self-contradictions and misapprehensions. It will further be shown that the n–p scattering cross-section based on this potential turns out to be much smaller than the experimentally observed value. It fails to account for the charge independence of nuclear forces. The slight observed difference between singlet n–p and p–p scattering lengths cannot also be accounted for by purely magnetic dipolar interaction[2].

The formula given in (I) for the attraction energy is

$$V = -2\mu_p\mu_n/r^3 \qquad (1)$$

This is also taken to be the total energy. It is not clear why the kinetic energy should be neglected. To estimate the binding energy from (1) Varshni calculates r by three methods. In the first, r is taken to be an integral multiple of the fundamental length:

$$r = n\hbar/Mc \qquad (2)$$

where M is the reduced mass of the n–p system. The ground-state corresponds to $n = 1$. This ground-state separation, of the order of 0·4 Fermi, disagrees with the observed value of 4 Fermis[3]. Also, it cannot be said that the charge centre separation in the deuteron is $4f$ while the magnetic moment centres are separated by $0{\cdot}4f$. Analyses of electron scattering data exclude such a possibility[4-6]. Moreover, such a separation would lead to a much larger quadrupole moment for the deuteron than is observed. The smaller value seems to have been chosen to get a value for V close to the experimental value of the binding energy (2·2 MeV). The observed neutron–proton separation would give a value a thousand times less.

The fundamental length is not a very well-defined concept, less so in the case of the nucleon. Various authors introduce it by various methods and get different values for it. If it is introduced to remove quantum mechanical divergences associated, for example, with cavity radiation its value is $5f$[7]. Other methods are due to Flint[8], who gets two fundamental lengths $\hbar/Mc$ (for field-quantum free case) and e^2/Mc^2 (with field) from a unified field theory coupled with a quantum theory of measurement, and Podolsky[9], who obtains it from an interpretation of the formulæ of electrodynamics.

The assumption that the orbital velocity $v \rightarrow c$ made in the second deduction of r_n contradicts Varshni's earlier statement that there is no orbital motion.

The third deduction depends on the similarity of deuteron with the hydrogen atom. Using Bohr's theory consistently to replace the electrostatic force Ze^2/r^2 by the assumed magnetic force $6\mu_p\mu_n/r^4$, we get for the radius:

$$r = 6M\mu_p\mu_n/n^2\hbar^2 = 6{\cdot}16 \times 10^{-16}/n^2 \text{ cm} \qquad (3)$$

Varshni uses the hydrogenic atom value $r_n = n^2\hbar/Mc$ and puts $\alpha = 1$, which is again self-contradictory because strong coupling has been completely ruled out by postulating magnetic interaction. Nevertheless, using the expression (3) for r_n, the total energy is

$$W = (Mv_n^2/2) - \hbar^6 n^6/(108M^3\mu_p^2\mu_n^2) = \hbar^6 n^6/(M^3\mu_p^2\mu_n^2)\ (1/72 - 1/108)$$

This being positive, binding is not possible.

It can be shown, using the uncertainty principle[10], that a particle subjected to a potential varying as r^{-3} will 'fall' to the centre. For if we confine the wave function to a small region of radius a, then the minimum uncertainty in momentum will be $\approx \hbar/a$. The mean kinetic energy in this state will be of the order $\hbar^2/Ma^2$ and the mean value of the potential energy is $-2\mu_p\mu_n/a^3$ where $2\mu_p\mu_n > 0$. Therefore the total energy $\hbar^2/Ma^2 - (2\mu_p\mu_n/a^3)$ can be made to take arbitrarily large negative values for sufficiently small a. The 'normal' state will therefore be of energy $-\infty$ and corresponds to the particle at the origin. If, however, we cut off the potential at a length a, then there can be binding only if $a < (2\mu_p\mu_n M/\hbar^2) = 10^{-16}$ cm, an improbably small distance.

It can also be shown that purely magnetic forces will give a very small value of the n–p scattering cross-section. The treatment given as follows[11] is general and not restricted to parallel dipoles only. In order to remove the formal difficulty of getting an indeterminate value of the interaction energy at the origin on assuming point dipoles, we assume a moment distribution[4] for one of the particles (proton for convenience). The current and moment densities in the proton are related by:

$$\text{curl}\ \overrightarrow{m(r_p)} = \overrightarrow{j_p(r_p)} \qquad (4)$$

The average interaction is:

$$V(\vec{r}) = \mu_n \vec{\sigma}_n \,.\, \overrightarrow{B(r)} \qquad (5)$$

where

$$\overrightarrow{B(r)} = -\int \frac{\vec{r}-\vec{r}_p}{|\vec{r}-\vec{r}_p|^3} \times \vec{j}_p \,\mathrm{d}\tau_p \qquad (6)$$

is the average magnetic field produced by the proton at the neutron co-ordinate r and the integration is to be performed over the volume of the proton.

The scattered wave function is:

$$\psi^{sc}(\vec{r}) = \psi^{sc}_{\frac{1}{2}}(r)\,\chi_{\frac{1}{2}} + \psi^{sc}_{-\frac{1}{2}}(r)\,\chi_{-\frac{1}{2}} \qquad (7)$$

where the χm_s are the spin eigenfunctions.

The Born approximation for the scattered wave is:

$$\psi^{sc}_{m_s}(\vec{r}) = -\,(M\mu_n/2\pi\hbar^2)\,(e^{ikr}/r)$$

$$\int e^{i(\vec{k}_0-\vec{k}).\vec{r}} \sum_{m_s'} \overrightarrow{B(r)} < m_s|\vec{\sigma}_n|m_{s'}> C_{m_s'}\mathrm{d}\tau \qquad (8)$$

Now on substituting the value of $\overrightarrow{B(r)}$ from (6) we have:

$$\int e^{i(\vec{k}_0-\vec{k}).\vec{r}}\, \overrightarrow{B(r)}\,\mathrm{d}\tau = -\,4\pi i(\vec{q}/q^2) \times \int e^{i\vec{q}.\vec{r}_p}\, \vec{j}_p \mathrm{d}\tau_p \qquad (9)$$

Substituting for $\vec{j}_p$ from (4) and using the long wavelength approximation followed by some simplification, the integral on the right of equation (9) reduces to:

$$-i\vec{q}\, x \int \overrightarrow{m(r_p)}\mathrm{d}\tau_p = -\,i\vec{q}x\vec{\mu}_p \qquad (10)$$

where μ_p is the proton magnetic moment.

Thus the total wave function is:

$$\psi(\vec{r}) = e^{i\vec{k}_0.\vec{r}} \sum_{m_s} C_{m_s}\chi_{m_s} + \frac{e^{ikr}}{r}\left[\frac{2M\mu_n}{\hbar^2}\frac{\vec{q}}{q}.\vec{\mu}_p\right.$$

$$\sum_{m_s',m_s} \chi_{m_s} < m_s|\frac{\vec{q}}{q}.\vec{\sigma}_n|m_{s'}> C_{m_s'}$$

$$\left.-\,\frac{2M\mu_n}{\hbar^2} \sum_{m_s',m_s} \chi_{m_s} < m_s|\vec{\mu}_p.\vec{\sigma}_n|m_{s'}> C_{m_s'}\right] \qquad (11)$$

The corresponding differential cross-section is:

$$\sigma(\theta) = (4M^2\mu_n^2/\hbar^4)\,[-\{(\vec{q}/q)\cdot\vec{\mu}_p\}^2 + |\mu_p|^2] \qquad (12)$$

For unaligned protons the average of the first term is zero and we have:

$$\sigma(\theta) = (4M^2\mu_p^2\mu_n^2/\hbar^4) \qquad (13)$$

This shows that the scattering is isotropic and independent of momentum transfer. The total cross-section is, however, negligibly small (10^{-7} barns) compared with the observed value of 20·2 barns.

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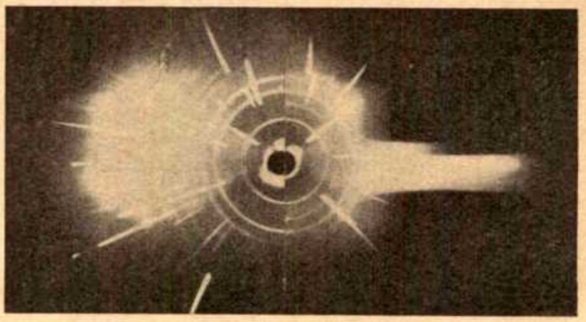
Structure of the High-pressure Phase of Indium Antimonide

THE transition under pressure to a phase showing metallic conduction in indium antimonide (InSb) was first found by Gebbie *et al.*[1] and confirmed by Jayaraman *et al.*[2]. These workers[2] gave a phase diagram with a triple point between InSb–I, InSb–II and liquid InSb, and showed that the conducting phase was solid. They also gave a minimum estimate of 20 per cent for the volume change on passing from InSb–I to InSb–II and suggested that, since tin lies between indium and antimony in the periodic table and InSb–I has the zinc-blende structure analogous to the diamond structure of grey tin, the new phase probably possessed the tetragonal white tin structure.

These predictions have been confirmed by X-ray diffraction photographs of InSb–II. The apparatus, which will be described in detail elsewhere, consists of two diamond hardness indenters which have had the points ground off to leave small flat surfaces. These are held in a framework and thrust together by a calibrated spring. Ignoring friction, a load of 100 lb. produces a pressure of 22·7 kilobar between the faces.

A Hilger and Watts micro-focus X-ray unit was used in conjunction with a single crystal camera. Three clear lines of the new phase were superimposed on those of the old phase. The d-distances derived from these were very close to those for β-tin, and gave a value for c/a of 0·517, with $a = 5{\cdot}92$ Å and $c = 3{\cdot}06$ Å. Fig. 1 shows the diffraction patterns at 1 kb. and 30 kb. juxtaposed.



1 kb. 30 kb.

Fig. 1. X-ray diffraction patterns of InSb at 1 kb. and 30 kb. pressure. (Molybdenum $K\alpha$-radiation, 6 m.amp, 45 kV, 12 h)

Table 1

	InSb–I	α–Sn	InSb–II consistent with	β–Sn
Structure type	$F\bar{4}3m$	$FD3m$	$I4_1/amd$	$I4_1/amd$
Atoms/unit cell	8	8	4	4
Cell constants (Å)	6·478*	6·489*	5·92, 3·06	5·831, 3·182*
c/a ratio (tetragonal axes)	1·414	1·414	0·517	0·545
Specific volume (c.c./g)	0·172	0·173	0·141	0·137
Observed spacings (Å)	—	—	2·96, 2·72, 1·98 (200) (101) (211)	—

* Taken from *ASTM Powder Data Index* (1960).

The density of the new phase is then found to be 7·09 ± 0·13 g/c.c., compared with a density of 5·70 g/c.c. at zero pressure and room temperature. This is equivalent to $\Delta v/v = -18{\cdot}5 \pm 0{\cdot}4$ per cent and may be compared with a value of −21 per cent for the $\alpha \rightarrow \beta$ tin transition[3].

Within experimental limitations no volume change in InSb could be detected before the transition. Fig. 2 shows the diffraction pattern at 22½ kb. pressure, in which the doublet due to the new phase has just appeared. If the zero pressure value of the compressibility[4] remains constant, a change in volume of 5 per cent would be observed. In practice, however, compressibility falls with increasing pressure so that 5 per cent is almost certainly a maximum value.

By applying the Clausius–Clapeyron relation to the volume change and the value of dT/dP from the phase diagram given in ref. 2, the entropy change for the solid I→solid II transition is found to be 0·0084 cal./g/deg., this indicating a latent heat at 13° C of 2·4 cal./g. This may be compared with a latent heat value of the corresponding tin transformation of 4·54 cal./g (ref. 3).

If the assumption is made that the entropy change on transformation is constant, then, since the phase boundaries are straight lines, the changes in volume, too, are constant. Making this assumption, the entropy and changes in volume close to the triple point may be deduced from the slopes of the phase boundaries given in ref. 2 and the measured values of volume and entropy changes on melting at zero pressure[5,6]. Ignoring volume changes due to thermal expansion, this process gives a value of $\Delta v/v$ at the solid I–solid II boundary of −17·5 per cent. This may be compared with the value of −18·5 per cent for $\Delta v/v$ reported here. The good agreement indicates that the entropy of melting is in fact nearly constant along the melting line from the melting point to the triple point.

The parameters for the low- and high-pressure forms of InSb are given in Table 1 together with corresponding data for white and grey tin.

We thank Mr. N. B. Owen for the design of the apparatus. The work described here is published by permission of the Director of the Laboratory.

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Gamma-Recoil Products from the Neutron Irradiation of Colloidal Silver

The colloidal state involves two or more components in two phases. One of the phases consists of particles distributed within another continuous medium. The diameters of the particles are roughly in the range 50 Å ($5 \times 10^{-3}\mu$)–2000 Å ($0{\cdot}2\mu$). This means that the lower limit is about the linear dimension of a molecule 30–40 atoms in length.

Within the range of the indicated particle size there is room for considerable variation in a number of factors: shape, composition, structure and surface area. It has been pointed out[1] that the division of a sphere 1 cm in diameter into spheres of 10^{-5} cm involves an increase in surface area by a factor of 10^5. Thus within the range of colloidal diameters the same quantity of a disperse phase can present surface areas varying by a factor of 40. In the following discussion I am concerned only with the aforementioned lower limit, that is, those particles having diameters of between 50 and 100 Å.

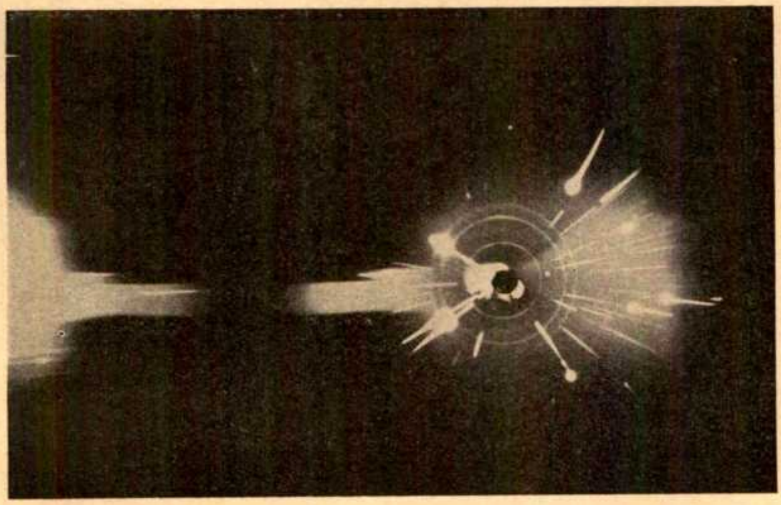

Fig. 2. X-ray diffraction pattern of InSb at 22·5 kb., showing first lines of the new phase. Exposure details as for Fig. 1

In the work reported here care was taken to select only those particles having diameters not larger than 100 Å from a commercially obtainable colloidal silver suspension. For the neutron irradiation of the colloidal suspensions the following procedure was developed and found satisfactory. A 10-ml. solution of colloidal silver, having a particle concentration of 0·5 mg/ml., was prepared and transferred to a special quartz irradiation container. The quartz container was then sealed ready for irradiation. A second sample was also prepared, in exactly the same manner, to constitute a control sample. Both samples were irradiated for a 1-week period in a neutron flux of 2×10^{11} n/cm²/sec in the *R*1 reactor in Stockholm producing the long-lived silver −110 m isotope ($t_{\frac{1}{2}} = 289$ days). On completion of the irradiation, the quartz capsules were opened and the liquid contents of one of them subjected to ultra-filtration. The ultra-filtration assembly consisted of a simple demountable funnel, made from lucite, with provision for a cellulose-acetate membrane having an average pore size of 24 Å. After assembly, the described arrangement was fitted to a conventional vacuum flask which was then evacuated to a pressure of approximately 0·1 mm mercury.

As a consequence of the membrane pore size, only those particles of less than 24 Å could pass through the membrane and into the vacuum flask. This takes into consideration any γ-recoil products which may be present in the solution, and, as will be shown, such products were present. The liquid was removed from the vacuum flask and a known amount of silver nitrate solution added as carrier. Any γ-recoil products were then precipitated, together with the nitrate, by the addition of hydrochloric acid, centrifuged, washed and finally deposited on a counting planchet. The control sample was not subject to ultra-filtration, but was otherwise treated in the same manner. Only the γ-radiation of the samples was measured, thus avoiding corrections due to the absorption of β-ray components by a thick sample. Out of twelve consecutive runs the activity of the filtered solution was found to contain 7–12 per cent of the total activity produced by the irradiation of a single sample.

I believe that the separable yield of recoil products is probably strongly dependent on the time-lapse between the irradiation of the sample and the eventual separation due to the fact that colloidal particles are known to carry an electric charge which most certainly contributes to the recombination of any γ-recoil products present with the original colloidal particles.

The method may be considered as a variation of the Szilard–Chalmers process[2] with the difference that in the present instance colloidal suspensions, rather than a solution, are made use of as the target material.

I thank Prof. Nils Ryde for his encouragement during the reported work.

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Packing of Equal O-Spheres

SMALLEY[1] has recently proposed the problem of determining the density of O-spheres distributed randomly on a line and has given some Monte Carlo estimates. In this communication I largely solve this problem, so far as the expectation of the density is concerned.

Without loss of generality, let the O-spheres have diameter unity and let $E(x)$ be the mathematical expectation of the number of spheres on an interval of length x. Suppose the O-spheres are forbidden to overlap each other or the end-points of the interval and that they are laid down sequentially according to independent random placing of their centres within the interval, forbidden spheres being rejected. My specification differs slightly from that of Smalley in its treatment of the end-points; this leads to a more succinct theory. We shall see that it does not change the asymptotic value of the mean density $\rho(x)=x^{-1}E(x)$ and, furthermore, that the solution of the problem with Smalley's boundary conditions follows at once from the solution with mine.

Consider now the position after one sphere has been laid on the line. The numbers, N_l and N_r, of spheres afterwards placed to left and right, respectively, of this first sphere are clearly independent random variables. If the centre of the first sphere is at $y+\frac{1}{2}$ from the left-hand end of the interval then the expectation of N_l+N_r is $E(y)+E(x-1-y)$. Hence we have:

$$E(x)=1+(x-1)^{-1}\int_0^{x-1}\{E(y)+E(x-1-y)\}\,\mathrm{d}y$$

$$=1+2(x-1)^{-1}\int_0^{x-1}E(y)\mathrm{d}y \qquad (1)$$

As $E(x)=0$ for $0\leqslant x<1$, this enables us to calculate $E(x)$ iteratively. An exact investigation for $x\leqslant 4$ does not encourage the hope that any simple general solution of (1) may be found. However, it follows from equation (1) that:

$$\frac{\mathrm{d}E}{\mathrm{d}x}=2(x-1)^{-2}\int_0^{x-1}\{E(x-1)-E(y)\}\,\mathrm{d}y \qquad (2)$$

and hence, by induction on x, $\frac{\mathrm{d}E}{\mathrm{d}x}\geqslant 0$ for all x except $x=1$, for which it does not exist. As, obviously, $E(x)\leqslant x$ always, we also deduce for $x>1$ that:

$$\left|\frac{\mathrm{d}E}{\mathrm{d}x}\right|\leqslant 2,\quad \lim_{x\to\infty}\frac{\mathrm{d}\rho}{\mathrm{d}x}=0 \qquad (3)$$

It is natural to suppose that, as $x\to\infty$, a limiting density exists and we now prove this using an auxiliary function $\varphi(x)=\rho(x)+x^{-1}$. From equation (1):

$$E(x+1)=x^{-1}+(1-x^{-1})E(x)+2x^{-1}\int_{x-1}^{x}E(y)\,\mathrm{d}y$$

$$\leqslant x^{-1}+(1+x^{-1})E(x)$$

where we used $\mathrm{d}E/\mathrm{d}x\geqslant 0$, whence:

$$\varphi(x+1)\leqslant\varphi(x)$$

As a function of n, $\varphi(x+n)$ is therefore monotonic decreasing and bounded below. So it tends to a limit as $n\to\infty$, which means that $\rho(x+n)$ does too. This proof leaves the possibility that the limit ρ_∞ may depend on the starting point x. Equation (3), however, shows the limit to be independent of it. As $\varphi(x)>\rho(x)$ for all x, we can easily deduce $\rho_\infty<1$. Conversely, using similar arguments to the foregoing, it follows from (1) that:

$$E(x+1)\geqslant x^{-1}\{1+(x-1)E(x)+2E(x-1)\}$$

whence by induction:

$$E(x)\geqslant\tfrac{1}{2}x$$

for $x>1$, which implies that $\rho_\infty\geqslant\frac{1}{2}$.

Equation (1) was solved numerically to give the values of E and ρ shown in Table 1, for integral values of $x\leqslant 10$. It was also solved for various other integral $x\leqslant 1{,}000$, these latter values of ρ being fitted to within 10^{-5} by the formula:

$$\rho(x)=\lambda+(\lambda-1)x^{-1} \qquad (4)$$

with $\lambda=0{\cdot}74759$. Clearly this λ is also the limiting density with, presumably, the same accuracy.

Smalley[1] used boundary conditions which were equivalent to placing spheres randomly on a closed 'circular' region. Let $F(x)$ be the expectation for such

Table 1. CALCULATED VALUES OF E AND ρ FOR INTEGRAL $x \leqslant 10$

x	E	ρ	x	E	ρ
1	0	0	6	4·2332	0·70553
2	1	0·5	7	4·9808	0·71154
3	2	0·66667	8	5·7284	0·71604
4	2·7425	0·68561	9	6·4759	0·71955
5	3·4851	0·69701	10	7·2235	0·72235

a region of length x. Then by considering the position after placing one sphere, we find:

$$F(x) = E(x-1) + 1$$

and hence the limiting density is the same as for the model discussed in the present communication. Using equation (4) we find the approximate relation $F(x) = \lambda x$. Smalley finds the two "observed values" of $x^{-1}F(x)$ of 0·78 and 0·75 for $x = 10$ and 50 respectively, in fair agreement with our calculated λ.

I thank the Director of the Cambridge University Mathematical Laboratory for making the computing service available and Mrs. M. O. Mutch for programming the problem.

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MINERALOGY

Appearance of Exothermic Peak in the Differential Thermal Curves of Vermiculites

THE following three Indian vermiculite samples: (i) Bagespura, (ii) Malavanghatta and (iii) Hazaribagh have been examined by X-ray and differential thermal analysis. The samples are of flaky habit and exfoliate on heating. All the samples give a basal reflexion of 14 Å, which collapses to 9·6 Å on heating to 750° C. In the differential thermal analysis curves (Fig. 1) there are two sharp water-loss peaks at 150° and 250° C, similar to true vermiculites. In each of the samples (i) and (ii) there is an additional endothermic peak at about 100° C, perhaps due to absorbed water. In all the samples there is an endothermic peak near 900° C.

The high-temperature exothermic peak present in Barshad's[1] samples 1 and 2 is absent in all these samples. Barshad's sample 4, which has been classed as hydrobiotite by Gruner[2] on the basis of X-ray analysis, has a differential thermal analysis curve more like that of (i), (ii) and (iii). As biotite is not responsive to differential thermal analysis, it seems that the vermiculite part of sample 4 is similar to (i), (ii) and (iii) in so far as the exothermic peak is concerned. Among the five samples examined by Barshad[1] samples 1 and 2 (natural) give an endothermic peak followed immediately by an exo-peak. However, when saturated with NH^+ and Na^+, the exo-peak becomes so small that only two endothermic peaks seem to be present, side by side, at 800° and 850° C respectively. Sample 2, when saturated with Ca^{2+}, Na^+ and Li^+, shows a similar effect. Sample 3 (natural) gives small endothermic peaks at 800°, 850° and 900° C, but no well-defined exothermic peak. It may be noted that an exothermic peak similar to that of sample 1 and 2 is present in chlorite. In the case of sample 5, this peak is attributed to chlorite, which, present as impurity, is characterized by a 600° C endothermic peak.

Thus it is observed that the exothermic peak of some of the vermiculites appears when saturated with certain cations but disappears when saturated with others. But since the different vermiculites saturated with the same cation do not behave alike, so far as the exothermic peak is concerned, the exchangeable cation does not seem to be a decisive factor in this regard. Barshad[1] suggests that the exothermic peak corresponds to the contraction of the lattice from the basal spacing 10·2 Å of moderately heated vermiculite to 9·4 Å on ignition. This suggestion does not appear tenable, since this contraction also occurs in samples in which the exothermic peak is altogether lacking. In view of the fact that the contraction occurs below the temperature of the exothermic peak, near about the region of the removal of the hydroxyl groups, the suggestion seems all the more unacceptable.

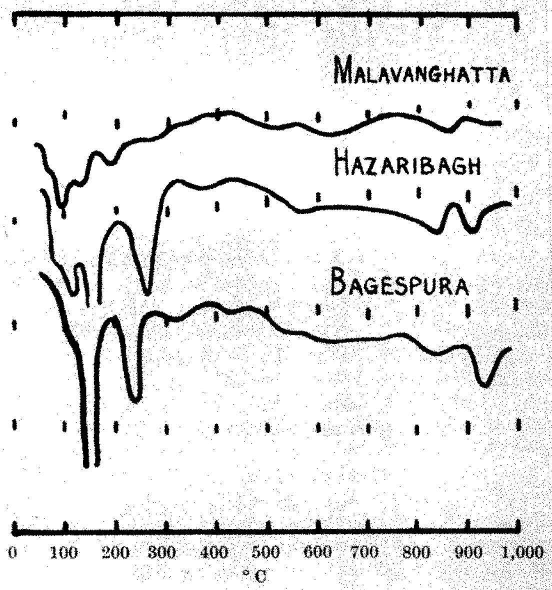

Fig. 1

From the results so far obtained, no conclusion can be drawn as to whether the exothermic peak is an essential feature of vermiculite or not. Three possibilities may be argued as follows:

(1) The exo-peak is not a characteristic feature of vermiculite but occurs due to an impurity (for example, chlorite) in the sample. Hence the absence of the peak would mean that the impurity is absent, so that the sample showing the other characteristic features of vermiculite is pure.

(2) The peak is really a feature of vermiculite. It may then be ascribed to a rearrangement of the lattice after the OH loss is complete (as in montmorillonite). If the peak is absent, it is possible to suppose that it is neutralized by an opposing reaction proceeding at the same temperature in an associated impurity.

(3) The two types may represent two polymorphic variations of the vermiculite group. Depending on the nature of substitution in the lattice, the exothermic reaction may be retarded.

It thus appears difficult to draw, on the basis of the data obtained so far, a valid conclusion regarding the true characteristic of the vermiculites in so far as the appearance of the exothermic peak is concerned.

I thank Prof. S. N. Bose for his advice. I also thank the Assam Oil Co., Ltd., for a scholarship and an equipment grant.

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CRYSTALLOGRAPHY

A Semi-bonded Compound in the Series SnI_4–S_8

DURING the course of investigations of solid solutions involving van der Waals solids it was found that sulphur and tin tetraiodide combine readily in carbon disulphide or benzene solution forming a stable compound, the formula of which was determined as $SnI_4.2S_8$. The substance is orange in colour, has a measured density of 2·85 g/c.c., and melts at 101° C. It crystallizes either as orthorhombic pyramids by evaporation of a carbon disulphide solution or as third-order prisms by cooling of a saturated benzene solution, leading to the assignment of the point group *mm*2. (A metastable monoclinic modification of density = 2·80 g/c.c. was also observed during rapid cooling of benzene solutions.)

Crystals of orthorhombic $SnI_4.2S_8$ are stable if kept in sealed glass containers, but hydrolyse slowly in water and slowly decompose on exposure to air. If dissolved in solvents such as carbon tetrachloride in which sulphur is practically insoluble, the compound is readily destroyed and the original components regenerated.

'Double salts' among van der Waals solids are rare and usually involve a more efficient packing arrangement than that of the separate constituents, but as the observed density of the compound was significantly less than the value of 2·93 calculated assuming the same packing efficiency as in cubic tin tetraiodide[1] and in orthorhombic sulphur[2], it appeared as though some factor other than packing efficiency was responsible for the existence of the compound, and an X-ray determination of the structure was undertaken to see what this might be.

Rotation photographs yielded the approximate lattice parameters $a = 20·8$ Å, $b = 21·7$ Å, $c = 11·6$ Å, indicating eight formula units per unit cell. Five degree and fifteen degree oscillation photographs were taken around the c and a axes, respectively, and the systematic absences in conjunction with the predetermined point group uniquely defined the space group as *Fdd*2.

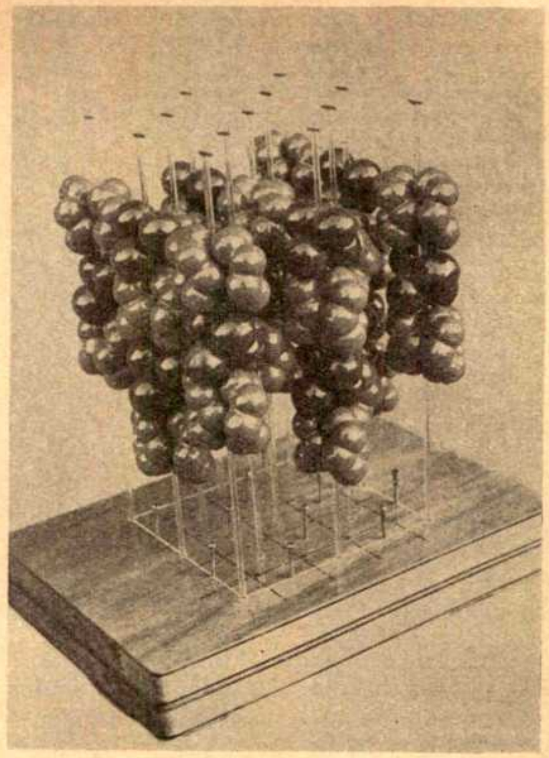

Fig. 1. Crystal structure of orthorhombic $SnI_4.2S_8$

The positions of the atoms in the cell were determined by trial. In this space group there is only one eight-fold position, the spatial requirement of which is that the lattice object must have a diad symmetry axis. As the virtual contact of iodine atoms in the SnI_4 unit precludes any significant distortion of tetrahedral configuration, the SnI_4 unit was placed in the cell so that its axis of four-fold rotary inversion was coincident with the diad axis of the space group. Various orientations of the SnI_4 unit about this axis were postulated and the *Okl* and *hkO* structure factors (sulphur ignored) were calculated and compared with the intensities observed on the films. It was found that agreement was obtained only with the mirror planes of the SnI_4 tetrahedron parallel to the a and b axes of the cell.

Table 1. ATOMIC CO-ORDINATES

Atom	x	y	z
Sn	0	0	0
I_1	0	0·93	−0·125
I_2	0·098	0	0·125
S_1	0·090	0	0·340
S_2	0·180	0	0·410
S_3	0·041	0·100	0·410
S_4	0·180	0·041	0·530
S_5	0·013	0·090	0·530
S_6	0·180	0	0·655
S_7	0·041	0·100	0·655
S_8	0·090	0	0·710

A scale model of the SnI_4 units in the cell was constructed and the S_8 units fitted in. Symmetry and steric limitations precluded arrangements other than that of two S_8 units arranged around a diad axis, one of the sulphur atoms of each ring fitting into the central hole of the other. The orientation of these sulphur ring pairs was fixed by the close-fitting voids in the SnI_4 framework.

Atomic co-ordinates (Table 1) were obtained for the tin and iodine atoms on the basis of the bond-lengths and van der Waals radii of Dickinson[1], and for the sulphur atoms on the basis of the data of

Table 2. COMPARISON OF CALCULATED AND OBSERVED INTENSITIES

h	k	l	I_o	$F^2\times10^{-3}$	h	k	l	I_o	$F^2\times10^{-3}$
0	4	0	*ms*	1077	6	4	2	*w*	163
0	8	0	*s*	2371	6	8	2	*m*	533
0	12	0	*s*	3541	8	2	2	*vw*	96
2	2	0	*s*	952	8	6	2	*m*	548
2	6	0	*mw*	270	8	10	2	*w*	382
2	10	0	*s*	2082	10	0	2	nil	77
4	0	0	*m*	322	10	4	2	*s*	1183
4	4	0	*s*	1618	10	8	2	*w*	337
4	8	0	*w*	175	0	4	4	nil	5
6	2	0	*vw*	23	0	8	4	nil	85
6	6	0	*s*	1602	2	2	4	nil	18
6	10	0	*vw*	88	2	6	4	*ms*	744
8	0	0	*s*	534	2	10	4	*mw*	190
8	4	0	nil	103	4	0	4	nil	37
8	8	0	nil	19	4	4	4	*s*	1773
10	2	0	*s*	2005	4	8	4	*ms*	640
10	6	0	*mw*	437	6	2	4	*m*	337
10	10	0	*s*	3298	6	6	4	*s*	1594
0	2	2	*m*	373	6	10	4	nil	31
0	6	2	*s*	2313	8	0	4	nil	106
0	10	2	nil	23	8	4	4	*w*	354
2	0	2	*m*	530	8	8	4	*w*	154
2	4	2	*m*	481	10	2	4	*mw*	220
2	8	2	*w*	123	10	6	4	nil	40
4	2	2	*s*	939	0	2	6	*w*	216
4	6	2	*w*	118	0	6	6	*s*	1535
4	10	2	*s*	1242	0	10	6	*w*	113
6	0	2	*s*	1921	0	0	4	*m*	746
					0	0	8	*s*	2045
					0	4	8	*mw*	200

Warren and Burwell[2], applied to photographs of the trial model, and with these co-ordinates the structure factors were calculated for the even-numbered planes having indices $\leqq 10$ (Table 2). The agreement between calculated intensities and those observed on the oscillation photographs verifies the correctness of the structure (Fig. 1).

A least squares refinement[3] of the lattice parameters based on twelve unequivocally indexed powder lines yielded the following values: a = 20·83 Å, b = 21·76 Å, c = 11·41 Å.

The structure consists of infinite chains of SnI_4 tetrahedra parallel to the c axis of the crystal, bonded together through double S_8 rings, each iodine atom being in closer proximity to a sulphur atom than can be accounted for on the basis of normal van der Waals radii. A similar interaction intermediate between that of covalent bonding and van der Waals attraction has been observed recently by Hassel *et al.*[4]. In many of the compounds examined by them covalently bound iodine atoms in otherwise electronically saturated compounds act as Lewis acids toward electron pair donors such as nitrogen, oxygen or sulphur atoms in other 'saturated' molecules, with a resultant shortening of the distance of closest approach between the atoms involved.

It appears that this charge transfer interaction is the principal source of the stability of $SnI_4 . 2S_8$. Another factor which could contribute to the stability of the compound is the dielectric effect of the S_8 rings in separating the SnI_4 units from each other, since, due to the partial ionic character of the Sn–I bond, there is a tendency toward mutual repulsion in the close packed arrangement of cubic SnI_4.

The effect of the charge transfer bond on the length of the c axis is striking: assuming normal van der Waals interaction the length of the c axis should be 12·06 Å, or 0·65 Å more than that observed. This effect is of the same order of magnitude as that observed by Hassel in iodoform–sulphur complexes and in dithian–sulphur complexes.

On the basis of the c axis shortening and the ability of this compound to exist at fairly high temperatures despite its unfavourable density, it would appear that the charge transfer bond can be a fairly strong type of force between otherwise saturated covalent molecules.

I thank Mr. J. F. Tilley and Mr. I. Munro for their help in this work.

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RADIOCHEMISTRY

Removal of Radiostrontium from Milk

In the past few years several methods have been proposed for the removal of radiostrontium from milk. Among the most elaborate methods is a procedure worked out by Migicovsky[1] and further improved by L. F. Emondson *et al.*[2]. This method is based on an ion-exchange process using a bed of resin charged with a mixed solution of calcium, potassium, sodium, and magnesium ions. In pilot plant experiments this process has proved able to remove about 90 per cent of the radiostrontium present in milk.

The procedure developed in our laboratory is a batch method using three treatments of the milk with cation exchange resins. In the first step 1 litre of milk is mechanically stirred for 20 min with 50 g 'Dowex 50 *W–X*8' (50/100 mesh) resin in potassium form plus 5 g of the resin in hydrogen form. After stirring, the resin is removed by filtration and the filtered milk is treated once more for 20 min with fresh portions of 50 g potassium resin and 5 g hydrogen resin. The milk is filtered and is finally stirred for 20 min with 55 g 'Dowex 50 *W–X*8' (50/100 mesh) resin in calcium form plus 5 g resin in hydrogen form. By this method it is possible in laboratory experiments to remove about 99·7 per cent of the radiostrontium from the milk.

The taste of the treated milk is good and a taste panel of 20 persons failed as a whole to distinguish any significant difference between treated and untreated consumer milk.

The used resin which contains the strontium activity removed from the milk is first washed with water and detergent, then sterilized with hot diluted nitric acid. The resin is then transferred to a column and converted to the sodium form by passing a strong sodium chloride solution through the column. The sodium resin is finally transferred to potassium resin, calcium resin, and hydrogen resin in three different columns by passing a potassium chloride solution, a calcium chloride solution, and a hydrochloric acid solution, respectively, through the sodium resin beds.

The milk used for the ion-exchange experiments was obtained from a cow of red Danish milk breed, which was intravenously injected with about 0·5 mc. strontium-85.

It is important to emphasize that the nutritional value and the chemical composition of the treated milk from these experiments have not yet been thoroughly examined; but the Food and Nutrition Division of the National Health Service has from the very beginning planned experiments at the State Vitamin Laboratory involving chemical analyses and investigation of nutritional value by pair-feeding experiments on rats. It may be mentioned that initial chemical determinations have indicated lack of certain vitamins.

Furthermore, the National Health Service planned experiments in pilot plant scale performed by the State Experimental Dairy in order to investigate whether it is practical to carry out the process on a technical scale while maintaining an acceptable efficiency of the ion exchange resin and a bacteriologically satisfactory quality of the treated milk.

We thank Mr. Mogens Simensen for carrying out the injections of the cows.

This work was initiated in 1961 by the Food and Nutrition Division of the National Health Service.

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CHEMISTRY

Ultramicrodetermination of Chemical Structure of Organic Compounds by Gas Chromatography

A new method has been devised for rapidly identifying in µg amounts the carbon skeleton and other structural features of a wide variety of compounds.

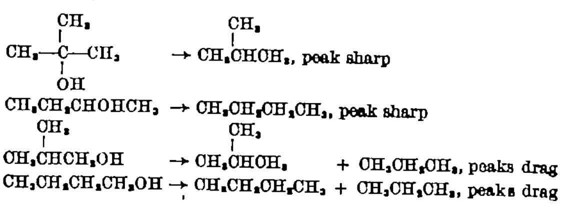
The procedure is the same as that used in gas chromatography except that a hot catalyst-containing tube is interposed between the injection port and the column of a flame ionization chromatograph which utilizes hydrogen as the carrier gas. Hydrogen, sweeping the injected sample past a hot palladium or platinum catalyst, saturates multiple bonds and replaces halogen, oxygen, sulphur, and nitrogen atoms in the compound. The resulting hydrocarbon passes into the chromatographic column and is identified by its elution time. The main product is either the parent hydrocarbon or the next lower homologue. In the latter case, for example, with aldehydes and acids, the parent compound may appear in a small amount and this serves as a further aid in identification. Types of compounds analysed and main products formed are listed in Table 1.

Table 1. Hydrocarbons obtained from Different Compounds

Type	Formula	Main product(s)
Paraffin hydrocarbons	$RCH_2CH_2CH_3$	$RCH_2CH_2CH_3$
Unsaturates	$RCH{=}CHR'$	RCH_2CH_2R'
Halides	RX, $R'X_2$, etc.	RH, $R'H_2$
Sulphides	RSH, $R'SSR'$, $\overline{R''S}$	RH, $R'H$, $R''H_2$
Esters	$RCH_2O\overset{\overset{\displaystyle O}{\parallel}}{C}R'$	RCH_3, $[R'H]$*
Epoxides	$\underset{\displaystyle O}{\underset{\diagdown\ \diagup}{RCH{-}CH_2}}$	RCH_2CH_3
Aldehydes	$RCHO$	RH
Alcohols, tertiary	$RR'R''COH$	$RR'R''CH$
secondary	$RR'CHOH$	$RR'CH_2$
primary	RCH_2OH	RH, RCH_3
Ketones	$R\overset{\overset{\displaystyle O}{\parallel}}{C}R'$	RCH_2R'
Ethers	RCH_2OCH_2R'	RH, $R'H$, RCH_3, $R'CH_3$
Acids	$RCOOH$	RH*
Anhydrides	$(RCO)_2O$	RH*
Amines	$RR'R''N$	RH, $R'H$, $R''H$
Amides	$RCONR'R''$	RCH_3, RH, $R'H$, $R''H$

* Obtainable with freshly reduced 1 per cent palladium on 'Gas Chrom P' at 260° or 5 per cent platinum on porous glass at 210°.

The shape of the elution pattern can be used to determine the position of substitution of a functional group. For example, it has been shown that secondary and tertiary alcohols give sharp peaks of the parent hydrocarbon; primary alcohols give 'dragging' peaks corresponding to the parent hydrocarbon and the next lower homologue. Thus the four butyl alcohols may be readily distinguished. Their patterns are shown in Fig. 1. The peaks from secondary or tertiary butyl alcohol are sharp, but the former appears at the retention time of butane, the latter at that of isobutane. Isobutyl alcohol gives two peaks which drag and appear at the retention times of isobutane and propane. Normal butyl alcohol also gives two dragging peaks which appear at the retention time of butane and propane. These reactions are summarized as follows:



$$CH_3{-}\overset{\overset{\displaystyle CH_3}{|}}{\underset{\underset{\displaystyle OH}{|}}{C}}{-}CH_3 \rightarrow CH_3\overset{\overset{\displaystyle CH_3}{|}}{C}HCH_3\text{, peak sharp}$$

$$CH_3CH_2CHOHCH_3 \rightarrow CH_3CH_2CH_2CH_3\text{, peak sharp}$$

$$CH_3\overset{\overset{\displaystyle CH_3}{|}}{C}HCH_2OH \rightarrow CH_3\overset{\overset{\displaystyle CH_3}{|}}{C}HCH_3 + CH_3CH_2CH_3\text{, peaks drag}$$

$$CH_3CH_2CH_2CH_2OH \rightarrow CH_3CH_2CH_2CH_3 + CH_3CH_2CH_3\text{, peaks drag}$$

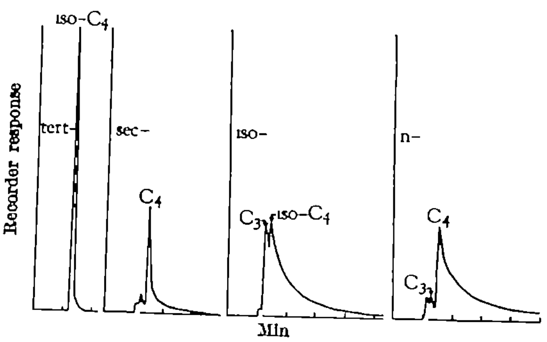


Fig. 1. Elution patterns of butyl alcohols

In general polar compounds react more slowly than non-polar ones and may give peaks that drag or 'tail'. Excessive dragging is associated with delayed retention times. By raising the temperature, dragging is reduced and the retention time of the main product more closely matches that of the expected hydrocarbon. Compounds giving hydrocarbons having as many as 9 connected carbon atoms have been analysed by this technique.

The present procedure is an outgrowth of the work of Thompson *et al.*[1,2], who used a special apparatus consisting of an aluminium reaction tube, a furnace for maintaining the tube at a desired temperature, a motor-driven syringe for injecting compounds at 1 µl./min, and a U-tube cooled in liquid air or dry ice. The hydrocarbon trapped in the U-tube is washed from the trap with solvent and analysed by gas-liquid chromatography.

The new apparatus is much simpler. It requires no cold traps or constant-drive syringe, requires practically no skill to operate, and is extremely sensitive, giving a good reading with amounts of 1–20 µg. No transfers are necessary nor is any special equipment needed to handle lower hydrocarbons. Analysis time is rapid, depending only on the retention times of the products.

The catalyst-containing aluminium tube (Fig. 2, *D*) is 9·5 in. long with an outside diameter of 0·5 in. and an inside diameter of 1/8–3/16 in. It is screwed on the injection port of the gas chromatograph by means of fitting *F*, which is a bolt, nut and stainless-steel tube silver soldered together as shown. A 'T' fitting holds the injection port assembly (*C*) and the hydrogen gas inlet (*B*). The temperature of the tube is regulated by means of a heating jacket (*E*) made by winding nickel-chromium resistance wire over asbestos tape on a glass tube, the heat output being adjusted by a variable transformer. The temperature is determined by a thermocouple embedded in tube *D*. *A* is magnesia pipe insulation. All gas connexions must be tight. A 15-ft. 3/16-in. (outer diam.) copper column containing 5 per cent squalane on 'Chromosorb *W*' (Johns Manville, New York) and operating at room temperature with a flow-rate of 20 ml./min is

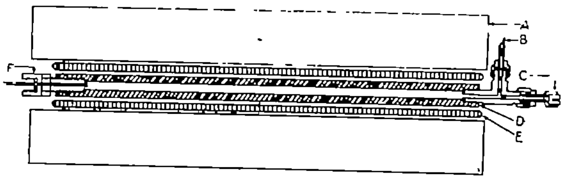


Fig. 2. Catalyst assembly. *A*, Insulation; *B*, hydrogen inlet, *C*, injection port; *D*, aluminium tube; *E*, heating jacket; *F*, fitting that attaches catalyst assembly to gas-chromatograph injection port

suitable for identifying almost all the C_1–C_7 hydrocarbons. Mixtures of known composition are injected periodically to establish appropriate retention times.

The catalysts were 1 and 5 per cent platinum or palladium on 60- to 80-mesh porous glass (No. 7930 Corning Glass Works, Corning, New York) or 1 per cent palladium on 'Gas Chrom *P*' (Applied Science Laboratories, State College, Pennsylvania). Palladium catalysts were usually maintained at 150°–260° C, the platinum catalyst at 120°–240°. The more active platinum catalyst, which is necessary for the analysis of amines and amides, may be used for compounds with carbon moieties up to six.

The method has wide applicability for determining structure of organic compounds yielding hydrocarbons with as many as 9 connected carbon atoms, especially those available in minute amounts, such as are isolated from natural products, degradation fragments, and gas chromatographic fractions.

I thank Rafael Sarmiento for assistance. Mention of proprietary products does not necessarily imply endorsement by the U.S. Department of Agriculture. The apparatus is being patented.

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Pyrolysis and Gas Chromatography of Benzene Clathrate

IN 1897, Hofmann and Kuspert[1] published a paper in which they described that when benzene is added to a solution of nickel cyanide in aqueous ammonia and containing acetic acid, a precipitate of the composition $[Ni(CN)_2.NH_3.C_6H_6]$ is formed. This compound, now known as benzene clathrate, was obtained with a slight change, using no glacial acetic acid, in the method described in ref. 2. The clathrate (referred to here as *VM*) on analysis gave: *C* = 45·86 per cent, H = 4·50 per cent, N = 20·86 per cent, and Hofmann and Küspert found: C = 45·07 per cent, H = 4·20 per cent, N = 20·37 per cent. The calculated analysis is: C = 46·60 per cent, H = 4·37 per cent, N = 20·39 per cent.

A small amount of *VM* was weighed into a capillary glass tube. The tube containing *VM* was connected to the platinum spiral shown in Fig. 1. A measured amount (2 μl.) of pentane was then pipetted into the glass bulb which was then immediately closed with a serum-cap. The spiral was heated by passing an electric current through it for a definite time. After 2 sec the platinum reached a dull red heat.

0·5–1·0 ml. of the vapour was taken from the bulb through the serum cap and injected into the gas-chromatograph. The added pentane and the decomposition products from *VM* were separated on to a column of 'Apiezon *L*' (20 per cent) and the eluted compounds detected by means of a hydrogen flame ionization detector. The pentane was added as an internal standard, so that quantitative determinations could be made independent of the accuracy with which the vapour sample was taken from the bulb.

The following blank experiments were carried out:
(1) 2 μl. pentane were pipetted into the bulb and after closing the spiral was heated for about 20 sec. 2 ml. of the vapour were injected into the gas-chromatograph. The chromatogram showed pentane was not decomposed in any significant amount under the conditions used. In particular no synthesis to benzene had taken place.

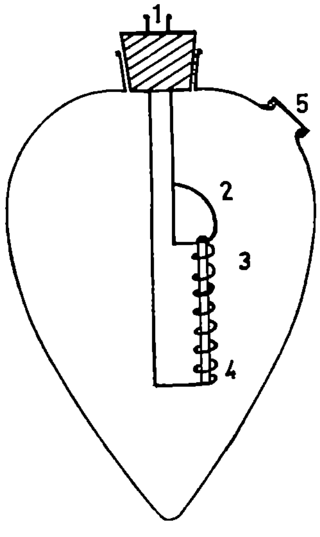

Fig. 1. Pyrolysis unit. 1, to heavy duty accumulator; 2, stainless steel wire to hold capillary in position; 3, glass capillary tube containing clathrate; 4, platinum spiral; 5, inlet closed with serum-cap

(2) 2 μl. of benzene were pipetted into the bulb and the spiral heated as in experiment (1). The final chromatograms again did not show any decomposition product from benzene.

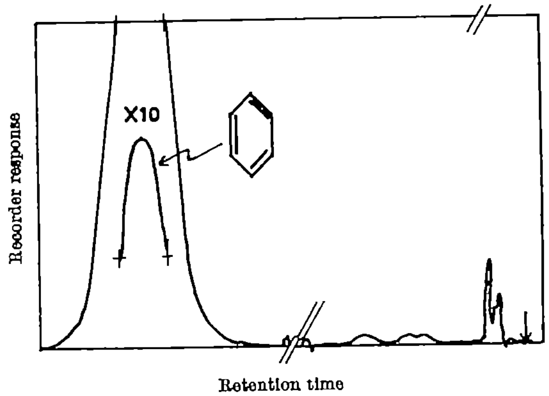

Fig. 2*a*. Chromatogram of pyrolysis products of clathrate

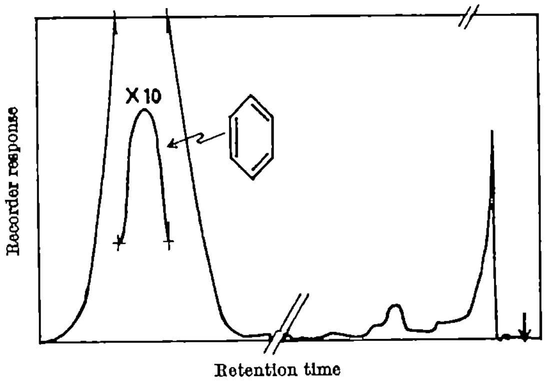

Fig. 2*b*. Chromatogram of benzene under the same conditions as Fig. 2*a*

For the qualitative experiments, 4·0 mg of *VM* were weighed in a glass capillary tube, mounted into position in the glass bulb (Fig. 1) and the spiral heated (10 sec). One ml. of the vapour was taken from the bulb and injected into the gas-chromatograph. The resulting chromatogram is shown in Fig. 2*a*. For comparison the chromatograms obtained by directly injecting a mixture of pentane and benzene in the gas-chromatographic column is given in Fig. 2*b*. The clathrate *VM* thus showed to give a peak with the correct retention time for benzene.

A characteristic property of benzene is that it is not pyrolysed under the conditions described by Dhont[3]. So before and after passing the vapour through the pyrolysis tube attached to the top of the chromatographic column, the retention time of the peak should be the same. This proved to be the case (Table 1).

Table 1. RETENTION TIME BEFORE AND AFTER PASSING THE PYROLYSIS TUBE FOR THE EXPECTED BENZENE PEAK

Retention time of benzene	2·14
Retention time of peak before pyrolysis	2 12
Retention time of peak after pyrolysis	2·16

The quantitative experiments were carried out as follows:

(*A*) Mixtures of known composition were prepared from benzene and pentane. From these, 2–5 μl. were pipetted into a glass bulb closed with a serum cap and allowed to evaporate completely.

0·5–2·0 ml. of the vapour was injected into the gas-chromatograph and the chromatogram recorded. The heights of the benzene and of the pentane peak were measured and the quotient:

$$Q = \frac{\text{height of the benzene peak}}{\text{height of the pentane peak}}$$

calculated.

Log *Q* was taken against the concentration of benzene per ml. of vapour and the graph in Fig. 3 constructed.

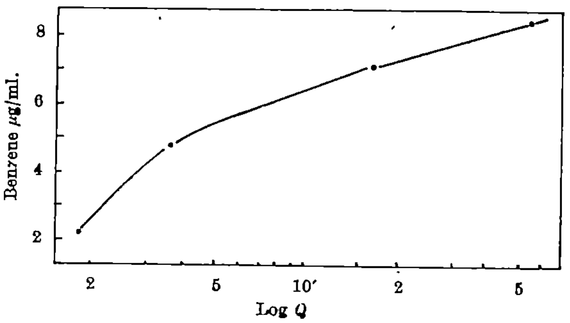

Fig. 3. Calibration graph

(*B*) For the quantitative measurements on *VM*, in the first series of experiments, a weighed amount of it was heated as described. The heating time was varied from 6·5 to 24·2 sec. From the chromatogram made from the resulting vapour it was clearly seen that the heating time had no influence on the amount of benzene liberated from *VM*. A number of experiments was then carried out using different amounts of *VM*. The results are shown in Table 2.

The figure in column 5 of Table 2 was calculated as follows: volume of the bulb: 166 ml., for 6·5 mg *VM* the amount of benzene expected is 78·1/205·9 × 6·5 = 2,470 mg.

Table 2

Amount of *VM* taken (mg)	Heating time (sec)	*Q* from chromatogram	Amount of benzene/ml. found from graph	Amount of benzene/ml. calculated from molecular formula and weight of sample*	Percentage of benzene found
3·1	12·6	0·85	4·7	7·2	51
5·4	14·3	0·81	6·1	12·5	49
8·8	11·5	5·10	8·2	19·4	42·5
6·5	10 0	3·30	7·8	14 9	52

* Vol. of the bulb used, always 166 ml.

Since the calibration graph has been constructed for the amount of benzene/ml., 2,470 was divided by the bulb volume.

2,470/166 = 14·9 μg; this is the figure recorded in Table 2.

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Acid-Base Equilibria in Dilute Aqueous Alcohols containing Mineral Acids

ALTHOUGH the early measurements of acid-base equilibria in dilute solutions of water in alcohols made by Goldschmidt *et al.*[1,2] showed that water is more basic than an alcohol in these conditions, the variation of acidity function with ethanol–water composition[3] showed that differences must exist between solutions of water in ethanol and solutions of ethanol in water. This has been supported recently by a kinetic investigation[4] of the retardation of oxidation in dilute acidic solutions of alcohols and ethers in water. The retardation is caused by the formation of an unreactive oxonium ion, with the alcohol or ether more basic than water, for example, for

$$\mathrm{H_3^+O} + R\mathrm{OH} \overset{K_b}{\rightleftharpoons} \mathrm{H_2O} + R\overset{+}{\mathrm{O}}\mathrm{H_2} \qquad (1)$$

$K_b' = [R\overset{+}{\mathrm{O}}\mathrm{H_2}]/[R\mathrm{OH}][\mathrm{H_3^+O}]$ = 2·2 at 0° C and 1·7 at 25° C for isopropanol in dilute sulphuric acid.

The spectrophotometric determination of acidity function by Braude and Stern[3] in ethanol–water mixtures was restricted to two concentrations of hydrochloric acid. A preliminary account is reported here of spectrophotometric measurements with *p*-nitroaniline in dilute solutions of isopropanol and methanol in water over a range of concentrations of hydrochloric, nitric, perchloric and sulphuric acids. In these conditions, if N and NH represent unprotonated and protonated *p*-nitroaniline respectively, and if the following equilibria are assumed to exist in addition to (1):

$$\mathrm{H_3^+O} + \mathrm{N} \overset{K_1}{\rightleftharpoons} \mathrm{NH} + \mathrm{H_2O} \qquad (2)$$

$$R\overset{+}{\mathrm{O}}\mathrm{H_2} + \mathrm{N} \overset{K_2}{\rightleftharpoons} \mathrm{NH} + \mathrm{ROH} \qquad (3)$$

where $K_b = [R\overset{+}{\mathrm{O}}\mathrm{H_2}][\mathrm{H_2O}]f_{R\overset{+}{\mathrm{O}}\mathrm{H_2}}\, f_{\mathrm{H_2O}}/[\mathrm{H_3^+O}][R\mathrm{OH}]$-$f_{\mathrm{H_3^+O}}\, f_{R\mathrm{OH}}$, $K_1 = [\mathrm{NH}][\mathrm{H_2O}]f_{\mathrm{NH}}\, f_{\mathrm{H_2O}}/[\mathrm{N}][\mathrm{H_3^+O}]f_{\mathrm{N}}$ $f_{\mathrm{H_3^+O}}$, $K_2 = [\mathrm{NH}][R\mathrm{OH}]f_{\mathrm{NH}}\, f_{R\mathrm{OH}}/[\mathrm{N}][R\overset{+}{\mathrm{O}}\mathrm{H_2}]f_{\mathrm{N}}\, f_{R\overset{+}{\mathrm{O}}\mathrm{H_2}}$ and *f* represents activity coefficient, it can be shown that:

$$\frac{cc_R}{c_R-c} = \frac{K_2F_2w}{K_1F_1r} \cdot \frac{c_0c_R}{c_0-c_R} + \frac{wc_0}{K_1F_1r}, \qquad (4)$$

where c_0, c_R and c represent the concentration of unprotonated *p*-nitroaniline determined spectrophotometrically in aqueous solution without added acid and alcohol, with added acid and alcohol and with added acid only, respectively: w = $[H_2O]$ before addition of alcohol, r = the total added alcohol concentration, $F_2 = f_N f_{R\overset{+}{O}H_2}/f_{NH} f_{ROH}$ and $F_1 = f_N f_{H_3^+O}/f_{NH} f_{H_2O}$. In dilute solutions of alcohol a plot of $c\, c_R/(c_R-c)$ against $c_R/(c_R-c)$ should give a straight line. Such plots are found to be linear, and examples obtained at an ionic strength $\mu = 1\cdot0$ are given in Fig. 1. For these plots, $K_b' = c_0w/rw_R \times$ slope, where w_R = $[H_2O]$ with the alcohol added. Table 1 shows that, except for sulphuric acid, reasonable agreement is obtained among the acids for a particular concentration of alcohol. Table 2 shows that little variation is observed among the acids in the range 2–10 per cent v/v isopropanol, except for sulphuric acid. Only a slight variation of K_b' with ionic strength has been observed: ionic strength was adjusted by adding the sodium salt of the acid (sodium sulphate with sulphuric acid).

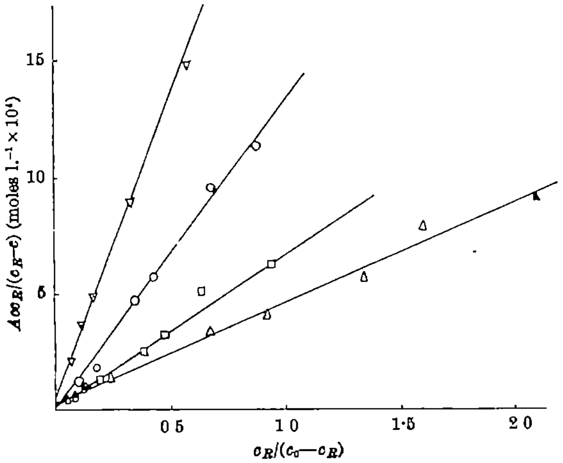

Fig. 1. Plots of $Acc_R/(c_R-c)$ against $c_R/(c_0-c_R)$ at $\mu = 1\cdot0$ with 5 per cent *v/v* alcohol. ○, Methanol + HCl, $A = 1\cdot0$; ⊡, isopropanol + HCl, $A = 1\ 0$; △, isopropanol + H_2SO_4, $A = 1\ 0$; ▽, isopropanol + $HClO_4$, $A = 0\cdot2$

Although, in these conditions, isopropanol and methanol are more basic than water, it is apparent that solutions of sulphuric acid behave in a different way to the other acids quoted in Tables 1 and 2. Moreover, values for K_b' for all the acids in Tables 1 and 2 differ from those found in sulphuric acid in the oxidation experiments[4]. However, K_b' can be calculated by a less direct method. Using equation (4), values of K_2F_2 can be calculated from the slopes and intercepts of lines similar to those in Fig. 1: from equilibria (1) and (3) values of K_b' can then be calculated. For all the acids and all the concentrations of isopropanol and methanol used in these dilute solutions, the apparent value of K_b' calculated in this way is independent of acidity, except for sulphuric acid, where K_b' increases with increasing acidity. This is illustrated in Table 3 for hydrochloric and sulphuric acids with 5 per cent v/v isopropanol. The values of K_b' in solutions of hydrochloric, nitric and perchloric acids agree with those determined directly from the slopes of Fig. 1, and the values of K_b' in sulphuric acid solution tend at low acidities to the values obtained directly from the slopes. One explanation of this is that F_2 and F_1 vary with HSO_4^- and SO_4^{2-} ions, but not with NO_3^-, ClO_4^- and Cl^- ions: this variation would cancel out for the determination of K_b' direct from the slopes in Fig. 1, allowing straight lines with sulphuric acid as with the other acids. This would account for the differences of the values of K_b' in sulphuric acid solutions from those in solutions of hydrochloric, nitric and perchloric acids in Tables 1 and 2. Moreover, the values of K_b' in sulphuric acid solution in Table 3 are comparable in the same region of $[H_2SO_4]$ with $K_b' = 1\cdot7$ at 25° C for isopropanol using picric acid and the rate of oxidation[4].

Table 1. K_b' FOR 5 PER CENT v/v ALCOHOL AT $\mu = 1\cdot0$ AND 25° C

Acid	Isopropanol	Methanol
H_2SO_4	0·54	0·18
HCl	0·36	0 093
$HClO_4$	0·45	0·10
HNO_3	0 29	—

Table 2. K_b' AT $\mu = 1\cdot0$ AND 25° C

per cent v/v isopropanol	HCl	$HClO_4$	HNO_3	H_2SO_4
2	0·21	0 46	—	0·42
5	0·36	0·45	0·29	0·54
10	0·37	0·38	0·34	0·67

Table 3. APPARENT K_b' IN 5 PER CENT v/v ISOPROPANOL AT $\mu = 1\ 0$ AND 25° C

[Acid]M	H_2SO_4	HCl
0·08	0 53	0 35
0 12	0·56	0·35
0 16	0 62	0 35
0 20	0·71	0·34
0 40	1·13	0·35
0 60	1·46	0 35
0·80	1 67	—
1 00	1·86	0·33

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Detection of Diols on Paper Chromatograms

BORIC acid forms complexes with compounds having adjacent OH groups in the *cis* position. The *p*K′ values of the complexes are lower than that of free boric acid, so that in the presence of diols it behaves as a stronger acid. This property has been used to detect *cis* diols on paper chromatograms. Spraying the dried paper with a 0·1 per cent solution of bromcresol purple in 0·5 per cent sodium carbonate solution which has been adjusted to *p*H 6·8 with hydrochloric acid reveals all acidic substances as yellow spots on a purple background.

These spots are marked and the wet paper is lightly sprayed with near-saturated borax solution adjusted to *p*H 6·9 with hydrochloric acid. Any freshly appearing yellow spots are due to *cis* diols. This method, in its rapidity and non-destructive effect on the compounds present, has some advantages over the more sensitive but cumbersome method of revealing diols devised by Baddiley *et al.*[1]. Preliminary work has shown that diols may be titrated in the presence of borax in an analogous manner to that in which formol titrations are performed.

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Heat Treatment of Carbon under Various Pressures

It has been said that the degree of graphitization of carbon depends chiefly on the highest temperature of heat treatment and that other conditions of heat treatment, such as holding time, are of secondary importance. However, it was previously reported[1] that the vacuum-evaporated carbon was scarcely graphitized in vacuum, although it was easily graphitized under atmospheric pressure. Therefore, we thought it necessary to study the effect of the gas phase of heat treatment on the graphitization of carbon. In order to investigate this effect, a calcined petroleum coke was heat-treated under various pressures and its lattice constant c_0 was measured by means of X-ray diffraction method using extra-pure silicon powder as an inner standard.

The change of lattice constant c_0 with the temperature of heat treatment is represented in Fig. 1.

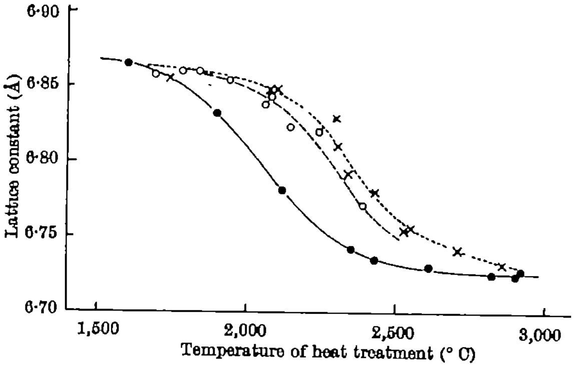

Fig. 1. Change of lattice constant, c_0, with temperature of heat treatment. ●, Heat treatment under atmospheric pressure; ○, under reduced pressure; ×, in vacuum

Heat treatment under atmospheric pressure was made in a carbon granular resistance furnace of a type similar to an industrial graphitizing furnace. The details of this heat treatment were reported elsewhere[2]. Heat treatment in vacuum of 10^{-3} mm mercury was made in a vacuum induction furnace. Heat treatment under reduced pressure (7 ~ 8 mm mercury) was made in a bell-jar type metallic chamber. In each case, the temperature of heat treatment was directly measured with the aid of an optical pyrometer.

The results of this work indicate that the relation between the degree of graphitization of carbon and the temperature of heat treatment is strongly affected by the gas phase of heat treatment. In this work, it was scarcely necessary to consider the catalytic action of metallic impurities, because the high-purity graphite (reactor grade) was used as a crucible. According to Mrozowski[3], powdered coke was less graphitized than unpowdered. Therefore, the particle size distribution of the coke sample was controlled to the range of 1·4 ~ 0·2 mm in order to eliminate the possible effect of particle size on the graphitization. Holding time at the highest temperature was about 15 min in each case. Heating-rate was about 200° C/min in the heat treatment both under atmospheric pressure and in vacuum, and about 2,000° C/min in reduced pressure. But it was already reported that heating-rate has no effect on lattice constants of heat-treated carbon[4]. Accordingly, it was not the difference of heating-rate that caused the difference in the behaviour of lattice constant shown in Fig. 1.

The degree of graphitization of carbon therefore depends not only on the highest temperature but also on the gas phase of heat treatment, although it is not decisive whether the total pressure of gas phase or the partial pressure of any kind of existing gas, such as nitrogen or oxygen, has any effects on the graphitization.

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Fractionation of Partially Isotactic Polypropylene Oxide

We have polymerized propylene oxide in dioxan with the zinc diethyl and water catalyst[1], dissolved the resulting high-molecular-weight polymer in iso-octane at 60° C and precipitated solid phases by lowering the temperature incrementally to 40° C. In this way we were able to obtain several fractions which were identical in average molecular weight and in the distribution of molecular weights about the average. By further lowering of the temperature we obtained liquid–liquid phase separation with fractionation occurring predominantly on the basis of molecular weight. A typical result of such a fractionation is shown in Fig. 1. Further analysis of that part of the polymer undergoing liquid–liquid phase separation (some 80 wt. – %), coupled with cryoscopic measurements on the fraction of lowest molecular weight, indicates that the ratio of the weight- to the number-average molecular weight for the polymer is greater than 1,000, the consequence of a large low molecular weight tail.

Other fractions produced in this way via solid-liquid phase separation from iso-octane have been investigated in more detail. Table 1 records, for

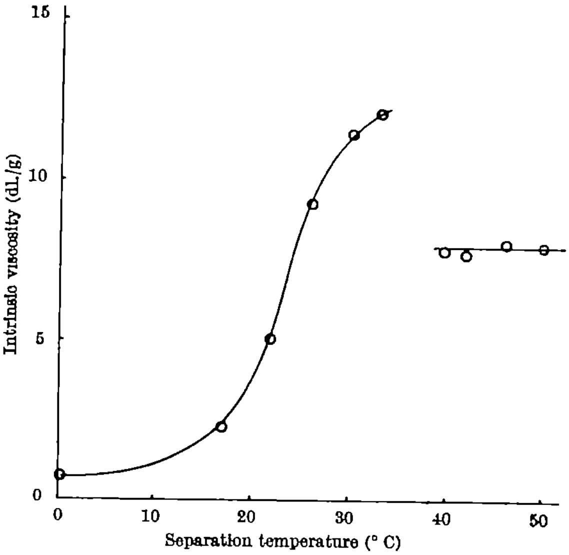

Fig. 1. The intrinsic viscosities (benzene at 25° C.) of fractions of polypropylene oxide versus their temperature of separation from iso-octane

Table 1

Fraction	Mol. wt. $\times 10^{-6}$	$A_2 \times 10^{-4}$ cm^3 g^{-2} mole	Melting point (° C)	Precipitation point (° C)	
				Iso-octane	Hexane
1	1·34	−1·75	—	55	—
3	1·18	0·31	68	50	38
5	1·37	0·45	68	46	34
7	1·13	1·43	62	42	29
8	1 00	2·31	56	39	24
Residue*	1·13	4·01	48	—	—

* Less the fraction of lowest molecular weight.

several fractions, the weight-average molecular weight and the second virial coefficient (A_2) measured by the light scattering method in hexane at 46° C, together with the precipitation temperature from iso-octane and hexane and the melting points determined on the hot stage of a polarizing microscope. These results show that we have separated from the polymer a succession of fractions differing only in their ability to crystallize under given conditions. The decreasing tendency of the later fractions to crystallize is presumably due to the increasing number of syndiotactic, and perhaps head to head, placements in an otherwise isotactic[1] polymer. For convenience, we refer to such polymers, which are neither tactic nor atactic as commonly defined[3], as partially tactic and to particular fractions as differing in degree of tacticity. We believe that polypropylene oxide made by our catalyst consists of a wide distribution of molecular weights superimposed on which (and independent of molecular weight provided it is not very low) is a large variation in the degree of isotacticity.

An interesting feature of Table 1 is the marked variation of the second virial coefficient from fraction to fraction when it is measured in hexane at 46° C. When A_2 was measured in a good solvent (toluene at 25° C) no large variation was detected[4]. Measurement of A_2 in a poor solvent would appear to be a convenient qualitative indicator of the degree of tacticity of polymer fractions of the type discussed here.

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Chemical Differences between Antibody Fragments as shown by Paired Label Studies

Porter[1] has shown that antibodies can be digested with papain to yield univalent fragments, molecular weight about 50,000, and an inactive fragment, molecular weight 80,000. The univalent fragments obtained are separable by chromatography on carboxymethylcellulose (CMC) into two fractions called Fraction I and Fraction II. Since then, Stelos *et al.*[2] have shown that the fragments in each fraction come from different antibody molecules (see also Palmer *et al.*[3]). We have also been able to show that fragments of Fraction I and fragments of Fraction II can be further fractionated and that the fragments in the various fractions appear to be different on a chemical basis also[2]. We showed that in the case of specifically purified antibody against the azobenzoate ion and azobenzenearsonate ion the rates of iodination of the fragments appearing in Fraction I are different from those appearing in Fraction II. This was demonstrated by iodinating the mixture of fragments and then chromatographing it. It was found that the iodine–protein ratio was different for the two fractions. On the other hand, the rates of iodination of the fragments of Fraction I and of Fraction II from normal γ-globulin were usually quite similar. The results of this experiment indicate that not only were there chemical differences between the fragments in Fraction I and in Fraction II from a specifically purified antibody, but also that these molecules differed from normal γ-globulin.

The communication describes experiments with a paired label technique in which fragments of one fraction were iodinated with iodine-125 and from another iodinated to the same level with iodine containing iodine-131 (ref. 4). The iodinated preparations were then mixed, digested with pepsin, and the resulting peptides separated by chromatography and high-voltage paper electrophoresis.

The ratio of ^{125}I/^{131}I was determined for each peptide spot. If the two preparations so treated yield a constant ^{125}I/^{131}I ratio for all spots then the proteins are identical by this test. However, if different ratios appear then the proteins are not identical. This procedure was used to compare the fragments of Fraction I and Fraction II*B* of specifically purified anti-X_p antibody and of the globulin fraction of anti-X_p antiserum.

Previously described methods were used for the preparation of rabbit anti-*p*-azobenzoate antisera[5], rabbit γ-globulin[6] and specifically purified anti-*p*-azobenzoate (anti-X_p) antibody[4].

Whole γ-globulin prepared from anti-X_p antiserum and specifically purified antibody from the same pool of antiserum were digested with papain[1] and the digests chromatographed on a 'CMC' column using a gradient previously described[7].

Proteins were iodinated with hypoiodite which was labelled with either iodine-131 or with iodine-125 to the extent of 25–50 μc. per atom iodine as previously described[4].

Mixtures of iodine-125 and iodine-131 labelled proteins were digested with pepsin in 0·5 M formic acid (10 mg protein per ml., protein : pepsin ratio. 30 : 1) for 14–18 h at 37° C. A portion of the digest. containing 0·5–1 mg protein, was subjected to descending chromatography (butanol : acetic acid : water. 4 : 1 : 5) on Whatman 3*MM* paper[8] and then to high-voltage paper electrophoresis at 22°–28° C using 4 per cent HCOOH as electrolyte (High-Voltage Electrophorator, Model *D*, Gilson Medical Electronics. Middleton, Wisconsin).

Autoradiographs were prepared on Kodak Industrial X-ray film, type *KK*. Areas on the chromatogram corresponding to the spots on the film were cut out and counted in an automatic recording dual channel gamma ray spectrometer (Packard Inst. Co.). The ^{125}I/^{131}I ratio for each spot was then calculated.

Fraction I fragments of specifically purified anti-X_p antibody were iodinated to the extent of 8·5 iodine atoms per fragment (mol. wt. 50,000) with iodine-125-labelled iodine and Fraction II*B* fragments[7] to the extent of 8·6 iodine atoms per fragment with iodine-

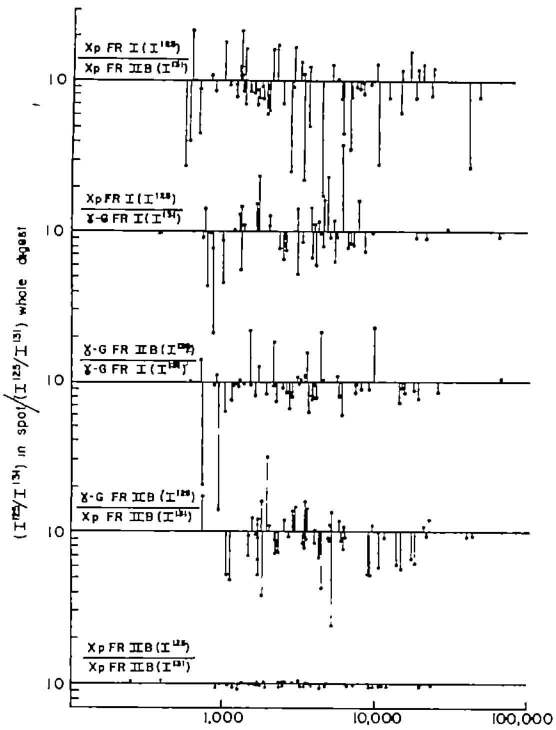

Fig. 1. Radioactivity present in iodine-containing peptides from peptic digests of paired labelled proteins, Fractions I and IIB of the γ-globulin fraction of anti-X_p antiserum and of the specifically purified antibody. The ratio of ^{125}I/^{131}I is shown for each peptide obtained from the proteins indicated (ratios were normalized by dividing by the ^{125}I/^{131}I ratio of the whole digest). The values are arranged in the order of increasing iodine-131 counts. Both axes are logarithmic. The bottom figure shows that when a single protein is carried through this procedure, no variation in ratios is observed

131-labelled iodine. Fragments prepared from whole γ-globulin of the same pool of anti-X_p antiserum were labelled similarly, that is, Fraction I, 8·9 iodine atoms per fragment, iodine-131-labelled; Fraction II*B*, 8·3 iodine atoms per fragment, iodine-125-labelled.

Five different pairs were mixed, digested and analysed for the ^{125}I/^{131}I ratio, namely:

(*a*) X_p Fraction I (^{125}I) and X_p Fraction II*B* (^{131}I),
(*b*) X_p Fraction I (^{125}I) and γ-*G* Fraction I (^{131}I),
(*c*) γ-*G* Fraction II*B* (^{125}I) and γ-*G* Fraction I (^{131}I),
(*d*) γ-*G* Fraction II*B* (^{125}I) and X_p Fraction II*B* (^{131}I), and
(*e*) X_p Fraction II*B* (^{125}I) and X_p Fraction II*B* (^{131}I).

The results are shown in Fig. 1 in the above order.

Control experiments (*e*) showed that when two portions of the same protein are carried through this procedure, the ratios for all derived peptides are the same. In all other cases the amounts of many iodinated peptides derived from each of the paired labelled fragments varied markedly. Thus the peptides derived from Fraction I of the specifically purified antibody differed from those of Fraction II*B*; those from Fraction I of the antibody were different from those from gamma-globulin Fraction I; those from Fraction II*B* of specifically purified antibody were different from those of II*B* of γ-globulin; and those of Fraction I of γ-globulin were different from those of Fraction II*B*.

The differences in amounts of each iodinated peptide could be due to several factors: (*a*) differences in amino-acid sequence resulting in different iodinated peptides, or (*b*) difference in amino-acid sequence adjacent to the derived peptide which affects the yield of that particular peptide during digestions, or (*c*) no difference in sequence but a difference in the rate of iodination of the tyrosine in the same sequence due to differences in availability of the tyrosine through folding (that is, secondary or tertiary structure), or (*d*) a combination of the former.

In no case, however, was there a spot observed which contained only one or the other isotope as might be the case if the proteins had different primary structures so that a particular iodinated peptide could be derived from one protein only. However, even if a unique peptide were isolated from one protein of the mixture this would not necessarily be due to a difference in primary structure, but might still reflect only a difference in folding.

In the work reported here we have shown definite chemical differences between fragments of Fractions I and II*B* of whole rabbit γ-globulin and from antibody specifically purified therefrom.

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β-D-1,6-Glucanase in Micro-organism

In the course of work on metabolic products of newly isolated moulds from soil, *Penicillium aculeatum* Raper and Fennell var. *apiculatum* Abe strain No. 2282 was found to produce a luteic acid-like substance of high viscosity[1]. The substance consisted of glucose polymerized by glucosidic linkage and malonic acid. As a result of periodate oxidation, almost all the glucosidic linkages of polysaccharide were of the β-1,6 type. Since the molecular weight of the polysaccharide calculated from a result of ultracentrifugal method (1·6 × 10^6) was greater than that of luteose, which was first reported by H. Raistrick and M. L. Rintoul[2], and consisted of 84 glucose units[3], it was found that the polysaccharide preparation is lutease of higher molecular weight.

An organism producing β-D-1,6-glucanase was isolated from dust, using medium containing luteose as a sole carbon source. The taxonomic position of this isolated organism is as yet uncertain.

The organism was grown in Czapek medium containing peptone and luteose as a sole carbon source at 30° C in the reciprocal shaker.

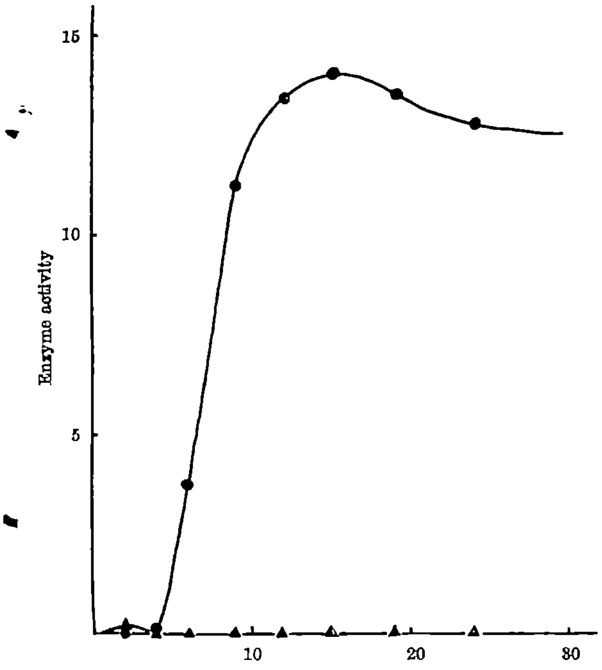

Fig. 1. Formation of luteose-degrading enzyme in a shaking culture. ●, Luteose added; ▲, glucose added. The organism was grown in Czapek medium containing 0·5 per cent peptone and 0 5 per cent glucose as a sole carbon source, and collected mycelia were washed with distilled water, and then incubated in *M*/30 phosphate buffer (*p*H 6·7) containing 50 mg of luteose and glucose (per medium 50 ml.) respectively. In mycelia, the low level of activity was observed even after incubation When the mycelia were incubated in phosphate buffer alone, no enzyme activity was observed

The β-D-1,6-glucanase activity was assayed as follows. The reaction system was composed of 10 ml. 0·2 per cent luteose, 5 ml. M/15 phosphate buffer (*p*H 6·7), 5 ml. enzyme solution. The system was incubated at 40° C for 1 h, and the reducing sugar produced was determined by a modification of Somogyi's method[4]. Enzymatic activity was indicated by the required volume ml. of N/20 sodium thiosulphate. If N/20 sodium thiosulphate was required up to more than 6 ml., the enzyme solution was diluted, and a dilution ratio was multiplied to the required volume of N/20 sodium thiosulphate.

The optimum *p*H for the reaction of this enzyme was about 6·7, and higher activity was observed at 40° C than at 30° C or 50° C in 1 h of incubation. Glucose, gentiobiose and oligosaccharides of β-1,6 linkage were detected at a beginning of the enzymatic hydrolysis of luteose by paper partition chromatography, and later only glucose and gentiobiose were detected. From this result it is considered that the degradation of luteose by this enzyme is random, similar to acid hydrolysis. The maximum degree of degradation of luteose by the enzyme was about 55 per cent as glucose. At the maximum of hydrolysis of luteose, some partial hydrolysis product may remain.

When the washed mycelia were incubated aerobically with phosphate buffer containing luteose, a large amount of the enzyme was formed, but scarcely formed in phosphate buffer plus glucose, as shown in Fig. 1. In the case of phosphate buffer alone the enzyme was not formed. This result suggested that the enzyme formation was induced with luteose, unlike the enzyme reported by E. T. Reese *et al.*[5].

Although starch and dextran are not degraded by the enzyme, carboxy methyl cellulose and laminarin are a little degraded. We propose to apply the name 'lutease' to this enzyme.

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6-O-Acetyl o-Nitrophenyl β-D-Galactoside

o-Nitrophenyl β-D-galactoside (ONPG), which was introduced by Lederberg[1] as a chromogenic substrate for β-galactosidases, has found widespread application in enzyme investigations. We have recently reported both a method for preparing partially acetylated glucose derivatives[2] and the action of esterases[3] on these compounds The synthesis was adapted to a galactose derivative—6-*O*-acetyl *o*-nitrophenyl β-D-galactoside (I)—with the view of examining both the specificity of certain β-galactosidases and esterases and the interdependence, if any, of these two enzyme groups.

46·9 g of tetracetyl-*o*-nitrophenyl β-D-galactoside (II), prepared according to Seidman and Link[4], were dissolved in 600 ml. cold acetone (−22°) and a mixture of cold 1 N potassium hydroxide (300 ml.) and acetone (300 ml.) was added over a period of 1 h. After standing a further 1½ h at −22° and 45 h at +3° the solution was concentrated *in vacuo* to 150 ml., cooled and filtered. The residue, 9 g of unchanged (II), was washed with water. The washings and filtrate were evaporated to a syrup which was partially dried by two evaporations with dioxan. The ensuing paste was four times extracted with dioxan. On standing, ONPG crystallized from the extract and was filtered off. The filtrate was evaporated. On crystallization from acetone 7·5 g of (I) was obtained. This was twice recrystallized from absolute alcohol to give 6 g white needles of (I): m.p. 155°, $[\alpha]_D = -26{\cdot}2°$ (*c.* 1·5; H_2O). Analysed for $C_{14}H_{17}NO_9$: C, 48·96; H, 5·23. Calc. C, 48·98; H, 4·99.

1 mol of ester bonding (as hydroxamate) was found per mol (I) (theoretical) and 1·8 mol of periodate were consumed per mol (I) (theoretical 2·0). This is compatible only with the acetyl at the C6 position. Infra-red spectrum analysis confirmed these findings: (I) had absorption bands at 5·77μ (C=O) and at 7·95μ (=C—O) but not at 10μ (—CH_2OH). (I) is light-sensitive, especially in solution, though to a lesser extent than ONPG.

(I) underwent acid, alkaline and enzymatic hydrolysis to give galactose, acetate and *o*-nitrophenol. The enzymes in pectinase, takadiastase, emulsin and *Escherichia coli* (strains *ML* 308 and *K*12) acted via two pathways, with ONPG and 6-*O*-acetyl galactose respectively as intermediates. The pathway followed depended on the enzyme acting and the *p*H prevailing.

(I), or its enzymatic breakdown products, slowly induced β-galactosidase formation in *E. coli* (*K* 12). Details of enzyme action on (I) will be published elsewhere.

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Pyrethrin and Gallotannin in the Flowers of Peony

FROM a light petroleum extract of dried petals of peony (*Peonia albiflora* Pall.) an oily substance (74·08 per cent C, 9·94 per cent H) was isolated by means of nitromethane extraction. It comprises 0·13 per cent of the dry weight of petals and 18 per cent of light petroleum extracts and shows in the ultra-violet spectral region maxima at 227 and 280 mμ —characteristic of pyrethrins[1]. Alkaline hydrolysis of this substance gives: chrysanthemic acid (oil. volatile in steam) and chrysanthemum dicarboxylic acid (m.p. 205–206°) in the ratio of 1 : 1. Paper chromatography in light petroleum saturated with methanol[2] extract of the compound investigated and of pyrethrins, isolated from *Chrysanthemum cinerariaefolium*, as well as of the products of their hydrolysis (acids and rhetrolones), afforded a proof of the identity of both substances.

In the methanol extract from defatted petals, gallic acid derivatives were found in great quantities. From this extract a substance, comprising 10 per cent of the dry weight of petals, was separated, being soluble in ethyl acetate and acetone, but insoluble in dry ethyl ether. It possesses tannin-like properties and after hydrolysis gives gallic acid as the only phenolic compound and glucose as the only carbohydrate, detected by paper chromatography. As the result of methanolysis[3] the methyl gallate was identified. Infra-red absorption of the compound, methylated with diazomethane, indicated the absence of free hydroxy groups, which indicated a structure of galloylated pentagalloylglucose for the compound investigated. The total amount of acid liberated on hydrolysis of the methylated compound was determined by titration, and the quantity of di-*O*-methylgallic acid found by measurement of the colour developed with Folin's reagent. The ratio of the two methylated gallic acids, 5 : 1, confirmed that the compound investigated is *m*-mono-galloyl-(pentagalloylglucose).

These results are interesting because it is the first observation of the occurrence of pyrethrins and gallotannins in a plant belonging to Ranunculaceae. The first of these compounds was hitherto found only in the *Chrysanthemum* genus (Compositae); the second one has not previously been found in flowers.

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Cross-dependence between Neamine and other Basic Antibiotics

THE emergence of streptomycin-dependent as well as 'persisters'[1,2] and resistant bacteria in an environment containing streptomycin is well known. Studies on the mode of action of streptomycin-dependence have been presented in a number of recent publications[3-6]. This communication adds to the present knowledge of antibiotic dependence and the relationship between structure and biological activity.

A strain of *Staphylococcus aureus FDA* 209*P* resistant to 1 mg neamine per ml. was obtained after approximately 20 serial sub-cultures into nutrient broth with increasing concentrations of neamine per transfer. After 20 additional transfers in nutrient broth containing 1 mg neamine per ml., the culture was neamine-dependent. Good growth occurred after 24 h at 32° C with concentrations of neamine between 20 and 1,000 μg/ml., light growth with 1–20 μg/ml. and no visible growth without neamine.

Tests were conducted to determine if the requirement for neamine could be fulfilled by other antibiotics. Each antibiotic was added to nutrient broth to concentrations of 1, 2, 5, 10, 20, 50, 100, 200 and 500 μg/ml. One-tenth ml. of a 1 : 100 dilution of a 24-h culture of the requiring strain was added to each tube containing 10 ml. and all tubes were incubated at 32° C. Growth occurred with streptomycin, paromomycin, zygomycin, neomycin C and neamine (Table 1). No growth occurred with neomycin B, kanamycin, erythromycin, spectinomycin and vancomycin. A response of growth indicated that the organism utilized the antibiotic as a substitute for its neamine requirement. Growth could not be interpreted as antibiotic resistance since growth did not occur in control tubes without antibiotic. Three control tubes without antibiotic were included in each broth experiment.

Tests for requirement were also conducted on plates containing nutrient agar seeded with 2 ml. of the dependent culture per 100 ml. of medium. Paper disks were impregnated with antibiotics and placed on the agar surface and the plates were incubated at 32° C for 24 h. Zones of growth around the disks were evidence of growth support. The agar tests confirmed the broth results. Fig. 1 illustrates the growth response of neamine and two other antibiotics.

Table 1 gives structures of the antibiotics tested and their effect on the growth of the neamine-requiring strain of *S. aureus*. Note that the common factors among the antibiotics that can substitute for neamine are: (1) aminohexoses; (2) nitrogen groups in the 2-carbon position on the hexoses. Basic antibiotics with no amino-sugars, such as spectinomycin (reported as actinospectacin in the literature) and vancomycin, and basic antibiotics with aminohexoses but with the nitrogen in the 3-carbon position, such as kanamycin and erythromycin, did not substitute.

Neomycin B did not substitute for neamine even though it contains 2,6-diamino-D-glucose, the same as neamine. However, the other amino-sugar is probably an active antibacterial site which may prevent the growth of the organism.

A streptomycin-dependent strain of *S. aureus* (derived from *P.S.* 53), which was obtained through the courtesy of Dr. Ruth Z. Korman at the School of Veterinary Medicine, Cornell University, was tested for cross-dependence. This strain acted

Table 1. STRUCTURES OF THE NEOMYCINES AND OTHER BASIC ANTIBIOTICS AND CROSS-DEPENDENCE WITH NEAMINE

Antibiotic (refs. in parentheses)		Structure	Can support growth of neamine-dependent *S. aureus*
Neomycin B	(7)	2,6-Diaminohexose—D-ribose—deoxystreptamine—2,6-diamino-D-glucose	No
Neomycin C	(7)	2,6-Diamino-D-glucose—D-ribose—deoxystreptamine—2,6-diamino-D-glucose	Yes
Neamine	(7)	Deoxystreptamine—2,6-diamino-D-glucose	Yes
Paromomycin	(8)	2,6-Diamino-D-glucose—D-ribose—deoxystreptamine—2-aminoglucose	Yes
Zygomycin	(9)	2,6-Diaminohexose—D-ribose—deoxystreptamine—2-aminoglucose	Yes
Streptomycin	(10)	Streptidine—streptose—2-methylamino-L-glucose	Yes
Kanamycin	(11)	6-Aminoglucose—deoxystreptamine*—3-aminoglucose	No
Erythromycin	(12)	Cladinose—erythronolide—3-dimethylamino deoxyhexose	No
Spectinomycin†	(13)	Three-ring structure with no amino-sugars	No
Vancomycin	(14)	Contains amino-acids and no amino-sugars	No

* 4,6-Di-substituted. All other deoxystreptamines except neamine are 4,5-di-substituted.
† Spectinomycin was formerly called actinospectacin.

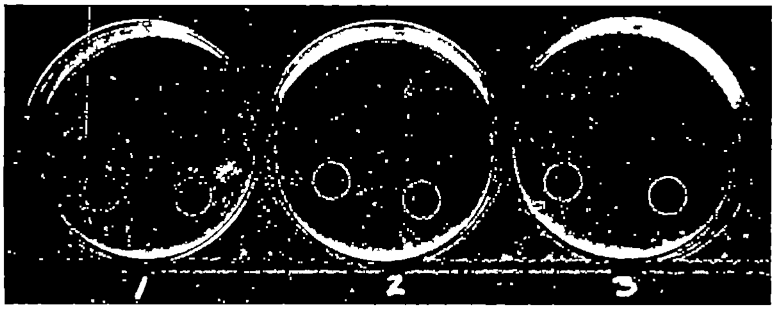

Fig. 1. The response of growth of neamine-dependent *S. aureus* to: (1) paromomycin; (2) neomycin *c*; (3) neamine. The top disk contains 1 µg and the lower 2 disks contain 10 µg antibiotic

similarly to our neamine-dependent strain in that it grew in the presence of streptomycin, neamine and paromomycin and did not grow with neomycin B, kanamycin or spectinomycin.

Two streptomycin-dependent *Escherichia coli* strains, *UC*684 and 312*Sd*1 (strain 312*Sd*1, a K_{12} derivative, was obtained from Dr. Ruth Z. Korman), and a streptomycin-dependent *Mycobacterium* (obtained from Dr. Michio Tsukamura of Obuso National Sanitarium, Japan) did not grow in nutrient broth with neamine. However, a streptomycin-dependent *Salmonella paratyphi B* obtained from Dr. Otto E. Landman of U.S. Chemical Corps did grow with 50, 100 and 200 µg neamine per ml. and not with concentrations less than 50 and greater than 200.

An interpretation of these results is difficult to make. Permeability does not seem to be a factor in the negative results with *E. coli* because neamine inhibited both strains in the presence of streptomycin. The *Salmonella* strain grew with 100 µg of neamine plus 100 µg of streptomycin as well as with 10, 20 and 50 µg paromomycin per ml. Why neamine could replace streptomycin for growth of *S. paratyphi* and not for growth of *E. coli* is puzzling since we assume that the species are related.

The possibility exists that streptomycin activity occurs both on the ribosomes as proposed by Spotts and Stanier[5] and on the cell membrane as suggested by Tsukamura[6], Landman and Burchard[4] and Engelberg and Artman[15]. As rational as the ribosome postulate seems, the reports of cell surface change cannot be ignored. If there are two active sites, ribosome and cell membrane, then neamine may not replace streptomycin at both sites. This may explain the negative results with *E. coli* and the *Mycobacterium*. There may also be a competition for the active site(s) between neamine and streptomycin.

A factor which we have not overlooked is the limits of the experimental design. Our methods for testing may not be optimal for showing cross-dependence.

We thank Drs. A. D. Argoudelis, D. G. Kaiser, G. S. Bradley and S. S. Cohen for their helpful suggestions.

Note added in proof. We did obtain growth of the streptomycin-dependent *E. coli* and *Salmonella* strains with neamine and paromomycin with the agar-disk technique similar to Fig. 1. There were zones of inhibition around the neamine and paromomycin disks surrounded by small zones of growth. This was interpreted to mean that there is a narrow range of concentration for these two antibiotics in which *SD* strains may grow. No zones of growth of the *Mycobacterium SD* strain occurred with neamine and paromomycin. Growth of all *SD* and *ND* strains including *Mycobacterium* was obtained with *U*-12898, a new basic antibiotic. Some biological and chemical properties of *U*-12898 were disclosed at the second Interscience Conference on Antimicrobial Agents and Chemotherapy, October 31–November 2, Chicago, Illinois.

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PHYSIOLOGY

Recording of Electrical Potentials from Specific Areas of the Retina

Electrical response obtained from the intact human eye[1] usually represents a gross summation of all sensory elements undergoing excitation by light. The levels of photic energy required for an electrical response are high; a good deal of light is scattered in the dioptric media[2]. As a result, considerably more retinal elements are stimulated than would be involved in excitation if the stimulus were at visual threshold level.

Effort was made to project precisely defined stimuli into specific regions of the retina in order to study the relationship between thresholds for visual and electrical response. To avoid complications by additional refractive surfaces of electrodes of scleral contact lenses which are not individually fitted, a corneal gold ring electrode[3] was used which did not interfere with the normal entry of light rays into the eye.

Instead of stimulation with a commercial photostimulator subtending a visual angle of 10° or more, a visual discriminometer[4] was used for the presentation of test fields. This instrument permits presentation of square test fields which may be varied in size, retinal position, brightness, and exposure time within a visual field of 35°–40°. The subject, in a sitting position, keeps his head stabilized with the aid of a chin rest. Accommodation is relaxed, with test field and fixation point appearing in the same plane, thereby excluding parallactic errors. With a binocular viewing system, uni- or bin-ocular testing is possible. The stimulus can be presented against a completely dark background (absolute threshold) or against any desired background luminance up to photopic levels. This technique permits a steady state of adaptation to be maintained and makes possible the study of differential thresholds. Since energies required for electrical response lie far above absolute visual threshold levels, it was found advantageous to keep the eye at a low photopic level of adaptation in order to avoid gross disturbances by the light exposure required for obtaining the electrical response.

For testing, a gold ring electrode is placed on the cornea of the preferred eye after instillation of two drops of dorsocaine hydrochloride, and the lids are held open with a plastic lid holder[5]. The subject sits at the discriminometer and sees with the prepared eye, through the eyepiece, a circular field of a luminance of 10·4 millilamberts. At the centre is a red fixation mark which must be sharp and clear. The test field is presented in the form of short exposures (0·04 sec) by means of a photographic shutter in the path of the light. During visual threshold determinations, the subject indicates by voice or taps whether he has seen the light stimulus. The operator increases or decreases the luminance of the test flashes by changing the position of a neutral graded density wedge until a level is reached at which the test flash is just perceptible (visual threshold). Five determinations are made in succession, and the mean of the luminance readings is taken as the threshold for visibility of the test field.

The corneal ring electrode (active electrode) and an electrode attached to the temple at the outer canthus (indifferent electrode) are connected with a Grass polygraph so that potentials may be recorded simultaneously with the test exposures. At visual threshold level no potential is obtained. The luminance of the test flashes is then raised in steps of 0·1–0·2 log unit until the first potential wave appears on the record. At that level, luminance is usually more than 2 log units higher than at threshold. The luminance is then raised in steps up to the maximum available, and potentials are recorded for each luminance-level in order to obtain relationships between height of B wave and luminance.

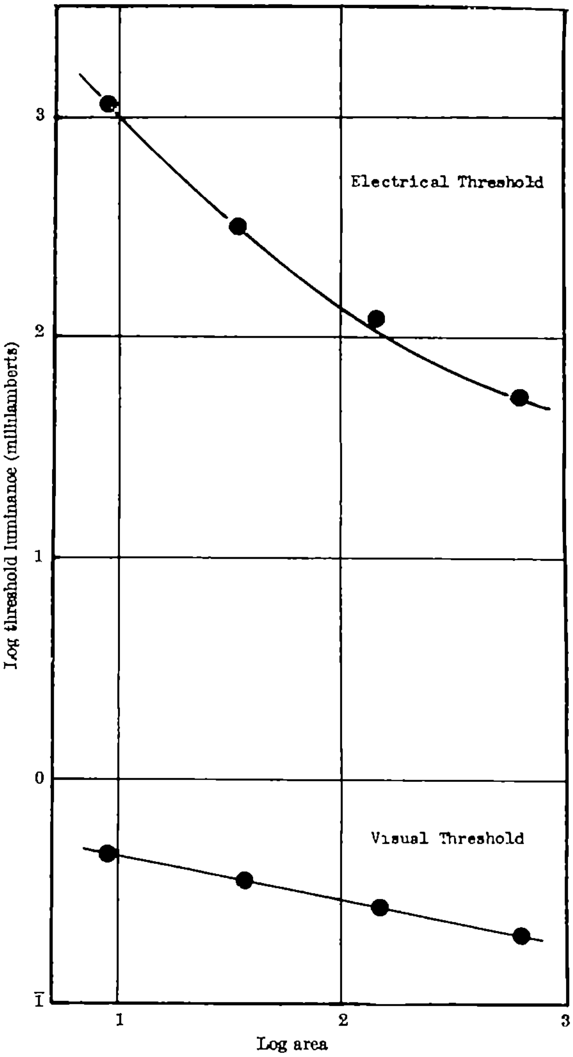

Fig. 1. Visual and electrical thresholds for centrally fixated square test fields of 3°, 6°, 12·5° and 25° angular subtense. Electrical thresholds lie 2–3 log units higher than visual thresholds. Test fields are presented against a background luminance of about 10 millilamberts

Fig. 1 shows results obtained with centrally fixated square test fields subtending 25°, 12·5°, 6° and 3° on a side. The logarithms of threshold luminance are directly proportional to the logarithms of test field area. The thresholds for electrical response lie approximately 3 log units above the visual thresholds. The relationship between log area and log luminance for electrical excitation are decidedly not linear and need further investigation.

Fig. 2 shows the relationship between test flash luminance and the height of the B wave. While the logarithm of flash luminance increases from 1·70 to

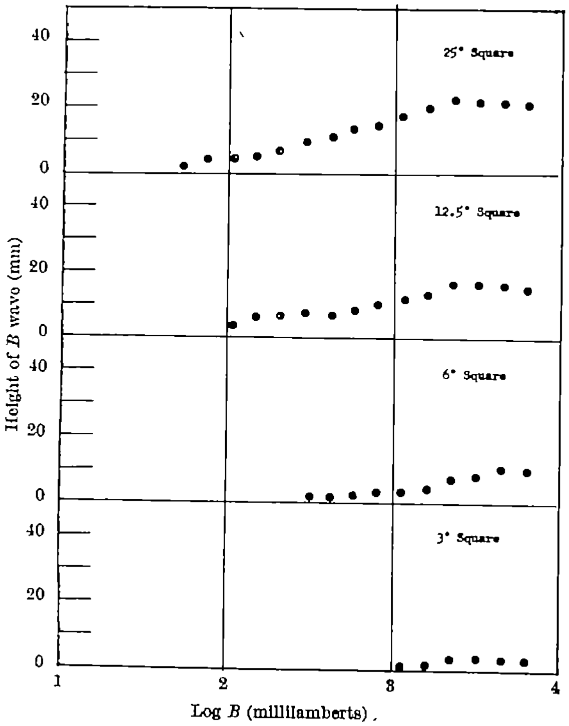

Fig. 2. Relationships between stimulus luminance and height of *B* wave for centrally located square test fields of 3°, 6°, 12·5° and 25° angular subtense. While threshold luminance decreases with increasing test field size, the size of the *B* wave becomes larger

3·80 millilamberts, the height of the *B* wave increases for a 3° test field from 1·5 to 4 mm, for a 6° test field from 1·5 to 10·5 mm, for the 12·5° test field from 3·0 to 11·5 mm and for the 25° test field from 2·5 to 24·0 mm. The greatest *B* wave height is reached below maximal luminance. If luminance is increased beyond a certain point the potential does not increase further and might even become smaller at excessive luminances.

In these tests foveal and parafoveal regions of the retina are involved in the excitation process. As the test area is increased and more and more rods participate in perception, threshold-levels for both visual and electrical responses are found at lower levels of luminance. The picture of the electrical response therefore reveals, to some degree, the relative cone and rod participation.

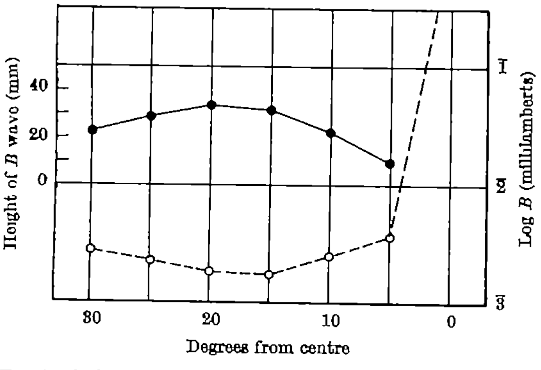

Fig. 3. Relationship of visual thresholds to height of *B* wave for test fields of equal size at various distances from centre. The height of the *B* wave is inversely proportional to threshold luminance

When test fields of equal size (5° angular subtense on a side) are presented in the retinal centre and at various distances from fixation, the electrical response gives further information on sensitivity of the retina. Fig. 3 shows results obtained when the test field was presented in the centre and in the temporal visual field at 5°, 10°, 15°, 20° and 25°. The open circles represent mean log luminances for visual thresholds. Thresholds are high at 5° from centre and drop considerably at 10°. The lowest thresholds lie between 15° and 20°. Farther laterally, they gradually rise to higher levels. This represents the classical picture of sensitivity distribution of peripheral retinal elements[4–9].

The potentials of the electroretinogram obtained at corresponding retinal locations are shown in the black circles of Fig. 3. At 5° from centre a relatively low *B* wave is obtained. As the test field is moved outward the height of the *B* wave increases and reaches its greatest height between 15° and 20° from centre. At 25° from centre the potential becomes again smaller. The electrical response is, therefore, inversely proportional to the visual threshold, and the height of the *B* wave appears to be an indication of the number of retinal elements activated by the light stimulus.

In studying electrical responses with test fields of different sizes presented in the four quadrants of the visual field, it has been found that the lower quadrants have lower thresholds for electrical response than the upper quadrants. In addition, differences between the nasal and temporal fields are indicated.

The preliminary results presented here show that by placing visual stimuli into precisely defined areas of the retina, quantitative measurements of bio-electrical responses can be obtained. These measurements are closely related to the structural and functional properties of the respective retinal areas. Application of this knowledge to the study of the electroretinogram in patients may help to a better understanding of retinal pathology through detailed evaluation of the electrical responses obtained from specific regions.

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Central Respiratory Chemo-sensitivity and its Relationship to Peripheral Nerve Carbon Dioxide-sensitivity

BECAUSE ventilation is proportional to the alveolar pCO_2 it is generally assumed that carbon dioxide has a direct excitatory effect on either the neurones in the 'respiratory centre'[1] or on a 'special chemo-receptor system' independent of the synapses in the respiratory reflex pathways[2]. An examination of the effect of carbon dioxide on the excitability of peripheral nerve suggests, however, another possible explanation. This has been investigated in 'normal' subjects in whom

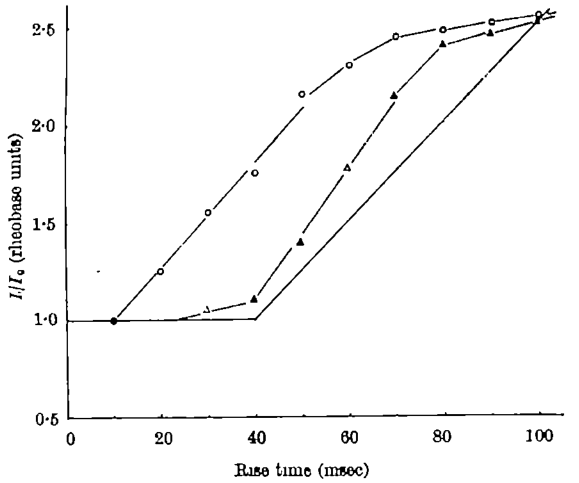

Fig. 1. Accommodation time-curves of the ulnar nerve of a 'normal' subject before hyperventilating for 6 min, continuous line; immediately after hyperventilating, circles, and 15 min after hyperventilating, triangles. The rheobase values are 0 85, 0·45–0·5 and 0 6–0 65 mA respectively

the alveolar carbon dioxide tensions were changed experimentally[3].

Excitability was determined by measuring the rheobase, the time at which nerve accommodation begins, the slope of the accommodation-time curve (rheobase units/sec) and the relative current strength (in rheobase units) at which accommodation breaks down. The ulnar nerve was stimulated with monopolar cathodal currents from a constant current stimulator which would produce currents rising linearly with different rates of rise. The minimum observable or palpable twitch of the small finger was taken as index of excitation.

The accommodation time-curve of the ulnar nerve of 'normal' subjects shows an initial delay time (Fig. 1) during which the stimulus current appears to be inducing threshold changes[4]. The slope of the curve reflects the rate of rise of the threshold evoked by the stimulating current. With longer rise times of stimulus current the slope gradually bends away, becoming more horizontal. This breakdown of accommodation coincides with the point at which the muscle contraction becomes drawn out, resembling a brief tetanus rather than a twitch, indicating that the nerve responds by discharging repetitively.

Experimentally lowering the pCO_2 in 'normal' subjects by hyperventilating for 6 min immediately lowers the rheobase, both the time at which accommodation begins and at which accommodation breakdown occurs sooner. The slope of the accommodation-time curve becomes slightly steeper (Fig. 1). Experimentally raising the end expiratory carbon dioxide tension above 57 mm mercury by breathing 5 per cent carbon dioxide in oxygen leads after 8 min to a rise of the rheobase and after 25-min breakdown of accommodation occurs earlier and at a lower current strength.

The rapid alterations in rheobase produced by lowering or raising the pCO_2 are due to changes in the threshold of nerve excitability resembling those found in isolated nerve preparations, and which are mainly due to changes in pCO_2 (ref. 5). The shortened delay-time and earlier breakdown of accommodation on lowering the pCO_2 are indicative of increased excitability. Whereas increasing the pCO_2 raises the threshold of excitability at an early stage, maintaining it at a high pCO_2 for a long period caused the breakdown of accommodation to occur earlier. This late increase in excitability is probably due to a secondary increase in the concentration of bicarbonate, which has been shown to increase impulse activity in isolated preparations[6]. Carbon dioxide and bicarbonate determine nerve excitability because adequate concentrations of both are required if action potentials of isolated nerves are to resemble the *in vivo* potentials[7]. The ratio of the bicarbonate–carbon dioxide concentrations of the fluid bathing a nerve rather than its pH, obtained with different buffers, determines excitability[6].

A comparison of the effects of carbon dioxide and bicarbonate on ventilation and nerve excitability shows that increasing the bicarbonate–carbon dioxide ratio decreases ventilation[8], but increases excitability and vice versa. Unless it can be shown that the carbon dioxide-sensitive cells respond to chemical changes in an opposite manner to that of most nerve cells it is difficult to accept earlier explanations of carbon dioxide-sensitive mechanisms. It is suggested instead that carbon dioxide exerts its effect on ventilation by acting mainly on specific inhibitory neurones which are in close contact with arterial blood and which in turn modify the activity of neurones in the 'respiratory centre'. Thus an increase in pCO_2 by decreasing the excitability of specific inhibitory neurones would lead to an increase in activity of the neurones in the 'respiratory centre' and hence to increased ventilation.

This explanation of the effect of carbon dioxide on ventilation takes into account experimental evidence on the effects of carbon dioxide on nervous excitability. It is consistent with other evidence: (*a*) that a negative feed-back is present in the brain-stem reticular formation[9], which is indicative of inhibitory neurones; (*b*) that an 'adequate' carbon dioxide tension (at least equal to the tension at which carbon dioxide stimulates ventilation) is required in order that evoked reticular activity should produce cortical activation[10], which indicates that carbon dioxide is involved in determining excitability at some synapses in the central nervous system. It also provides a theoretical basis for the view that altered respiratory responses to carbon dioxide found when the resting arterial blood gas tensions and acid-base concentrations fall outside the 'normal' range are due to changed excitability of the carbon dioxide-sensitive mechanism.

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Coiled Cathode Oxygen Polarograph

Blood oxygen tension (P_{O_2}) may be measured polarographically by shielding the cathode with a membrane[1]. Existing electrodes have two types of cathode, large and small. The large cathode of 1–2 mm diameter passes a current of 1–2 μamp at a P_{O_2} of 150 mm mercury. Unfortunately reduction at the cathode produces a local zone of oxygen depletion and a steady reading can only be obtained by vigorous stirring[2]. Stirring inevitably requires a rather large cuvette and a blood sample which is too large for repeated estimations on the infant and small experimental animal.

The small cathode, 40μ in diameter, is protected from zonal oxygen depletion by diffusion within the blood. The current passed is reduced to a twentieth of that from the larger cathode and requires expensive screened equipment for its measurement.

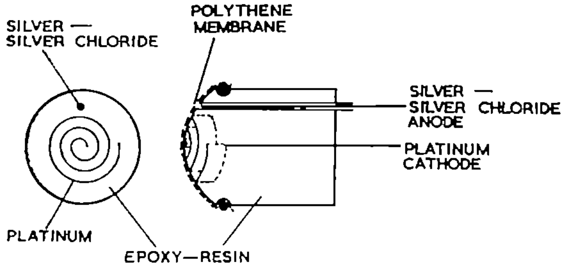

Fig. 1. Constructional details of the electrode

We have constructed an electrode in which the cathode takes the form of a spiral of platinum 40μ thick. It is embedded in epoxy resin and one edge is exposed by turning and grinding (Fig. 1). In this way we have obtained the area and current of a large cathode which is sufficiently dispersed to avoid zonal oxygen depletion without stirring (Fig. 2). This combination of the advantages of the two previous types of cathode enables cuvettes to be at least as small as 0·2 ml. without the constructional difficulties of stirring, and the current may be measured satisfactorily on a laboratory mirror galvanometer.

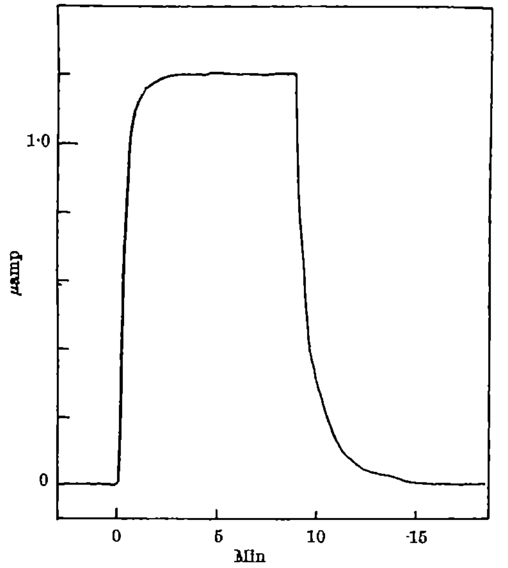

Fig. 2. Response of the electrode shown in Fig. 1. Air-equilibrated water was introduced into the cuvette at zero time and left unagitated for 9 min when the cuvette was flushed with nitrogen

The response of this prototype electrode is rather slower than the Clark cell. This may be due to the solubility of oxygen in the epoxy resin. Glass may be a more suitable material for supporting the electrode.

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HÆMATOLOGY

Comparative Metabolism of Hæmoglobins A and F in Thalassæmia

Inquiry into the comparative metabolic behaviour of the various normal and abnormal types of hæmoglobin seems likely to yield important knowledge about the pathogenesis of inherited disorders of hæmoglobin synthesis. The following study is submitted as a preliminary report which demonstrates a difference in the metabolism of hæmoglobins A and F in a patient with thalassæmia intermedia.

The patient was a 33-year-old male with an established anæmia at a hæmoglobin level of 9·0 g per 100 ml. The spleen was intact. He has not required transfusions. Red cell morphology was typical for thalassæmia. Chromium-51 red cell survival was only slightly shortened (half-life 23 days). His hæmoglobin composition was 36 per cent F, 5·9 per cent A_2, and the remainder A.

Two hundred μc. of α-^{14}C-glycine were given intravenously in a single dose. Blood samples were drawn periodically for 122 days. The separation of hæmoglobins A, F, and A_2 was done chromatographically, using an initial separation on 'Amberlite *CG* 50' (ref. 1), followed by additional purification of the F on carboxymethylcellulose[2]. The samples were concentrated to small volumes by ultra-filtration, dialysed against water, and lyophilized. Radioactivity of the weighed samples was determined by liquid scintillation counting after combustion to $^{14}CO_2$ by modification of the Schoeniger technique[3].

The results (Fig. 1) demonstrate a clear difference in the metabolism of hæmoglobins A and F. During the initial period of the curves, the specific activity of A rises to 1·3 times that of F. In addition, hæmoglobin A is seen to have the shorter mean life-span (73 days

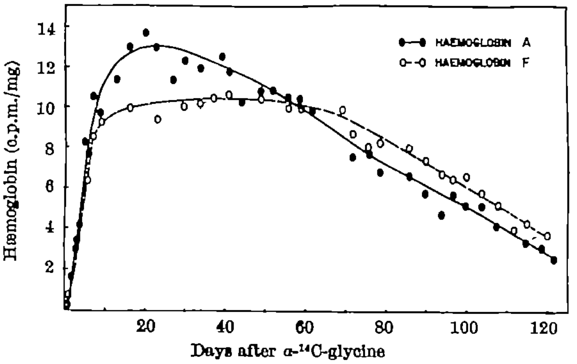

Fig. 1. Comparison of the specific activities of hæmoglobins *A* and *F* during a period of 122 days following an intravenous injection of α-^{14}C-glycine in a patient with thalassæmia intermedia

compared with 104 days for hæmoglobin F). Hæmoglobin A_2 was found to follow a pattern similar to that of hæmoglobin A.

These findings suggest that in this patient the hæmoglobins A and F tend to be segregated in different populations of red cells. They further indicate that the synthetic rates of these hæmoglobins may not be proportional to their concentrations in the peripheral blood.

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Adsorption of Human and Bovine Thrombin

ALTHOUGH certain metallic compounds are known and used to adsorb prothrombin and factors VII, IX and X from blood, hitherto the effect of these adsorbents on thrombin has not been reported.

The following observations are derived from the experiments presented in Table 1.

Table 1. ADSORPTION OF BOVINE AND HUMAN THROMBIN

Compound used*	Clotting-time of substrate (sec)† Human thrombin	Bovine thrombin	Species adsorbed
Saline (control)	22	22	—
Aluminium hydroxide	99	>120	both
Aluminium phosphate	43	22	human slightly affected
Aluminium silicate (kaolin)	69	>120	bovine more than human
Bismuth carbonate	36	120	
Calcium phosphate	21	>120	bovine
Barium carbonate	22	86	bovine
Barium sulphate	22	22	neither
Magnesium hydroxide	24	26	neither
Magnesium oxide	29	25	neither
Magnesium trisilicate	22	21½	neither
Magnesium carbonate	57	40	both slightly affected
Polyaminostyrene	22	22	neither

* To 10 volumes of thrombin solution (clotting substrate in 20 sec) was added 1 volume of the suspension to be tested. After incubating the mixture for 5 min (with frequent shaking) the compound was precipitated by centrifugation and the thrombin activity of the supernatant tested as follows.

† To 0·4 ml. of the substrate (fibrinogen 200 mg/100 ml.) at 37° C was added 0·1 ml. of the solution tested for thrombin activity, recording the clotting time.

(*a*) A compound which adsorbs prothrombin is not necessarily a thrombin adsorbent. Barium sulphate is an example of this.

(*b*) Thrombin exhibits species specificity; the adsorption of the bovine material is different from that of the human reagent.

Further investigations demonstrated that intrinsic thrombin formed in recalcified citrated plasma or blood was similarly affected as shown by the deposition of fibrin on the suspended adsorbent particles and fragmentation of the clot. Accordingly, care should be exercised in choosing the alkali and the species of thrombin used in the control of upper gastrointestinal bleeding.

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PATHOLOGY

Production of Hyaluronic Acid in Tissue Culture of Rous Sarcoma

ROUS chicken sarcoma has been known for a long time to produce large quantities of hyaluronic acid[1-3]. Rous sarcoma homogenates were shown to contain all the enzymes necessary to synthesize hyaluronic acid *in vitro*[4]. No information, however, was available as to whether or not Rous sarcoma cells produce hyaluronic acid in tissue culture as they do in the tumour. Such information may have a bearing on the question of identification of the Rous sarcoma cell. Also it was hoped to find a link between the enormous increase of hyaluronic acid synthesis in cells of a virus-induced tumour and certain aspects of virus-cell interaction, such as the increased synthesis of non-viral RNA in cells of a RNA-virus tumour.

1–2 tenths of a ml. of various dilutions of a standard virus (10^{-1}–10^{-8}) were injected subcutaneously in the wing web of 5- to 7-day old White Leghorn chicks. At various times after injection chicks were killed and the tumours were removed aseptically, washed, minced with scissors and suspended in an equal volume of saline. After 24 h at 4° C, and after centrifugation a more or less sticky supernatant fluid was obtained, and was tested for the presence of hyaluronic acid. From parts of the tumours cultures were prepared. The glass floor of the culture vessel was coated with a thin layer of heparinized plasma just enough to make the explants stick to the glass and about five tissue fragments per cm^2 were explanted. After several hours at 37° C the fluid medium was added consisting of Eagle's minimum essential medium with 10 per cent horse serum, 1 per cent glutamine and 5–10 per cent half-diluted embryo extract. One hundred units potassium penicillin and 100 µg dihydrostreptomycin sulphate per ml. were added. Renewal of the medium was carried out three times a week and the used medium of each culture was separately tested for the presence of hyaluronic acid. In most cultures the explants were detached after several feedings, leaving behind continuous monolayer cultures. The mucin clot test, which is the method of choice for testing small samples of native non-purified fluids containing hyaluronic acid, was used[5-7]. The concentration of hyaluronic acid was estimated by a sample dilution method. Assuming that the lowest concentration of high-polymer hyaluronic acid required for formation of mucin clot is about 30 µg per ml. (ref. 5), multiplication by thirty of the minimal amount of fluid in ml., which prevents formation of mucin clot, allows a rough estimation of the concentration of hyaluronic acid in the original undiluted sample. The supernatant fluids obtained from centrifugation of the minced tumours were mostly sticky and on addition of a few drops of 1 N acetic acid to about 1 ml. sample yielded a tight mucin clot. The formation of the clot could be prevented by hyaluronidase which was obtained from rat testes. The endpoint of dilution which gave a mucin clot on acidification was 1 : 72 in two tumours; 1 : 68 in four tumours; 1 : 40 in four tumours; and 1 : 23 in one tumour. Since an equal volume of saline was added to the minced tumour for extraction, the highest dilution which gave a positive mucin clot test in the first two sarcomas was 1 : 144. The concentration of hyaluronic acid in two tumours was thus roughly as high as 144 × 30, or 4,320 μg/ml. The concentration of hyaluronic acid in Rous sarcoma was mostly higher than that in synovial fluid in which the concentration has been found to vary between 2,160 and 2,040 μg/ml. in samples from four patients with uncomplicated knee trauma. In addition to eleven sarcomas with high concentration of hyaluronic acid, three sarcomas were found to contain very low concentrations of hyaluronic acid. These tumours were smaller at the day of their removal than they were at the preceding days and evidently were regressing.

Unlike hyaluronic acid in synovial fluid and hyaluronic acid produced in tissue culture which is relatively stable, hyaluronic acid in Rous sarcoma extract was spontaneously depolymerized and lost the property of forming mucin clots. The speed of depolymerization was dependent on temperature. Increase of reducing sugar suggested enzymatic degradation[8]. Addition of protein to the samples to be tested did not restore the quality of formation of mucin clot, which suggested that the loss of the quality of formation of mucin clot was not caused by proteolytic enzymes which would digest the protein necessary for the formation of a mucin clot with acetic acid.

The used media of cultures in which Rous sarcoma cells were growing for 48–72 h were tested for the presence of hyaluronic acid. Nine experiments with 134 cultures were carried out. After the second change of medium, the supernatant of 109 cultures yielded a tight mucin clot with acetic acid the formation of which could be prevented by testicular hyaluronidase. The highest dilution which gave a positive mucin clot test ranged from 1 : 7 to 1 : 10. The same was found at the third change of medium. At the fourth change of medium about 70 per cent of cultures gave a positive mucin clot test and the end-points of dilution were 0–1 : 3. After the seventh change of medium 30 per cent of cultures gave a mucin clot when undiluted. After 34 days in culture the supernatant of only 8 per cent gave a mucin clot when undiluted. It is evident that the Rous sarcoma cell is a fibroblast regardless of morphological changes because only fibroblasts are known to produce hyaluronic acid in tissue culture and because production of large quantities of hyaluronic acid by Rous sarcoma cells in tissue culture can only be conceived as increase of an activity normally present in the stem cell of the tumour.

In cultures of normal fibroblasts production of hyaluronic acid *in vitro* was found to parallel cell growth[6]. On the other hand, in tissue culture of Rous sarcoma it has been observed that in cultures with a high rate of growth, Rous sarcoma cells very soon lost the ability to synthesize hyaluronic acid *in vitro*, while in cultures with poor or no growth Rous sarcoma cells produced hyaluronic acid up to 34 days despite unusually large vacuoles in the cytoplasm.

In virus-induced tumours, the synthesis of cell nucleic acid of the same nature as that of the virus appears to be increased[9,10]. An excess of cytoplasmic non-viral RNA was found in cells of stationary Rous sarcoma cultures[9]. This is consistent with the finding that, unlike normal fibroblasts, Rous sarcoma cells in tissue culture produce more hyaluronic acid in stationary cultures than in fast-growing cultures. Since a common pool of nucleotides provides building blocks both for viral and for non-viral RNA, there must be more competition for those building blocks in Rous sarcoma cells than in normal fibroblasts in which only non-viral RNA is manufactured. Hence RNA-building blocks may be a limiting factor in hyaluronic acid synthesis in fast growing Rous sarcoma cells producing both viral and non-viral RNA and not in normal fibroblasts producing only non-viral RNA.

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Induction of Lysogenic Bacteria as a Method of detecting Potential Antitumour Agents

It is known that certain antitumour agents are capable of inducing lysogenic bacteria. Thus, Jacob[1] showed that nitrogen mustard had this property. Gots *et al.*[2] reported the same for azaserine, and Otsuji *et al.*[3] for mitomycin *C*. It was thought that this property might afford a relatively simple, rapid and convenient method of detecting potential antitumour agents produced by micro-organisms and might also serve as a suitable assay for following extraction procedures. Accordingly, a quantitative method of measuring the induction of λ phage in *E. coli* strain K_{12} has been worked out. It consists essentially of inducing a young culture of bacterial cells in *M*9 medium[4] for a period of 1 h with the agent and growing the induced cells in nutrient broth for 2¼ h. Phage counts are then done on plates seeded with the indicator strain *E. coli*, *W*3001. At the same time, counts are made of the bacteria since it has been found that, when assaying broths which are rich in nutrient materials, growth of the lysogenic strain can lead to increased phage titres and confuse the results of the test. From the results, the induction index, which is the ratio of phage count to bacterial count, is calculated.

Fig. 1 illustrates the results obtained using different concentrations of the agent mitomycin *C*. It will be seen that the curve has three slopes. At concentrations between 0·06 and 0·5 μg/ml., the induction index rises slowly. From 0·5 to 2 μg/ml., the slope increases. Above this level, the curve rises steeply because of killing of large numbers of organisms due to the antibiotic action of the agent. Because of the complex nature of the curve, several different dosages are used for quantitative assays. Controls without any agent are run in all experiments. An induction index is considered significant if it is at least 10 times greater than the control index in the absence of killing of the *E. coli*, K_{12}, cells.

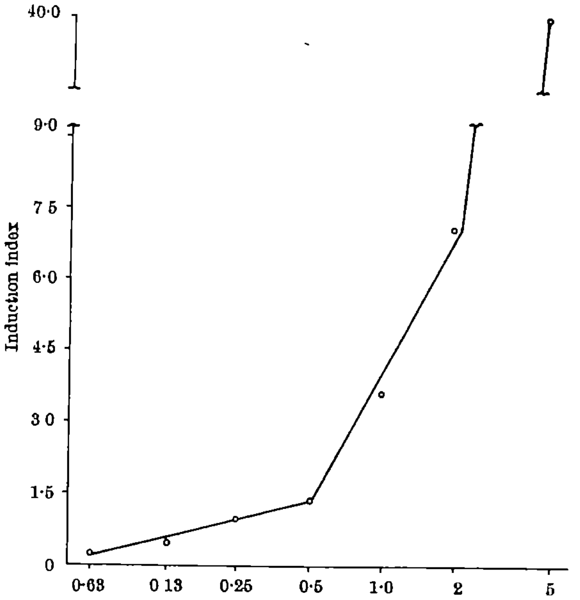

Fig. 1. Effect of different concentrations of mitomycin C on induction index

In order to check the specificity of the test, a variety of known antibiotics were tested. In Table 1 are listed those agents which gave significant increases in the induction index. Also indicated in Table 1 are the published results, when available, of antitumour tests of these materials using rodent transplanted tumour systems. It is evident from Table 1 that all agents which have a significant induction index also display antitumour activity in the cases where such results are available. In addition to the antibiotics listed, a total of 125 were tested which did not give a significant index. Several of these are known to have antitumour activity. Thus, as might be expected, this test detects only certain classes of antitumour activities.

As an indication of the usefulness of this test, it may be noted from Table 1 that there was no antitumour information available on the antibiotic phleomycin when the induction test was done. Since this antibiotic showed a positive induction index and was available in these laboratories, it was tested against three transplanted tumours of mice, Sarcoma 180, Adenocarcinoma 755, and Leukæmia 1210, using the standard procedures of the Cancer Chemotherapy National Service Center[5]. Significant antitumour activity was found against Sarcoma 180 at doses of 0·5–16·0 mg/kg/day and against Adenocarcinoma 755 at doses of 0·3–8 mg/kg/day. The test thus detected antitumour properties of an antibiotic not previously suspected.

The method has been applied to fermentation broths of actinomycetes to determine if agents were present which would give a positive test. Of the broths tested, 2·2 per cent gave a significant induction index. Positive broths are now undergoing extraction on the basis of this technique in order to isolate the active agent in pure form.

Table 1. ANTIBIOTICS WHICH HAVE A POSITIVE INDUCTION INDEX AND THEIR ANTITUMOUR ACTIVITIES

Antibiotic	Antitumour activity
Azaserine	+
Gancidin *A*	+
Griseolutein	+
Mitomycin *C*	+
Phleomycin	No results available
Porfiromycin	+
Pluramycin	+
Streptonigrin	+
Streptozotocin	No results available

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IMMUNOLOGY

Effect of Neonatal Thymectomy on the Immune Response in the Chicken

THE function of the mammalian thymus has recently become a matter of considerable interest in the field of immunology. It is now reasonably clear that the presence of the neonatal thymus is essential if the animal is to develop the capacity to reject homografts. This has been shown by Miller[1] and by Arnason *et al.*[2], who thymectomized mice and rats respectively within 24 h of birth and demonstrated that a skin homograft placed on the animal several weeks later was either permanently accepted[1] or showed a prolonged rejection time[2]. This has lent considerable support to the view, originally proposed by Fichtelius[3], that the thymus is a primary organ of lymphocytopoiesis which, during the first week of life, sends out lymphoid cells to colonize other lymphoid organs, and where on appropriate antigenic challenge will be stimulated into immunological activity. However, some recent results from Miller[4] have indicated that the influence of the thymus on the development of immunological competence may not be purely cellular, but may be of a hormonal nature.

It was shown by Mueller *et al.*[5] that removal of the bursa of Fabricius in the chicken, by either hormonal[6] or surgical means on the first day of life, rendered the bird incapable of antibody production following an antigenic challenge six weeks later.

In previous communications[7,8] we have confirmed this observation and have also shown that if the thymus of these bursa-less birds is morphologically normal, then they will reject skin homografts in a normal time. If, however, the thymic cortex showed a complete atrophy (this occurring in about 10 per cent of bursa-less birds hatched from hormone-treated eggs) then these birds accepted a skin graft until the time of death (usually 6–8 weeks). It was concluded, therefore, that a dissociation of immunological responsiveness existed in the chicken, and that the presence of the neonatal thymus is essential if homograft immunity is to develop, but that the organ does not play any part in the eventual development of antibody-producing cells, this latter function being determined by the bursa. A recent article by Papermaster *et al.*[9] has shown that bursaless birds hatching from hormone-treated eggs are unable to reject a massive llular homograft. It is stated that these birds have lly meagrely developed thymuses, and it is possible at these birds correspond to our own bursa-less birds owing a cortical atrophy of the thymus, which were apable of rejecting a skin graft[9].

o confirm our interpretation, we have surgically mectomized chickens within 24 h of hatching, the sa being left untouched. The nature of the cken thymus is such that it is extremely difficult to nove all the bottom lobe on each side, and subse- ent examination of the birds after testing showed at about 5–10 per cent of thymic substance had en left. These results, therefore, present the effect a 90–95 per cent neonatal thymectomy. The ults are given in Table 1. Out of eleven birds, two ected their grafts in normal time, seven retained ir grafts for 13–18 days (2–3 times normal rejec- n time), the remaining two taking 5 and 9 weeks espectively to reject. All control birds rejected their rafts in no longer than 8 days.

able 1. IMMUNOLOGICAL RESPONSE OF NORMAL AND THYMECTOMIZED CHICKENS

Type of reaction	Normal chickens	Chickens thymectomized at 1 day
mograft rejection: verage time of ejection	7 ± 1 day (10)	< 15 days: 6, 8, 13, 13, 13 > 15 days 17, 18, 18, 18, 35. 60 (11)
ibody production HGG· ncidence of positive itres:	100 per cent	100 per cent
ean reciprocal titres	5,120 (20)	3,200 (11)
n No. of small mphocytes × 10^6/ml.	10·5 (10)	Homograft rejected < 15d : 7 0 Homograft rejected > 15d . 2·8 (11)

Figures in parentheses show the number of birds tested.

From blood counts made on the day of grafting, it n be seen that there is a rough correlation between homograft rejection time and the number of ulating small lymphocytes—which probably re- ts to some extent the amount of residual active mus. This last finding is supported by the results ained (Table 2) from a second skin graft on the ls which had taken longer than twice the median vival time to reject their first graft. The second ft was made at a time when the number of circu- ng small lymphocytes had reached a normal ue, and it can be seen that these homografts were ected in normal time.

le 2. RELATIONSHIP OF CIRCULATING LYMPHOCYTES AND HOMOGRAFT REJECTION TIMES IN THYMECTOMIZED CHICKENS

Bird No.	First graft Rejection time	Small lymphocytes × 10^6/ml. (normal range 4·8–15·5)	Second graft Rejection time	Small lymphocytes × 10^6/ml. (normal range 12·0–24 0)
580	60 days	1·0	9 days	21·5
583	35 days	2·9	7 days	18·0
586	17 days	1·4	7 days	12·0
587	18 days	5·8	9 days	19·2
891	18 days	2·2	9 days	12·0

All birds were thymectomized within 24 h of hatching. The first raft was applied at about 30 days and the second at 70 days.

Neonatal thymectomy has had no influence at all on the production of antibody to a classical protein antigen (human γ-globulin).

These results infer that removal of about 90–95 er cent of the neonatal chicken thymus considerably olongs the rejection time of skin homografts, and at a normal rejection time is not reached until the idual 5–10 per cent of thymus has had sufficient e to exert its influence, probably by the produc- n of sufficient numbers of appropriate immunologically competent cells. Taken together with the fact that antibody is produced in normal amounts in these birds, these results support our hypothesis that a dissociation of immunological responsiveness exists in the chicken, in that the presence of the neonatal thymus is essential if the bird is to develop homograft immunity, and the neonatal bursa is essential for the development of potential antibody producing cells.

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HISTOLOGY

Thermoelectric Apparatus for the Preparation of Fresh-Frozen Tissue Sections

THE application of the Peltier effect in the preparation of frozen histological sections has been described by Ioffe[1] and Brown and Dilly[2], who cooled the microtome stage by means of thermoelements constructed of semi-conductor materials. Sections of fixed material can be obtained in this way; but it is not possible to cut sections of fresh unfixed tissue by merely cooling the microtome stage. For this purpose it is necessary to cool not only the stage, but also the knife, and although this can be done with solid carbon dioxide the method is not very satisfactory.

Recently, in conjunction with Semiconductor Thermoelements, Ltd., we have used series-connected 'Frigistor' thermoelements to cool both microtome stage and knife and have been able to cut satisfactory frozen sections of fresh tissue with this equipment.

The microtome stage is formed by a thin anodized aluminium plate, 20 mm × 20 mm, bonded to the top surface of a bank of eight thermoelements. A water-cooled heat sink attached to the lower surface ensures a high rate of heat extraction from the thermoelements. Two banks of thermoelements, also fitted with anodized aluminium plates and water-cooled heat sinks, are clamped 2 in. apart on each side of the microtome knife. The equipment has been used on the standard Reichert and Leitz sledge microtome, but can be readily adapted for use with other makes. A single power unit supplies a variable direct current of up to 15 amp at 1 V to the stage, and of 15 amp at 4·8 V to the thermoelements which cool the knife.

The thermoelements efficiently reduced the temperature of the microtome stage and knife. A current of 15 amp cooled the stage to − 36° C in 30–40 sec and

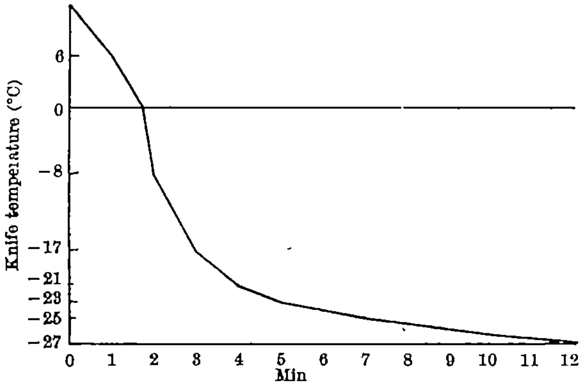

Fig. 1. Rate of cooling of 160-mm microtome knife at 15 amp

the knife to −20° C within 5 min. Fig. 1 illustrates the rate of reduction in knife temperature at an average ambient temperature of 20° C, the mean temperature of the water entering the heat sinks being 17° C.

Frozen sections of a variety of rat tissues have been cut at a knife temperature of −16° C to −20° C and a stage temperature of −8° C to −20° C. The most satisfactory results were obtained when the knife temperature was a few degrees lower than the stage and the sections were as good as those prepared by conventional freezing methods. The method revealed that different tissues require to be frozen to slightly different temperatures before optimal cutting conditions are attained.

The new method of cooling is the only one whereby the temperature of the stage and the knife can be accurately controlled and held at the optimum cutting temperature indefinitely. Serial sections can be cut and a further advantage is that solid carbon dioxide or carbon dioxide gas is not required for cooling.

We thank Dr. J. H. Ashby, of Semiconductor Thermoelements, Ltd., Langley, Bucks, who designed the knife-cooling attachment for us.

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RADIOBIOLOGY

Disappearance of Tritiated Thymidine and Tritiated Water from the Anterior Chamber of the Rabbit Eye

Tritium-labelled thymidine (^{3}Hth), a DNA precursor, has been widely used for examining cells which have recently received stimulation for reduplication. The ^{3}Hth, which can be localized at the cellular level by means of autoradiography, is reputedly incorporated almost exclusively by cells which are undergoing chromosome duplication in preparation for cell division[1].

A number of investigations on ocular tissues, in which the ^{3}Hth was injected directly into the anterior chamber of the rabbit eye, have indicated that many cells in the lens, cornea and other tissues are undergoing synthesis of DNA. Furthermore, the distribution of DNA-synthesizing cells can be greatly altered following mechanical injury[2]. For sor investigational purposes it is important to know hc long the ^{3}Hth is available to the cells following injection into the anterior chamber (for examp when two injections of ^{3}H-thymidine are mad order to determine the distribution of incorpora cells at two different times)[2]. In the experin reported here, the rate of disappearance of ^{3}Hth fr the anterior chamber was determined. Para experiments were performed to determine the rate disappearance of tritiated water under similar c ditions.

Young American Dutch rabbits were used in experiments. After pentobarbital anæsthesia 0·1 of aqueous humour was withdrawn from the anter chamber, and 0·1 ml. of ^{3}Hth, concentration 1 µc./ in Eagle's basal medium, specific activity 1·90 c./m or tritiated water at 40 µc./ml. (control), was inject After various predetermined periods of time, paracentesis was performed of the anterior chamb The right eye of all the animals was sampled 5 n after injection and this was taken as a reference val in the experiments. The time at which the left e was sampled varied from 2·5 min to 120 min. 7·5 n of scintillation fluid plus the aqueous sample we counted in a Tri-carb scintillation spectrometer. internal standard was used to correct for quenching.

Three series of experiments were performed. Ser I and II were on ^{3}Hth, and series III on tritia water. A dilution of 1 : 2 and 1 : 3 occurred when ml ^{3}Hth was injected. The results show a ra disappearance of ^{3}Hth from the anterior chamb The values for series I fell from 5,917 c.p.m. at 5 m to 2,382 at 20 min; from 5,767 at 5 min to 1,300 40 min; and from 6,722 at 5 min to 659 at 1 h. T values for series II fell into a similar pattern, fall from 5,726 at 5 min to 2,180 at 20 min; from 6, at 5 min to 991 at 40 min; from 7,239 at 5 min 482 at 60 min; and from 6,815 at 5 min to 48 at 2 Another experiment showed a 2-h reading approx ately the same as in the previous 2-h experiment (

A graph of these values for series I and II shows disappearance of ^{3}Hth to be at an apparently ra rate (Fig. 1). A plot on a semilogarithmic scale of values expressed as a percentage of the correspon 5-min reference values indicates that there may be exponential rates of disappearance for ^{3}Hth: a m rapid earlier component and a somewhat slower co ponent (Fig. 1, insert *A*). The rates were read direc from the graph as the slopes: 6·9 per cent/min fr 5–20 min, and 3 7 per cent/min from 20–120 min.

In the control experiment (series III) very ra disappearance of tritiated water from the ante chamber was noted. Five-minute values in the f experiments ranged from near 47,000 to near 54, whereas 0·1 ml. of the tritiated water solution injec gave a value near 207,000 c.p.m. A semilogarith plot of the series III values expressed as per cent of 2·5- or 5-min values suggests that more than exponential rate of disappearance is also present tritiated water. There is a rapid rate of loss at and a lower rate of loss afterwards. The value 19·7 per cent/min for tritiated water for the 5–20- interval compares favourably with the overall v of 18·5 per cent for deuterium oxide in this ra obtained by Kinsey *et al.*[3]. Therefore, the metho obtaining the values for the rate of disappearanc tritium may be assumed fairly accurate, and values obtained plausible.

As indicated here, the experiments on ^{3}Hth sug that the rate of disappearance of tritium lies betw

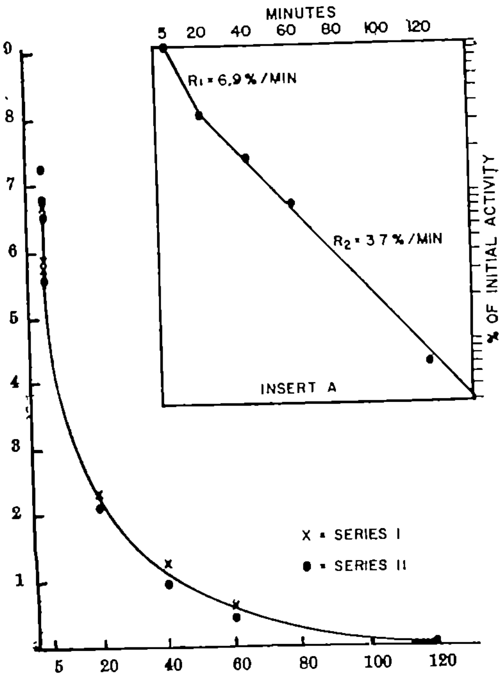

Fig. 1. Disappearance of tritium-labelled thymidine (³Hth) from the anterior chamber of the eye

lues of 3·7 and 6·9 per cent/min. It can thus concluded that the availability of ³Hth in the ueous humour, following injection into the anterior amber, is of short duration. The possibility remains, wever, that ³Hth (or another tritium-labelled DNA ecursor derived from ³Hth)[1] may be retained within e tissue for longer periods of time.

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Use of Radioisotopes in Laboratory Energy Assimilation Investigations with *Daphnia*

In most zooplankton–phytoplankton grazing studies, assimilation by the grazers (which is the true measure of energy transfer in a food web) has been inferred indirectly from measurements of phytoplankton cell concentration and zooplankton filtration, egestion, growth, and respiration rates. A more rapid and critical method of assessing zooplankton assimilation is to measure it directly with a radiosotope tracer and translate radioactivity into energy nits. Energy units (calories) are the logical choice r measurement of such rates, as all matter capable being assimilated by an organism can be converted eadily into a caloric 'common denominator' for asy comparison.

In controlled grazing experiments, assimilation of *Chlamydomonas* sp. (10,000 cells/ml. labelled with phosphorus-32) by *Daphnia pulex* was measured directly by 'counting' animals which had been allowed to graze on non-radioactive carmine particles after grazing periods of successively longer duration in the labelled algæ concentration. The animals were kept in the carmine suspension until all radioactive algæ in the digestive tract had been forced out. Dry weights of the animals were obtained at each time period, so as to assess assimilation as counts/min/mg dry weight. All counts were corrected for background, isotope decay, and direct absorption and adsorption from the water, and 'normalized' to a cell concentration of 10,000 cells/ml. The number of calories assimilated per milligram dry weight of *Daphnia* was calculated for each time period by multiplying 'normalized' c.p.m. at time *t*/mg *Daphnia* (cleared gut) by the number of *Chlamydomonas* cells/radioactive count (mean of experimental results) by the number of calories/cell (from mean weights and mean caloric values of *Chlamydomonas* concentrations)[1].

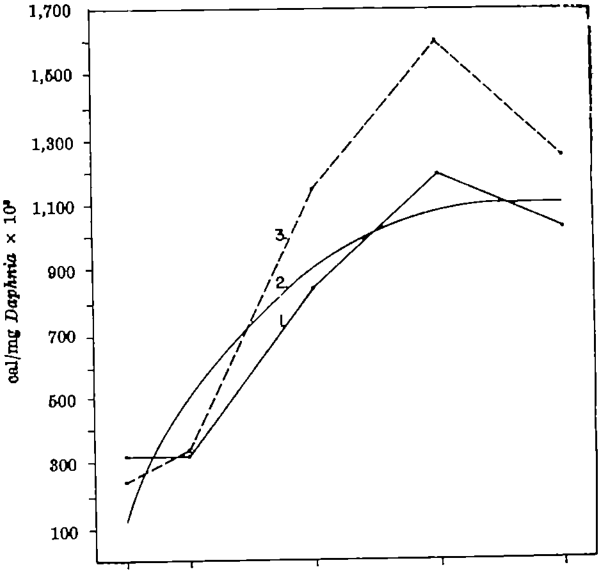

Fig. 1. Assimilation-rate (1), theoretical assimilation-rate (2) and 'ingestion'-rate (3) for *Daphnia* grazing for 24 h on *Chlamydomonas* labelled with phosphorus-32 (10,000 cells/ml.)

The solid line joining the points in Fig. 1 depicts a 24-h time-sequence energy transfer from 10,000 *Chlamydomonas* cells/ml. to *Daphnia*. Assimilation efficiency (assimilation-rate/'ingestion'-rate) can be computed between any two time periods if we assume both rates are linear between any two periods. 'Ingestion'-rates were determined by 'counting' animals without first clearing their digestive tracts, and plotting these counts to establish the broken-line curve in Fig. 1. A point on the 'ingestion' curve at time *t* represents energy assimilated plus the energy content of food in the gut at time *t*, not total ingestion to time *t* (which would be energy assimilated plus 'energy' egested plus 'energy' still in the gut). The curve nevertheless describes the rate of supply of potentially assimilable energy, and can be used to compute assimilation efficiency. Assimilation efficiency between 6 and 12 h grazing time was (0·08835 cal/mg/h)/(0·13010 cal/mg/h) = 68 per cent, while between 12 and 18 h it was 0·05873/0·07440 = 79 per cent. Assimilation efficiency of *Daphnia* grazing in 25,000 cells/ml. for 40 days was 31 per cent[1], while efficiency of *Calanus finmarchicus* was 88–99 per cent (grazing on 620–62,000 *Chlamydomonas pulsatilla* cells/ml.)[2].

A theoretical assimilation-rate was computed for comparison to the actual rate, by assuming that the increase in assimilation with time is described by the equation:

$$A = A_0 \, [1 - e^{-t(\lambda+\mu)}]$$

A = assimilation (cal/mg) at any time t, A_0 = assimilation at 'steady state' (about 18 h), t = time (h), λ = radioactive decay constant (0·693/phosphorus-32 half-life in h), and μ = constant for biological half-life[3]. In theory a straight line is obtained when log $(A_0 - A)$ is plotted against time, the equation being:

$$\log(A_0 - A) = \log A_0 - [0{\cdot}434t\,(\lambda + \mu)]$$

In this work, a straight line was fitted to the logarithmically transformed assimilation difference, $\log(A_0 - A)$, by least squares. Each log difference was adjusted afterwards to the line, and then converted back to $(A_0 - A)$. Adjusted assimilation at each time period, A, was obtained by subtracting $(A_0 - A)$ from A_0, and these values were plotted in Fig. 1. It is seen that the smoothed theoretical curve closely approximates the actual assimilation curve.

With the preliminary results indicating the feasibility of obtaining energy assimilation-rates by direct and rapid isotope-labelling techniques, investigations of energy relationships and interactions can proceed using combinations of phytoplankton food (after establishing caloric constants) and zooplankton grazers under controlled light, temperature, and nutrient conditions.

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BIOLOGY

Specific Gravity Changes during the Development of the Sheep Fœtus

In work concerned with pre-natal growth measurements in sheep and their relation to patterns of mammalian growth[1] it was necessary to obtain estimates of changes during development in the specific gravity of the fœtus. Information on such changes does not appear to have been given previously although the specific gravity of mutton carcasses has been recorded and the results used to estimate fat content[2,3].

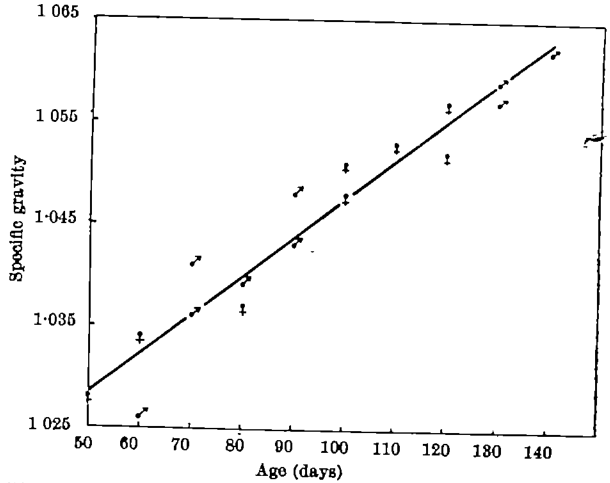

Fig. 1. Changes in the specific gravity of the whole fœtus with increase in fœtal age. ♂, Ram fœtuses; ♀, ewe fœtuses, ——, least squares regression of specific gravity on age

Fœtuses were obtained from fine-wooled, Australian Merino ewes mated to a ram of the same strain, and fœtal ages, which were calculated from the last day of the œstrus in which conception occurred, ranged from 50 to 140 days. Fœtuses were taken at ten-day intervals within this period and, usually, two fœtuses were obtained at each age. The specific gravity of the whole fœtus was obtained from the weight in air and in water at room temperature (20°–25° C). Next, the carcass was dissected and the specific gravities of six different bones and four different muscles were obtained by the same method. In addition the composition of the whole carcass in terms of percentage dry matter, fat and ash was found from chemical analyses.

The results from this work are summarized in Fig. 1 and Table 1. The specific gravity of the whole fœtus, shown in Fig. 1, increased steadily from a value of approximately 1·03 at 50 days to about 1·06 at 140 days. The relationship did not show significant departures from a linear regression of specific gravity on age although plots of the means suggested a tendency towards a curve.

The specific gravities of the bone and muscle tissues are shown in Table 1, except that the specific gravity measurements taken at 50 days, and also the measurements taken on the muscles at 60 days, were inaccurate and have not been included. The specific gravity measurements are given for the individual bones as these showed quite large differences, and, in addition, for each bone there were differences between fœtuses at any one age. The specific gravities of the muscle tissues, however, were all fairly uniform and only the mean specific gravity for the four muscles, longissimus dorsi, latissimus dorsi, supraspinatus and biceps femoris has been given in Table 1. As shown, the average specific gravity of the fœtal bones increased

Table 1. Specific Gravities of the Bone and Muscle Tissues of the Body of the Sheep Fœtus

Age (days)	No. of fœtuses	Specific gravities of the individual bones						Mean bone specific gravity	Muscle specific gravity (means of 4 individual muscles)
		Scapula	Humerus	Fore-cannon	Femur	Tibia–fibula	Hind-cannon		
60	2	1·080	1·078	1 110	1 082	1·109	1 133	1·096	—
70	2	1·112	1·120	1·194	1·101	1 174	1·150	1·146	1·030
80	2	1·157	1 139	1 172	1·122	1 161	1·160	1·151	1 029
90	2	1·164	1·158	1·180	1·137	1·183	1 180	1·167	1·035
100	2	1 185	1·165	1·184	1·150	1 185	1·179	1·174	1·038
110	1	1 198	1·179	1 205	1·164	1 210	1·203	1 193	1·044
120	2	1 200	1 183	1 208	1·175	1·206	1·211	1 197	1·047
130	2	1·199	1 201	1 216	1 190	1·219	1 216	1 206	1·053
140	1	1·197	1·212	1·226	1 198	1·231	1·248	1 218	1·057

steadily with increases in fœtal age. Similarly, muscle tissue increased in specific gravity, but at a much slower rate than bone, and there were no marked differences between individual muscles or between the muscle tissues of different fœtuses at the same age. As expected, the specific gravity of the bone was much higher than that of the whole fœtus while the specific gravity of the muscle was lower.

The results from the chemical analyses showed that the percentage of dry matter in the fœtuses increased during pre-natal life from about 10 per cent to 20 per cent. It was not possible, however, to obtain reliable estimates of the composition of the dry matter at the time the rest of the work was carried out, and the results from the stored material were not particularly accurate. Nevertheless, it was evident that the amount of fat in the dry matter increased markedly over the age period studied, while, at the same time, there was a fall in the percentage of ash.

These results show that, in spite of an increase in fat content, the specific gravity of the whole fœtus increased markedly during the later stages of development and growth. The two interrelated causes of this effect appear to be the fall in the water content and the marked increase in the specific gravity of the bone, muscle, and possibly other tissues of the body. Bone and muscle make up by far the greatest proportion of the total weight of the carcass and the percentage of bone appears to increase slightly at later fœtal ages[4]. Also, muscle tissue probably comprises a fairly large proportion of the internal organs, or offal parts, and besides the carcass, the organs comprise the other major component, by weight of the body[4].

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Mitochondrial Changes during the Process of Awakening from Hibernation

Liver mitochondrial succinoxidase (SO) activity of hibernating hamsters is markedly lower than that of cold-acclimatized awake controls when assayed at the body temperature of the hibernating hamsters, 7° C[1,2]. However, when the same mitochondrial preparations are assayed at the body-temperature of awake hamsters, 37° C, no such difference is observed[1,2]. It is apparent that an SO-inhibiting substance or mechanism in the mitochondria of the hibernating animals operates at 7° C. but not at 37° C.

To find out if this characteristic inhibition is still present just after hamsters awaken from hibernation, SO assays were made simultaneously on mitochondria from three groups: animals killed during hibernation (Group I), controls which had been kept in the same 5° C cold-room but had not hibernated (Group II), and hamsters killed just after being awakened from hibernation (Group III). The results of these studies are presented in this communication.

Adult male hamsters (*Mesocricetus auratus*) weighing 90–110 g were caged individually with wood-chip beds in a cold-room (5° ± 2° C). They were supplied with water and 'Purina Chow' pellets *ad libitum*. After 8 weeks some of these hamsters hibernated. In hamsters which had been hibernating 2–5 days, initiation of the awakening process was induced by gentle prodding with a pencil eraser for a few seconds. Then these animals were left undisturbed until they had fully awakened—about 3½ h. The heart-rate, breathing-rate, and general activity of these newly awakened hamsters are slightly above prehibernation rates[3].

The liver mitochondria were isolated, and SO activity and associated phosphorylations measured by standard techniques described in detail in previous work[1]. The Q_{O_2} (µl. oxygen/mg dry wt./h) values recorded in Table 1 were obtained with an acceptor system consisting of hexokinase and glucose in the reaction mixture. For comparison, the 'per cent temperature effect':

$$\left(\frac{Q_{O_2} \text{ at } 37^\circ \text{ C}}{Q_{O_2} \text{ at } 7^\circ \text{ C}} \times 100\right)$$

for each group is recorded in Table 1.

Table 1. Mitochondrial Succinoxidase Activities, P : O Ratios and per cent Temperature Effects

Group	No. of hamsters	Q_{O_2} assayed at 37°	P : O at 37°*	Q_{O_2} at 7°	P : O at 7°	Per cent temperature effect
I	14	73 ± 9	1·3 ± 0·3	5·2 ± 0·6	1·6 ± 0·4	1,420 ± 172
II	12	84 ± 6†	1·5 ± 0·2	9·6 ± 0·7†	1·7 ± 0·2	874 ± 63†
III	13	81 ± 9	1·3 ± 0·3	8·6 ± 0·5†	1·5 ± 0·5	945 ± 81†
IV	6	76 ± 6	1·4 ± 0·5	5·5 ± 0·5	1·5 ± 0·4	1,382 ± 164

* There were no statistically significant differences in the P : O ratios (moles of phosphate esterified per gram atom oxygen consumed) among the four groups at either 7° C or 37° C.

† Statistically different from group 1, $P < 0·01$.

The results given in Table 1 show that distinct mitochondrial changes occur during the awakening process. The low Q_{O_2} at 7° C characteristic of mitochondria from hibernating hamsters does not appear in the Group III mitochondria. Moreover, the Group III mitochondrial per cent temperature effect is close to, if not equal to, that of control (Group II) mitochondria.

The mitochondria of the hibernating animals (Group I) never became warm between the time the animal entered hibernation and the time of assay at 7° C, since isolation is performed at about 1° C. The fact that when these mitochondria are assayed at 37° C their Q_{O_2} is relatively high suggested the possibility that the SO-blocking mechanism which operates at 7° C is simply eliminated by the higher temperature.

To determine the extent of heat-lability of the inhibitory mechanism, aliquots of Group I mitochondrial suspensions were put in tubes, heated to 37° C, held at this temperature for 6 min, then cooled back down to 1° C. Group I mitochondria thus treated are designated as Group IV mitochondria in Table 1. For comparison, other aliquots of the same Group I preparations were held at 1° C, never being permitted to become warm. SO assays on the different aliquots were then conducted at 7° C and 37° C. At neither assay temperature were significant differences observed (Table 1). From this *in vitro* study, it appears that the factor in Group I mitochondria which inhibits SO at 7° C is not eliminated simply by short-term heating. Consequently, it is concluded that the *in vivo* disappearance of this factor probably does not result simply from the mitochondria reaching a critical temperature as the body of the awakening hamster returns to 37° C.

However, the period of awakening is more than 3½ h, and it is possible that this longer period of time during which mitochondrial temperature gradually increases is influential in eliminating the inhibitory factor.

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Chromatophoric Responses of Eyestalk-less Shrimps

REMOVAL of the eyestalk from a shrimp, *Palaemon serratus*, induces complete dispersal of the bichromatic chromatophores of the body. Afterwards, the general coloration of the shrimp becomes red. The bands appear as marked and continuous lines. This coloured aspect is reached a few hours after the bilateral removal and eyestalkless shrimps keep it permanently, in spite of the background colour.

In the experiments reported here, I noticed that the chromatophoric behaviour of eyestalkless shrimps did not settle completely after bilateral ablation.

Twelve *Palaemon serratus*, in the *C*-stage of their intermoult, were previously kept on white background under illumination for two weeks. Eyestalks were then cut off, on February 1, and the shrimps were set again on white background, in individual containers, supplied with running sea-water. Animals were fed twice a week. Water temperature was about 13° to 16° for two months (February and March). During this time, the shrimps moulted twice.

After removal of the eyestalks, they darkened quickly and remained red throughout February. Early in March, the colour became paler, by loss of pigment and by concentration of chromatophores. These operated animals became as white as control animals, which were maintained on illuminated white background for the same time. The band chromatophores were dispersed at intermediate stages, and those scattered on the body strongly concentrated. On March 19, six of these eyestalkless *Palaemon* were removed from their white background and put before a dark one. Six controls were treated the same. Chromatophores of the eyestalkless shrimps began then to expand for 24 h, so that shrimps were finally pale red, slightly pink. This colour was quite different from the white colour of the other blind shrimps left on the white background. The normal animals, placed on dark background, darkened too: their chromatophores were less expanded than those of the operated shrimps, but their coloured aspect was undoubtedly more evident, because of the blue diffusion and of normal rate of pigmentation in cells. All these animals were once more set again on white background, after three days of black adaptation. Chromatophores of these six eyestalkless *Palaemon* did not seem to concentrate or became so sluggish that concentration was no longer apparent. Intact animals changed and became pale normally.

In this case, a darkening substance acts, which does not depend on the eyestalk. Knowles, Carlisle and Dupont-Raabe[1] separated dispersing and concentrating substances from post-commissural organ extracts by electrophoresis, and Brown, Webb and Sandeen[2] have pointed out that the highest ratio of red dispersing hormone to concentrating substance is found in the abdominal cord. But we may suppose, in this special assay of adaptation, existence of photoreceptors, different from eyes, or a direct and secondary action of light, either on chromatophores (Fingerman *et al.*[3] gave examples of responses of melanophores of eyestalkless *Sesarma reticulatum* to incident light) or may be the abdominal nerve cord. On the other hand, these results must be compared with the experimental analysis of the responses of chromatophores of *Palaemonetes vulgaris* to long adaptation[4]. This experience gave evidence of stocking of chromatophoric substances, which were not used for adaptation, in post-commissural organs. Here the previous white adaptation may have led to storage of darkening substance. Finally, I must refer to the persistence of activators of pigment in supracesophageal and circumphageal connectives, established by Fingerman and Mobberly[5] in a cave-blind crayfish, *Orconectes pellucidus australis*. It demonstrated that "the loss of the controlling mechanism takes longer than the loss of the end organ". I found a similar situation here.

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Photophore and Eye Movement in the Euphausiid *Meganyctiphanes norvegica* (G. O. Sars)

THE complex structure of the light-emitting organs of euphausiids has long been known[1,2], though their precise function has eluded investigators. While examining further this problem, it has become clear that these organs, all of which are movable except those on the eye-stalks, do not operate independently but may be co-ordinated. *Meganyctiphanes* has 10 photophores, one incorporated in the eye-stalk below each eye, 4 in the thoracic region at the bases of the 2nd and 7th pairs of appendages, and one between each of the first 4 pairs of pleopods. The eye-stalks, bearing the large compound eyes and photophores, project laterally and are closely approximated at their base in such a way that the eyes, though they may be moved independently to some extent, may be turned together in the sagittal plane, the amount of possible rotation being greater than 90°. The thoracic and abdominal photophores can turn through nearly 180° to face forwards, ventrally, or backwards.

Fresh specimens may be seen to flash brilliantly when handled in the dark[3], and they may afterwards glow at a lower intensity for variable periods, with all the photophores in action. The stimuli inciting the different luminescent reactions are at present under investigation[4].

The co-ordinated movement of the eyes and photophores may be seen by observing luminescing specimens retained ventral side uppermost in a dark room in red light. When making these observations the animals were held by threads on a 'Perspex' plate and were viewed with a binocular microscope or hand-lens.

A microscope light and red filter were used for illumination: it is likely that some light of wave-lengths other than red also reached the animal. When viewed as here, all photophores, including those on the eye-stalks, are usually facing ventrally or postero-ventrally. When the light is shaded all the photophores can be seen to flick forwards, and then back as the light is again directed on the animal. This was repeated when the light was shaded approximately once a second. Specimens that are not luminescing may still show the movement of the eyes: the latter turning forward when they are shaded, so that the photophores on the eye-stalk, previously facing postero-ventrally, point forwards. Dark adapted specimens subjected to an electronic flash will not show this response immediately, the delay lasting for approximately 2 min. These movements in response to changes in light intensity are being investigated further.

The abdominal photophores are each linked by 4 strands to a ligament running either side of the ventral abdominal artery; this system will be described elsewhere. Chun figured nerves to these photophores in *Stylocheiron;* they are also present in *Meganyctiphanes*.

I thank R. H. Kay and my father for confirming these observations.

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Oxygen Dependency of *In vivo* Photophosphorylation

The mechanism by which ATP is formed when the intact green is illuminated is not yet fully understood. ATP formation in isolated chloroplasts has been shown to be dependent on illumination[1] and has been shown to proceed by either a cyclic or a non-cyclic process depending on the oxido-reduction catalysts used[2]. Chloroplasts are capable of photophosphorylation at low rates in the absence of catalysts. This endogenous activity has been demonstrated to be oxygen dependent[3,4]. Ion-exchange chromatographic studies of the ATP content of intact barley seedlings show that the steady-state level of ATP in light is several fold that in dark[5]. The level with illumination drops if the leaves are exposed to nitrogen or to carbon monoxide–oxygen mixtures. This was interpreted as evidence that formation of ATP in barley seedlings in light was an oxidative process, using molecular oxygen as a terminal electron acceptor.

Uptake of glucose or sucrose by floating leaf disks has been shown to be dependent on the presence of oxygen and to be inhibited by 2,4-dinitrophenol[6]. It has also been shown that sections of tobacco leaf can utilize glucose from solution to produce starch in darkness in air but not in nitrogen[7]. This process would require at least three high-energy phosphate bonds for storage of one mole of glucose as starch if starch is formed in the plant via the uridine diphosphate pathway by which glycogen is now known to be formed in animals. Thus the rate of starch formation from glucose can be used as an index of the rate at which an intact leaf can form ATP. Machlachlan and Porter have used this device, and have claimed that starch is formed as rapidly by tobacco disks floated on glucose solutions *in vacuo* in light as in air in light[7]. They interpret this as evidence that cyclic photophosphorylation occurs in the leaf disks. This communication reports similar experiments performed under a stream of highly pure nitrogen in which the opposite results from those of Machlachlan and Porter were obtained.

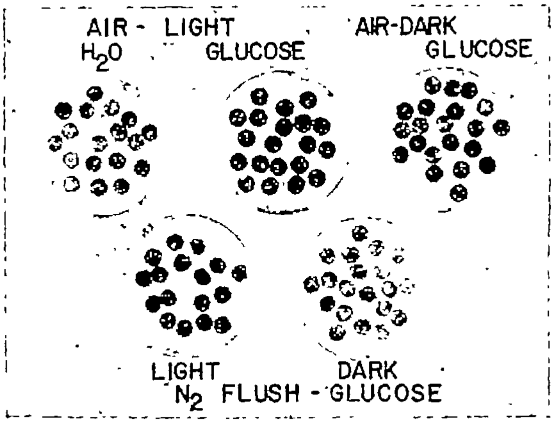

Fig. 1. Starch formation by tobacco leaf sections floated on water or 5 per cent glucose. The three upper experiments were run in air, the two lower experiments under a stream of Matheson 'Prepurified' grade nitrogen. Temperature, 23° C. Time, 24 h. Illumination, > 500 ft.-candles white light

Tobacco leaf disks were cut from large, thin, tobacco leaves made free of starch by storage in dark in air with the petiole immersed in water. Starch content was tested by immersing the boiled, ethanol-extracted, leaf sections in the usual iodine–potassium iodide solution. The leaf sections were cut with a sharp cork borer and floated on water for a few minutes before floating on 5 per cent glucose or sucrose solutions. Preliminary experiments showed that exposure for 24 h was necessary in order to obtain complete blackening of leaf sections in air in light. Presence or absence of carbon dioxide in the air and flushing the sample with air made no difference in the degree of blackening obtained. Exposure to air in darkness always gave less starch formation than exposure to light in air. The experiments illustrated by Fig. 1 show the results obtained by these treatments. The lack of starch formed when leaves are floated on water demonstrate that the starch is formed from glucose and not by photosynthetic uptake of carbon dioxide.

The almost complete lack of starch formation by the sections illuminated in a stream of nitrogen demonstrate the requirement for oxygen of this process. When the leaf sections are exposed to 99·5 per cent nitrogen while they are illuminated, they formed less starch than those illuminated in air, about the same as those exposed to air in dark and considerably more than those exposed to pure nitrogen in light. Thus only a small amount of oxygen is required for the activity of the oxidative enzyme involved in photosynthetic formation of ATP. The much more rapid formation of starch in light than in dark in air fits well with our earlier observation that ATP-levels are several fold higher in illuminated than in darkened leaves.

In other experiments, we have found that 3-*p*-chlorophenyl-1,1-dimethyl urea, an agent known to inhibit non-cyclic but not cyclic photophosphorylation[8], completely blocks starch formation in air in light. This means that the *in vivo* photophosphorylation leading to starch formation in tobacco leaves is a non-cyclic process rather than cyclic process and that it utilizes molecular oxygen as a terminal electron acceptor.

It is not known with certainty why our results differ from those of Machlachlan and Porter. Flushing with high-purity nitrogen is probably more effective than evacuation as a means of removing small traces of oxygen that would be formed by accompanying photosynthetic fixation of carbon dioxide produced by anaerobic metabolism of glucose.

These results must not be interpreted as meaning that all ATP formed during photosynthesis is produced by a process utilizing oxygen as a terminal oxidant, even though that might prove to be the case eventually. The rate of starch formation here is probably far lower than the maximal rate of fixation of carbon dioxide attainable by the plant. These experiments indicate only that the ATP utilized for glucose uptake and starch formation is not formed by a so-called cyclic or anaerobic process in the intact tobacco leaf, but by a phosphorylation coupled to an electron transport system utilizing oxygen as a terminal acceptor.

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Taxonomic Disposition of *Allium*

THE rust fungi evolve in genetic balance with their host plants, and inevitably reflect their interrelationships. Although rust investigations usually merely confirm accepted classifications, occasionally, like studies in comparative biochemistry, they may suggest modifications of accepted ideas. A few such suggestions have already been presented[1], among them the indication that the occasional occurrence of *Puccinia asparagi* on *Allium* threw doubt on Hutchinson's[2] transfer of Allieae to Amaryllidaceae, a disposition followed by several recent authors. Hutchinson maintained, with apparent reason, that the umbellate inflorescence with an involucre of bracts, seen in Allieae and typical Amaryllidaceae, was of greater significance than the position of the ovary. However, new and more significant evidence from the rust fungi requires a reconsideration of this disposition.

I have recently shown[3] that the rusts of *Allium* form a complex evolutionary series, with one group mainly in the North American Cordillera and a second, still inadequately studied, in Eurasia. The series illustrates a repeated tendency toward simplification of the life-cycle and the development of dispersive teliospores, but three separate morphological characters show the species to be related: the occurrence of numerous scattered germ-pores in the urediniospores; the occurrence of delicate and widely spaced ridges, tending to anastomose above, on the teliospore walls; and the very rare occurrence, or generally complete lack, of germ-pores in the teliospores. The first of these characters is not uncommon in *Puccinia* and *Uromyces*, but the others are so unusual as to command attention even if they did not occur together.

No rust is known on any amaryllidaceous host which shows these characters, singly or in combination; but, attacking *Bellevalia*, *Hyacinthus*, *Muscari* and *Scilla*, of the tribe Scilleae in Liliaceae, we find *Uromyces muscari* (with several *formae speciales*) and the closely related *U. circinalis* and *U. algeriensis*. These rusts are essentially short-cycled; but urediniospores are occasionally found in the telia and prove to have several scattered germ-pores. The teliospores are generally somewhat more distinctly sculptured than those of the *Allium* rusts, but show the same pattern of widely spaced ridges anastomosing toward the apex of the spore; and the teliospores lack germ-pores, germination taking place through an irregular split in the spore wall. The correlated occurrence of these characters, two of them practically unique in the rust fungi, is convincing evidence that these rusts are actually closely related to those on *Allium*. However, the shortened life-cycle, and the fully deciduous and more conspicuously sculptured teliospores, make it clear that the *U. muscari* complex is more highly evolved than are the *Allium* rusts.

It appears from this evidence that *Allium* is related to, but more primitive than, *Scilla* and related genera. If the inflorescence characters emphasized by Hutchinson are a reliable indication of relationship to the amaryllids, the most satisfactory disposition of Allieae seems to lie in restoring the group to family rank. Alliaceae may then be regarded as close to the immediate common ancestor of Liliaceae and Amaryllidaceae. Hutchinson's arrangement, with Amaryllidaceae derived from Liliaceae and containing Allieae, is unrealistic, both because it denies the proximity of *Allium* to Liliaceae, and because it suggests that Allieae are more modern than Liliaceae whereas the rust relationships indicate the reverse.

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VIROLOGY

Hæmadsorption to Single Cells in Suspension

IN work on the reproduction of viruses there is often need to determine the number of cells reaching a certain stage in the multiplication cycle at a given time. Techniques involving fluorescent antibody, autoradiography, staining for inclusion bodies or hæmadsorption satisfactorily identify the infected cells in a monolayer culture, but the negative cells are almost impossible to count with certainty because their borders tend to be indistinct. Moreover, it is not possible to make continuous observations on a single population of cells since a culture must be killed every time a reading is made.

These difficulties may be overcome by maintaining the cells in suspension culture[1], and examining samples at intervals. We have used hæmadsorption to single cells in suspension as an index of viral infection. Following inoculation with virus, samples are mixed with red cells and counted in a hæmocytometer. Hæmadsorbing cells stand out in marked contrast to the others, and both can be counted with ease and accuracy (Fig. 1).

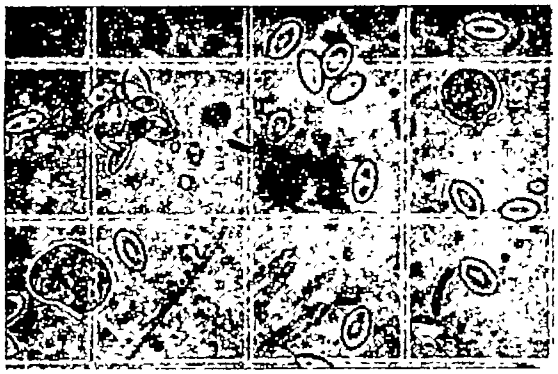

Fig. 1. Hæmadsorption to single HeLa cells in suspension. The cell at top left is positive, the other two negative

The cells may be grown initially in suspension culture or grown as a monolayer then resuspended. The single cells are then continuously agitated at 36° C in a maintenance medium based on Eagle's calcium and magnesium-free medium for suspension culture[2]. Neither silicone-coated glassware nor methylcellulose has been found necessary. Virus is inoculated directly into the culture.

We have applied the technique specifically to the problem of delay in 'initiation' of infection[3,4]. It has been established that influenza virus experiences this delay as a necessary step in its normal cycle of multiplication. We are now studying a variety of types of abortive infection to determine whether or not they also involve this step.

In HeLa cells influenza virus undergoes an abortive cycle of multiplication leading only to the production of non-infectious hæmagglutinin[5]. Because no second cycle of infection occurs, this is an ideal system in which to measure the parameters of delay in initiation. We have found hæmadsorption to occur quite normally under these conditions and have used it as an indirect index of the time at which initiation has occurred in any given cell.

Dilutions of influenza virus, strain *MEL*, dispersed by ultrasonic vibration, were added to HeLa cells in suspension. At 30 min intervals the cells were sampled, mixed with fowl red cells at a final concentration of 0·1 per cent, kept at 4° C for 0·5 h, then counted in a hæmocytometer. The percentage of HeLa cells becoming hæmadsorption-positive in each 0·5-h interval is plotted in Fig. 2.

Following inoculation at all multiplicities in excess of unity about 80 per cent of the cells eventually became infected, the remainder being refractory. At high multiplicities all susceptible cells almost simultaneously acquired the capacity to adsorb red cells.

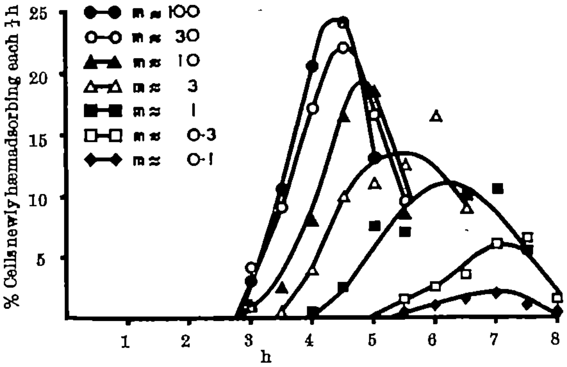

Fig 2. Time of acquisition by HeLa cells of capacity to adsorb red blood cells. HeLa cells were infected in suspension with influenza virus at different multiplicities of infection (*m*) The percentage of cells becoming hæmadsorption-positive in each 0 5 h interval is plotted

When the multiplicity was dropped to one or less, marked asynchrony between cells in the acquisition of this property became apparent, the mean delay being of the same order as that shown by suspended allantois-on-shell on infection with the same virus[6], namely, about 4 h. This clearly reflects delay in initiation of infection.

The method should be applicable to any of the hæmadsorbing viruses[7], vaccinia, influenza, para-influenza, NDV, mumps or measles, and to any cell capable of being maintained for short intervals in suspension.

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MICROBIOLOGY

Divergent Pathways of Indole Metabolism in *Chromobacterium violaceum*

THE first observations of violacein formation by bacteria were reported almost a century ago[1]. Since then, occasional investigations of the production and chemistry of this pigment were made without revealing much information on its nature[2]. A significant contribution to this problem was made only recently by Ballantine *et al.*, who elucidated the structure of this pigment and confirmed it by chemical synthesis[3]. Their results showed that the violacein molecule consists of a 5-hydroxyindole, an α-pyrrolidone and an oxindole unit, and suggested that all three units could be derived from tryptophan. Indeed, results of a study, which was also made in recent years by DeMoss and Evans, established that this amino-acid was the only substance required for the biosynthesis of violacein[4].

In our investigations on the biogenesis of this pigment by a non-gelatinous strain of *Chromobacterium violaceum* A.T.C.C. 533 (ref. 5), we confirmed that this organism readily converted L-tryptophan to violacein, and found that it did not utilize this amino-acid for growth. This observation served as a basis for the development of a novel microbiological assay which is specific for L-tryptophan and in which the quantity of violacein produced is a function of the amount of L-tryptophan present in the test sample[6]. The D-isomer of this amino-acid was not utilized by the bacterium.

Nutritional requirements of *C. violaceum* were found to be modest, since any one of the following L-amino-acids alone provided carbon and nitrogen for growth: alanine, phenylalanine, tyrosine, histidine, glutamic acid (and, to some extent, also arginine and aspartic acid). (The medium consisted of (mg/100 ml.): $MgCl_2.6H_2O$ 10; K_2HPO_4 60; K_2SO_4 50; $FeSO_4$ 0·1; amino-acid 100–200, *p*H 7·2.) In the presence of these compounds, the cells grew for 1–2 days without elaborating any pigment. When incubated longer, small amounts of violacein always appeared in the cells, probably as a result of a low rate of L-tryptophan synthesis from these amino-acids.

For the study on the formation of this pigment, 16–20-h-old colourless washed cells were used. (The cells were inoculated in 250 ml. flasks, each containing 100 ml. of a medium consisting of (g/100 ml.) glucose 0·5; Difco yeast extract 0·1; K_2HPO_4 0·1; $MgSO_4.7H_2O$ 0·02; and adjusted to *p*H 7·0. After an overnight incubation at 28° C in shaken flasks, the medium became slightly acidic (*p*H 6·0–6·7), under which conditions no pigment was formed. The cells were collected by centrifugation and washed once with water.) They were resuspended in phosphate buffer (0·05 M, *p*H 7·2–7·5) which contained L-tryptophan (1 mg/ml.). Since atmospheric oxygen is required for violacein formation[4], they were then incubated in aerated (shaken) flasks at 28° C for 24–48 h. Pigmented material, which was synthesized in the cells during that time, was extracted with ethyl acetate and analysed by thin-layer chromatography (Silica Gel *G* or Aluminium Oxide *G*; Merck, A. G., Darmstadt). As expected, violacein was found to be the main pigment, and deoxyviolacein was a minor component. When this experiment was repeated with lyophilized preparations of washed cells, an indole-like odour was noted within 24 h of incubation. (The drying had to be completed in 2–3 h. Longer lyophilization, or drying at room temperature, gave inactive preparations.) The material was identified as indole in the following way: The suspension was adjusted to *p*H 9·0 and extracted with methylene chloride. The extract was dried over sodium sulphate and evaporated *in vacuo*. The residue had three absorption maxima which were identical with those of indole: 272, 278, and 288 mμ (in methylene chloride). In programmed vapour-phase chromatography on a silicone polymer column, the main component of the residue had the same retention time as indole. A single compound with R_F values of indole was detected in this residue by descending paper chromatography (0·26 in *cyclo*hexane saturated with formamide) and by thin-layer chromatography on silica gel *G* and aluminium oxide *G* (0·41 and 0·48 resp. in *cyclo*hexane/benzene (1 : 1) solvent system) when sprayed with Ehrlich's reagent.

On the basis of this finding, washed cells of the bacterium were incubated in an atmosphere of indole vapours, which provided the bacterium with indole in sub-inhibitory concentrations. Again, violacein with small amounts of deoxyviolacein was rapidly formed. The ratios of these two pigments and their rates of formation were similar to those obtained in the experiments with L-tryptophan, thus strongly indicating that indole was the first intermediate in the synthesis of violacein from L-tryptophan. Further insight into the metabolism of this micro-organism was obtained by using the same lyophilized preparations as already mentioned here (and already described). When L-tryptophan was incubated with such preparations, indole was transiently formed and, after a prolonged (48-h) incubation, a deep-blue pigment was synthesized. Indole vapours were also metabolized under the same conditions to a pigment of the same colour. The pigmented material, which had been extracted in both cases with hot chloroform, contained neither violacein nor deoxyviolacein. It was identified as indigo on the basis of its colour, absorption spectra (maxima at 285 and 602 mμ in methylene chloride) and R_F values in thin-layer chromatography on silica-gel *G* developed with chloroform and butyl acetate. Another micro-organism, *Pseudomonas indoloxidans*, was reported to form indigo from indole but not from tryptophan[7], while a mutant of *Schyzophillum commune* synthesizes this pigment from ammonium ions by a completely unknown reaction sequence[8].

None of the following compounds, which are related to L-tryptophan and indole, yielded any colour when incubated with washed cells of *C. violaceum*: Tryptamine, 5-hydroxytryptamine, 5-hydroxyindole, indolyl-propionic acid, indolyl-acetic acid, 5-hydroxyindolyl-3-acetic acid, skatole, anthranilic acid, 3-hydroxyanthranilic acid, isatin, L-kynurenine and oxindole. 5-Hydroxytryptophan, which was described to be formed from L-tryptophan by the parent organism[9], was equally inactive in this respect. On the other hand, indoxyl acetate was rapidly condensed to indigo. The latter observation indicates that indoxyl is an intermediate of the indigo pathway in this bacterium, and is comparable with the indoxyl→ indigo condensing system which operates in rat liver mitochondria[10] (Fig. 1).

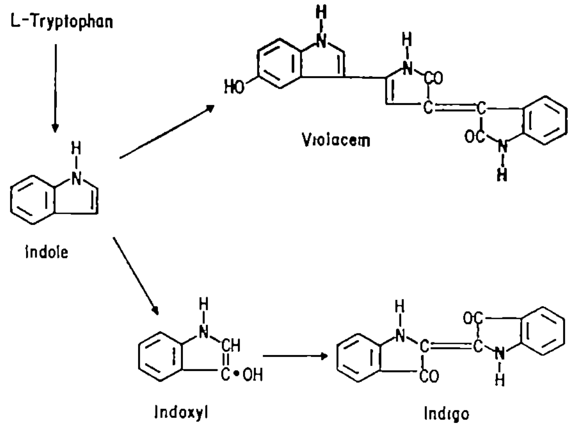

Fig. 1. Proposed metabolism of L-tryptophan and indole in *C. violaceum*

Our findings thus suggest that: (1) *C. violaceum*, which is not considered to form indole[11], metabolizes L-tryptophan to indole by the action of tryptophanase (or tryptophan synthetase); (2) growing and washed cells of this micro-organism synthesize violacein not only from L-tryptophan but also from indole (the violacein pathway); (3) rapid lyophilization of washed cells inactivates enzyme(s) of the violacein pathway and, as a result, both L-tryptophan and indole are metabolized to indigo; (4) indoxyl is a likely intermediate in the indole→indigo condensation; and (5) hydroxylation is not the first step in the violacein synthesis, but takes place at a later stage in the pathway.

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Deoxyribonucleic Acid Base Composition and Taxonomy of some Protozoa

THE characteristics by which organisms are recognized are the expressions of the information contained in their nucleic acids. As such, a knowledge of their DNA base composition, or if possible the base sequences of some organisms, should be a valuable asset in their classification. Lee, Wahl and Barbu[1] were the first to recognize the taxonomic importance of analysing the average DNA base compositions of micro-organisms. While the overall base compositions of some unrelated organisms may be the same, this equivalence would appear to be a minimum requirement for extensive base sequence homologies[2] and genetic compatibility (except in certain cases of *F*-duction[3]). Thus, similar DNA base compositions in two organisms might indicate close relationships whereas dissimilar base compositions would indicate that the organisms are unrelated.

Bacteria, fungi and algae and possibly the protozoans are particularly well suited for an examination of the correlation between their DNA base composition and their taxonomic positions since the DNA compositions of lower organisms vary widely[4–6], whereas those of higher plants and animals lie within a narrow range[7,8].

The taxonomy of protozoa has presented a challenge and has led to major differences in classification of groups as well as individual organisms[9,10]. The protozoa are difficult to classify because of many transitional forms of uncertain relationship[10], confusion of primary and secondary characters[11], and, in the case of the Sporozoa, because of polyphyletic origin[12]. The present investigation was undertaken to examine whether DNA base composition could be a useful aid in protozoan taxonomy.

The mean mole per cent guanine plus cytosine (G + C) content of DNA from representatives from taxonomically related ciliates (Ciliata), from plant and animal-like flagellates (Phyto- and Zoo-mastigophora) and from the 'amoeba'-like slime mould (Rhizopoda) were determined.

The DNA base compositions were determined by cæsium chloride density gradient centrifugation[13], and from their thermal denaturation temperature[14]. The buoyant density of DNA in cæsium chloride is linearly related to its G + C content and its determination requires only small quantities (in the range of 1–2 μg) of DNA either in the purified state or as the cell lysate[15]. It has also been shown that the base composition of purified DNA is linearly related to its thermal denaturation temperature[14]. Purified DNA, when heated in solution, shows a sharp increase in its extinction coefficient at the temperature at which the transition from the native, double-stranded form to the denatured state takes place. The temperature corresponding to the mid-point of the absorbance rise, the T_m, is related to the G + C content of the DNA.

For the determination of the density of the DNA, a purified DNA sample (isolated by Marmur's method[16], for example), a partially purified sample, or a cell lysate can be used. A lysate can be prepared by suspending the cells in saline ethylenediamine tetraacetate (EDTA) solution (0·1 M EDTA plus 0·15 M sodium chloride, *p*H 8·0) and adding sodium lauryl sulphate to a final concentration of 3 per cent. The sample is centrifuged with a DNA standard in a cæsium chloride solution adjusted to a density of 1·700 ± 0·010 g/c.c. at 44,770 r.p.m. in the Spinco model E analytical ultracentrifuge until equilibrium is attained (usually 22 h). The banded DNA is then photographed using ultra-violet absorption optics, the negatives traced with a Joyce-Loebl microdensitometer and the density of the sample determined by establishing its position relative to that of the standard DNA of known density. All buoyant densities in cæsium chloride are related to that of *Escherichia coli* DNA, which is taken as 1·710 g/c.c.

For determination of the T_m, approximately 20 μg/ml. samples of purified DNA in saline–citrate solution (0·15 M sodium chloride plus 0·015 M sodium citrate) contained in glass-stoppered quartz cuvettes were placed in the Beckman *DU* spectrophotometer, the temperature of which could be raised by circulating hot water through two thermal spacers. The optical density, corrected for thermal expansion, is divided by the value at 25° C, and this ratio is plotted versus the temperature of the solution.

The flagellates examined in this investigation have G + C contents within the range 46–61 per cent, while the ciliates, represented only by the Holotrichs, range within 22–35 per cent G + C (Table 1). *Chlamydomonas rheinhardii*, which can be classified as Phytomastigophora, has a G + C content of 64–66 per cent[6,17], a value consistent with the high G + C composition of flagellates.

Within the flagellates, certain zooflagellates contained a minor 'satellite' DNA band when banded in cæsium chloride. A purified sample of DNA from *Crithidia fasciculata* was found to have a satellite band with a density of 1·698 g/c.c. (39 per cent G + C). The DNA of *Strigomonas oncopelti* was found to have at least one and possibly two satellite bands with densities of 1·693 g/c.c. (33 per cent G + C) and 1·701 g/c.c. (41 per cent G + C) respectively.

Table 1. TAXONOMY AND DNA BASE COMPOSITIONS OF SOME PROTOZOA

Class*	Subclass	Genus and Species	Density (g/c.c.)	Per cent (G+C)†	2σ‡	T_m^+	DNA preparation
Flagellata	Phytomastigophora	*Euglena gracilis*	1·705	46	7·3	90	purified
		Isochrysis galbana	1·720	61			part purified
		Prymnesium parvum	1·717	58			part purified
	Zoomastigophora	*Crithidia lucilliae*	1·716	57	8·7	94·1§	purified
		Crithidia fasciculata	1·717	58	8·1	97·4§	purified
		Strigomonas oncopelti	1·713¶	54	8·7	95·5§	purified
		Leishmania tarentolae	1·714	54			
		Trypanosoma lewisi	1·719	59			
Ciliata	Holotricha	*Tetrahymena patula* LFF-1	1·684	22¶	5·2	77	purified
		Tetrahymena pyriformis W	1·690	30		81·2	purified
		Paramecium aurelia 51.7s	1·689	29	9·3		part purified
		Colpidium colpidium carolina	1·691	32	5·0		lysate
		Colpidium colpidium burbank	1·692	33	5·0		lysate
		Colpidium camphylum	1·691	32	4·0		lysate
		Colpidium truncatum	1·694	35	3·9		lysate
		Glaucoma chattoni	1·693	34	6·2		lysate
Sarcodina	Rhizopoda	*Dictyostelium discoideum* NC-4	1·681	22¶		79 5	purified

* The taxonomic system used is that proposed by Corliss, J. O., "Taxonomic Procedures in Classification of Protozoa", *Twelfth Symp. Soc. Gen. Microbiol.*, edit by G. C. Ainsworth and P. H. A. Sneath (Camb. Univ. Press, New York, 1962). All the above organisms were grown in axenic medium except *Dictyostelium*, grown in the presence of *Aerobacter aerogenes.*

† Estimated from the buoyant density.

‡ Estimates of standard deviation of compositional distribution expressed in terms of mole per cent G+C These values were determined from the profile band in the cæsium chloride gradient The molecular weight contribution was not subtracted from the total variance, so that these are estimates for the maximum compositional variance.

§ Estimated from bimodal heating curve (Mandel, M., and Stidham, H. D., unpublished results).

¶ Determined from the relative amounts of triphosphate incorporated into DNA when the samples were used as a primer for *E. coli* polymerase

+ In standard saline-citrate (0 15 M sodium chloride plus 0·015 M sodium citrate).

All the zooflagellates examined belong to the trypanosomid flagellates, characterized by the presence of Feulgen-positive kinetoplasts (or parabasal bodies)[10]. In addition to the kinetoplast, *S. oncopelti* contains cytoplasmic particles called bipolar bodies[18] which appear to be bacteria or bacteria-like organisms[19]. The relation of the minor DNA components which band in cæsium chloride to the microscopically observed cytoplasmic structures is not known at the present time.

Within the ciliates, variation of the G+C in the genus *Tetrahymena* was found to be 23–30 per cent, confirming the range reported by Sueoka[5]. The G+C content found for the DNA from *Tetrahymena* and the slime mould *Dictyostelium* are among the lowest so far encountered from any source when compared with the major DNA components of other organisms.

The range of compositional heterogeneity of DNA molecules in terms of G+C content (expressed as twice the standard deviation) for certain ciliates examined was comparable with that found for bacteria (less than 6 per cent). Higher organisms have a wider range (6·2–9·6 per cent).

It is of interest to note that the diatoms, the chemically determined base composition of which have been investigated, have a range of DNA G+C content of 36–40 per cent, the green algae, 54–66 per cent and the one brown alga analysed, 58 per cent[4].

With more extensive investigation of representative organisms, the molar G+C content may serve as a valuable aid in establishing the taxonomic position of major groups and perhaps of controversial transitional forms the G+C content of which are within the appropriate range. The G+C content may also be an aid in studying the phylogenetic origin and relation of algae and protozoa.

We thank the following: Drs. G. W. Kidder and V. C. Dewey for supplying cultures of *Tetrahymena patula, Tetrahymena pyriformis, Strigomonas oncopelti, Colpidium camphylum, Colpidium truncatum, Colpidium colpidium carolina, Colpidium colpidium burbank, Glaucoma chattoni, Crithidia fasciculata,* and *Crithidia lucilliae*; Dr. L. Provasoli for supplying *Prymnesium parvum*; Dr. S. Hutner for supplying *Isochyrsis galbana*; Dr. W. Trager for supplying *Leishmania tarantolae*; Dr. M. Sussman for supplying *Dictyostelium discoideum*; Dr. J. Schiff for supplying *Euglena gracilis* and Dr. I. Sherman for supplying *Trypanosoma lewisi.* We also thank Drs. S. Hutner, A. Wilson and I. Sherman for useful discussions.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, November 26

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W 12), at 4 p.m.—Dr. Brian Ackner. "Alcohol Addiction".*

British Society for the History of Science (in the Council Room of the Science Museum, Exhibition Road, London, S.W.7), at 5.30 p.m.—Prof. Herbert Dingle "A Hundred Years of Spectroscopy".

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C 1), at 6 p m.—Mr. J. Wilson and Mr. A W. Comer: "Transistorized Television Camera Chain".

Institution of Mechanical Engineers, Hydraulic Plant and Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W 1), at 6 p m.—Discussion on "Pump Test Codes".

Institute of Metals (at the Royal School of Mines, South Kensington, London, S.W.7), at 7 p.m.—Prof. R. F. Mehl: "A Few Problems about Pearlite and Bainite".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Eric Shipton, O.B.E.. "Land of Fire".

Tuesday, November 27

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 1.15 p.m.—Mr. D. J. Furley: "The Invention of Atoms".*

Royal Institution of Naval Architects (at the Institute of Marine Engineers, 76 Mark Lane, London, E.C.3), at 5.30 p m.—Mr J. W. Landis. "Operating Experience with Nuclear Propulsion".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. P. G. H. Gell. "Can Hypersensitivity Cause Disease "* (Thirteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Automobile Division (joint meeting with the Lubrication and Wear Group and the Internal Combustion Engines Group, at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Oil Drain Periods".

Research Defence Society (in the Physiology Lecture Theatre, University College, Gower Street, London, W.C.1), at 6 p.m.—Sir Derrick Dunlop· "The Safety of Drugs" (Thirty-first Stephen Paget Memorial Lecture).

Society for Analytical Chemistry, Physical Methods Group (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6 30 p.m.—Annual General Meeting, followed by meeting on "Recent Improvements in Separation Techniques".

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Prof. R. N. Haszeldine: "Progress in Fluoro-Polymers".

Society of Instrument Technology, Automation Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. D. Cameron Small: "Automation in the Process Industries with particular reference to the Biscuit Industry".

Royal Institute of Chemistry, London Section (joint meeting with the South-East Essex Technical College Chemical Society. at the South-East Essex Technical College, Longbridge Road, Dagenham, Essex), at 7 p.m.—Prof. J. A. V. Butler, F.R S.: "Nucleic Acids and Heredity".

Wednesday, November 28

Institute of Personnel Management Institute of Office Management (at the Connaught Rooms, Great Queen Street, London, W C.2), at 9.15 a.m. to 4.45 p.m.—Conference on "The Electronic Computer's Challenge to Personnel Management".

Plastics Institute (at the Corrosion and Metal Finishing Exhibition, Olympia, London), at 2.30 p.m—Mr. K. V. Pepper: "Plastics in Corrosion-resistant Applications", followed by a discussion forum.

Institute of Fuel (at the Central Electricity Research Laboratories, Leatherhead, Surrey), at 5.30 p m.—Paper on "Work of C.E.R.L. on Combustion and Related Topics".*

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. J. A. Sykes. "Southern Region Electrification"

Society of Apothecaries of London, Faculty of the History of Medicine and Pharmacy (at Apothecaries Hall, Black Friars Lane, London, E.C 4), at 5.30 p.m.—Mr. W. S. C. Copeman: "The History of Gout" (The Sydenham Lecture).

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m—Prof. J. Lederberg (Stanford): "The Resolution of Bacterial Genes".*

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C 2), at 6 p.m.—Mr. J. E. H. Davies. "Pipelines".

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at p.m.—Mr. G. B. Booth. "Sweep Random Vibration"

British Industrial Biological Research Association (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 6.30 p.m. —Prof Harold C. Hodge (U.S.A.). "Research Needs in the Toxicology of Food Additives".*

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p m.—Meeting on 'Food Technology in Ireland".

Wednesday, November 28—Thursday, November 29

Iron and Steel Institute (at the Federation of British Industries, Tothill Street, London, S.W.1, and at the Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1)—Autumn General Meeting, and the Forty-fifth Meeting of the Iron and Steel Engineers Group.

Zoological Society of London (at Regent's Park, London, N.W.1), at 10 a.m. daily—Symposium on "Cardiovascular Anatomy and Pathology".

Wednesday, November 28—Friday, November 30

British Plastics Federation (at the Café Royal, Regent Street, London, W.1)—Third International Reinforced Plastics Conference.

Thursday, November 29

Institution of Electrical Engineers, Science and General Division P.G.S.8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Training for the Profession of Electrical Engineering" opened by Mr. S. E. Goodall.

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m—Dr. J. Pepys: "Immunological Responses in Man to Dusts of Vegetable Origin".* (Fourteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Lubrication and Wear Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m. —Discussion on "Lubrication of High-Speed Rolling Bearings".

Royal Institute of Chemistry, London Section (joint meeting with the Battersea College of Technology Chemical Society, at the Battersea College of Technology, Battersea Park Road, London, S.W.11), at 7 p.m.—Dr. D. J. Millen. "Some Developments in Microwave Spectroscopy".

Friday, November 30

Royal Society (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p m.—Mr. K. D. C. Vernon. "The Foundation and Early Years of the Royal Institution".

Saturday, December 1

London County Council (at Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss Olga Tufnell: "Dress and Ornament in Biblical Times".

Monday, December 3

University of London (in the New Engineering Building Lecture Theatre, University College, Torrington Place, London, W.C.1), at 5 p.m.—Prof. S. A. Schelkunoff (Columbia University): "Generalized Telegraphist's Equations and Their Applications".* (Further lectures on December 5 and 7.)

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. H. Woodger: "Lopsidedness, its Cause and Cure".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Plugs, Sockets and Interconnections" opened by Mr. N. E. Hyde.

Royal Institution, Library Circle (at 21 Albemarle Street, London, W.1). at 5 30 p m.—Mr. J. S. Chapman: "English Explorers and Their Books".

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. G. C. Bunn, Mr. D. M. Pilcher and Mr. T. W. Ball· "Reconstruction of Sewers for the Metropolitan Borough of Shoreditch".

British Society for International Health Education (at the Draper's Hall, Throgmorton Street, London, E.C.2), at 6 p.m.—Dr. Brock Chisholm. "New Horizons in Health".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—Mr. Jens Bjerre "Yendumu" (Colour films with commentary).

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S W.1), at 6.30 p.m.—Mr. J. E. Davis. "Recent Developments in the Gas Industry".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned·

Lecturers (graduates with good honours degrees) in (*a*) Pure and/or Applied Mathematics; (*b*) Physics, and (*c*) Botany at University College of Cape Coast, Ghana—The Assistant Registrar (Overseas), University of Ghana, 15 Gordon Square, London, W.C.1 (November 28).

ASSISTANT LECTURER IN ZOOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (November 30).

RESEARCH ASSISTANT (science graduate), to take part in a programme designed to study human chromosome abnormalities—The Secretary, Institute of Cardiology (University of London), 35 Wimpole Street, London, W.1 (November 30).

LECTURER IN THE DEPARTMENT OF LOGIC—The Secretary, The University, Aberdeen (December 1).

LECTURER IN THE DEPARTMENT OF PHYSICAL METALLURGY—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (December 1).

LECTURER (with research experience) IN THE DEPARTMENT OF PHYSICAL METALLURGY—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (December 1).

SENIOR LECTURER IN POLYMER CHEMISTRY; and a LECTURER IN ORGANIC CHEMISTRY—The Staff Officer, College of Advanced Technology, Gosta Green, Birmingham 4, quoting Ref. 11/62/31 (December 4).

LECTURER (well qualified physicist, and preferably with experience of the application of physics in industry) IN PHYSICS—The Registrar, Department 48/1, Bradford Institute of Technology, Bradford 7 (December 7).

LECTURER IN PSYCHIATRY—The Registrar, The University, Sheffield (December 8).

LECTURER (medically qualified) IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, quoting Ref. No. CV/206/N (December 8).

TRAINEE BIOCHEMIST (with a degree or equivalent in chemistry, and experience in, or wishing to take up, clinical biochemistry) at the Welsh National School of Medicine (University of Wales)—The Professor of Pathology, Cardiff Royal Infirmary, Cardiff (December 8).

LECTURER or ASSISTANT LECTURER in each of the following: PARASITOLOGY: PHARMACEUTICS at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 15).

LECTURER or SENIOR LECTURER (with a degree in applied science or chemical engineering, or a degree in pure science with suitable experience) IN APPLIED CHEMISTRY at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

SCIENTIFIC OFFICER (with a good honours degree in chemistry, and preferably experience in classification and fertility work on soils), to relate chemical characteristics of soil profiles to their productivity; and an ASSISTANT EXPERIMENTAL OFFICER (graduate in chemistry, biology or horticulture), to study soil fertility problems through glasshouse experiments—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (December 31).

PROFESSOR OF PHYSICS; and a PROFESSOR OF CHEMISTRY at the University of Adelaide, Adelaide, South Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, January 16).

GEORGE SWEET MEMORIAL FELLOW IN ECONOMIC GEOLOGY, to carry out practical work in geology calculated to develop primary and secondary industries based on the use of non-metalliferous deposits in Australia—The Dean of Graduate Studies (Mr. J. S. Rogers), University of Melbourne, Parkville, N.2, Victoria, Australia (January 31).

LECTURER IN BIOCHEMISTRY at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 31).

ADULT LABORATORY STEWARD (with an interest and preferably practical experience in biological work) IN THE NATURAL SCIENCE DEPARTMENT—The Registrar, Derby and District College of Technology, Kedleston Road, Derby.

SENIOR LECTURER (honours graduate with research and teaching experience, and preferably some industrial experience) IN INORGANIC CHEMISTRY—The Principal, College of Further Education, Flatt Walks, Whitehaven, Cumberland.

TECHNICAL LIBRARIAN (Chartered Librarian with a technical library background, and with experience in classifying (U.D.C.), abstracting and editing of reports), to take charge of an established library and information service at a research station near Cheltenham—Mr. H. Cooke (X114B), National Coal Board, Coal Research Establishment, Stoke Orchard, Cheltenham, Glos.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Royal Society. British National Committee for Oceanic Research. United Kingdom Scientific Programmes during the International Indian Ocean Expedition, 1961–64. Pp. 22. (London: The Royal Society, 1962.) [269

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Mathematics By Visual Aids Pp. 48. (London: British Association for Commercial and Industrial Education, 1962.) 6*s.* (5*s.* to members of the Association.) [110

Planning. Vol. 28, No. 464, (1 October, 1962): The Negotiations on Political Union. Prepared by Michael Palmer. Pp. 295–326. (London: Political and Economic Planning, 1962.) 3*s.* 6*d.* [110

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Bulletin of the British Museum (Natural History). Botany. Vol. 21, No. 11: The Athyroid Ferns of Ceylon. By W. A. Sledge. Pp. 275–323+plates 30–32. (London: British Museum (Natural History), 1962.) 16*s.* [110

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World Meteorological Organization. Technical Note No. 44: Numerical Methods of Weather Analysis and Forecasting. (Report prepared by a working group of the Commission for Aerology.) Pp. xi+31. (WMO–No. 118. RP.53). 4 Swiss francs. Technical Note No. 45: Performance Requirements of Aerological Instruments: an Assessment based on Atmospheric Variability. By J. S. Sawyer. Pp. x+29. (WMO–No. 119, TP.54). 4 Swiss francs. (Geneva: World Meteorological Organization, 1962.) [249

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Durban Museum Novitates. Vol. 6, Part 15, (31st August, 1962): Miscellaneous Taxonomic Notes on African Birds, 19. By P. A. Clancey. Pp. 181–194. 3 cents. Vol. 6, Part 16, (31st August, 1962): On the Geographical Variation in the Black-headed Oriole *Oriolus larvatus* Lichtenstein of Africa. By Walter J. Lawson. Pp. 195–202. 25 cents. (Durban: Durban Museum, 1962.) [110

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THE MANAGEMENT PROBLEM IN BRITAIN

QUITE apart from Lord Hailsham's condemnation, when addressing the third International Machine Tool Design and Research Conference at the University of Birmingham last September, of the amateur in British industry, particularly in reference to the management of large-scale industry, whether in the private or public sector, the quality of British management has been brought into question both in relation to the National Productivity Year which started in November and to Britain's proposed entry into the Common Market as well as by present-day unrest in industry. The long series of unofficial strikes in the motor industry, even since the Minister of Labour two years ago instituted an inquiry into the causes of these frequent stoppages, has provoked concern that bad management may be one cause even if a tradition of indiscipline on the shop floor and defective leadership in the trade unions may be others. Again, the unrest in British railways arising out of the closing of branch lines and workshops on the face of it seems to indicate some failure in industrial relations which could be due to poor management at some level. In a broadcast discussion on sluggishness in British industry generally, Mr. F. Zweig recently expressed the opinion that, while management at the middle level was perfectly good and satisfactory, at the upper level it lacked imagination and at lower levels was lacking in leadership. Supervision was a weak point and needed strengthening all round, and there was too much rigidity in the factory.

Lord James of Rusholme also suggested in a broadcast discussion that some of the difficulties arose from the way in which for generations, while Britain had supplied many of her ablest young men for service in the Commonwealth, comparatively few had entered careers in technology. He thought that much more positive leadership was required from the Government in explaining to the nation what were the real needs and priorities to-day and what was involved in meeting them. Otherwise, excessive rigidity might result in Britain getting the worst of both worlds. Lord James also stressed the need for professional representation in management if real efficiency was to be achieved. Britain's move to produce a more equalitarian society might fail to provide what is much more important—a more efficient society, and the technologists and right type of civil servants to run it.

In this context, fresh interest is being taken in the provision of higher education for business, on which, *inter alia*, the Robbins Committee is to report next year. Meanwhile, some debate is proceeding between those who advocate the old practice of what was little more than learning on the job, possibly supplemented by evening or correspondence study for the examinations set by one of the professional bodies, and those who support a broader education for all young people who promise to do well later on in one branch or another of business, particularly in middle management. This later approach has been stimulated by the agreement of some of the professional bodies for the first time to exempt from all or part of their intermediate examinations students who have taken a general course in a college of further education. The colleges of advanced technology clearly have an important opportunity here, though the Welsh College of Advanced Technology at Cardiff claims to be the only one so far offering courses leading to a diploma in business administration. Much will depend, however, on how far firms are prepared to encourage such further education for future businessmen and on whether the professional bodies will follow the engineers by allowing academic teachers a greater share in shaping the coming generation of managers.

The action by the Ministry of Education and some of the technical colleges and colleges of commerce, as well as some professional bodies, in fostering a new system of awards in business studies parallel with the awards in technology is only a beginning. Apart from the question of how far industry itself and the professional bodies are prepared to recognize the new awards there is the matter of co-operation. Anything in the nature of 'sandwich' courses cannot be organized without active co-operation from industry quite apart from readiness to recruit those who successfully complete the course and to offer them appropriate opportunities. Moreover, as Prof. W. J. M. Mackenzie has pointed out, there is the question of providing adequate facilities and support to ensure teaching of high quality both in the universities and in the colleges of technology for such courses. It would be unwise in present circumstances to take the necessary financial resources for granted, or to disregard the risk of depreciation in standards of diplomas in this field if they are withdrawn from the control of the relevant professional bodies.

One general criticism that has been made of British education for business and management is that it is too indifferent to what is being done elsewhere. As a corrective to insularity alone, Prof. Granick's recent book, *The European Executive**, is useful. It is the outcome of a research fellowship at the University of Glasgow, and, although at one point Prof. Granick draws on the survey made in 1956 by the Acton Society Trust of management succession, in the main it is based on his own visits to Great Britain, France, Belgium and Germany and the information he gathered from those inquiries. Much of this, too, is as personal as his interpretations of the data, but his comparisons are often suggestive and could correct some present-day illusions about methods of choosing managers and the relative importance of the various factors, of which education is only one.

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* *The European Executive*. By Prof. David Granick. Pp. 384. (London: Weidenfeld and Nicolson, Ltd., 1962.) 30s. net.

Prof. Granick is concerned essentially with the quality of management at its highest level, and in this connexion he notes that the prestige of business leadership in Britain, as in France, is low—the lowest in Europe. Britain in 1960, moreover, he asserts, quite apart from variations in income tax structure, which are of major importance, too, was a far more egalitarian country than France, Belgium, Germany or the United States, and generally through his study he is fully aware of the implications of social policy and conditions in determining whether or not men of first-class ability will choose to enter on a business career. Obviously, at the level of management with which he is primarily concerned, provision of management training of the most impeccable quality will fail in its purpose if other factors deter first-class men from entering such a career.

This is repeatedly illustrated in Prof. Granick's comparison of conditions in the four countries he elected to study, in the relations between business and the Civil Service, his comments on relations between the engineer and technologist and those who have no technological skill or training. He has shrewd comments on the Administrative Staff College and the British Institute of Management, but he is not impressed by postgraduate studies in the universities at present. In his chapter on management and education he points out that the amateur tradition in Britain is the stronger, and suggests that the success of the Administrative Staff College derives very largely from the way in which, so far, it has been able to attract students of the highest ability who essentially educate themselves. He does not appear at all optimistic about the development of the professional tradition, and it is to be noted that he entitles one of his chapters "Great Britain: the Home of the Amateur", in which, however, he shows marked respect for the amateur theory in practice.

There is much food for thought in Prof. Granick's comparisons between Britain and the other three Western European countries he visited, though, as regards the several Civil Services and the ways in which they are recruited, Brian Chapman's study of the public service in Europe, published three years ago under the title *The Profession of Government*, is a useful supplement which makes a little clearer some of the points over which Prof. Granick passes rather cursorily. What stands out, indeed, is the value of mobility and interchange and the importance of conditions which foster or hinder them. Prominent among such factors are public esteem and prestige, and if it is true that a country gets the Civil Service it deserves, it is largely true also that the quality of management reflects the esteem and respect which management enjoys in the public mind.

Where this is particularly important is in regard to enterprise and initiative on which the success of the Productivity Year will very largely depend. Prof. Granick clearly regards British management generally as deficient in this respect and he attributes this in part to the failure of business to attract sufficient men with really first-class minds. He suggests it is due also in part to the tendency for large organizations to model administrative practice on the Civil Service and in part to the comparatively low public esteem which business enjoys—which, of course, is a contributory cause in his first factor. Curiously enough, while commenting elsewhere that the scientist or technologist has poorer prospects of reaching board-level than the arts graduate who has taken up an industrial career, he makes no remark here on the value of a scientific training, for example, its bearing on the ability to take a calculated risk or evaluate technical factors accurately. This, at least, is an element in such a training which should give the scientist who also possesses administrative ability a potential advantage over those who have not had such training, and it would seem that Sir Noel Hall's conception of the change from the specialist to general manager, as set forth by Prof. Granick, slightly overstates the case. The ability to take a calculated risk is something fostered by practice, and the decision to do so or not depends largely on the ability to assess and interpret correctly the advice given by different specialists. This is not a quality to be thrown on one side when a scientist or technologist takes on the widest administrative responsibilities: on this point the Zuckerman Report seems to be sounder than Prof. Granick.

None the less, his book contains much that the promoters of the National Productivity Year could wisely ponder as well as professional associations, trade unions and all concerned with the conduct or extension of education for management. What is unmistakably clear is that no expansion of management education is likely to succeed in its ultimate objective, if the social factors are ignored and if no attention is given to the problems of mobility and general esteem which are so decisive in deterring or attracting entrants of the highest ability.

Here the publicity associated with the National Productivity Year affords an unmistakable opportunity for educating public opinion. Even if courses in management consistently attract students of the highest quality, the benefit to the country will be slight unless firms are willing to use the students so trained. In this connexion it should be remembered that the trade unions are as backward as many of the firms the management of which they are prone, quite justifiably, to criticize. Few unions so far have shown any eagerness to engage trained administrators for their management, despite the increasing responsibilities and complexities which confront them, or to appreciate the effect on recruitment of the raising of the school-leaving age and the wider opportunities for entering a university or college of technology. However valid the criticism which is levelled at management in Britain, whether in private or nationalized industry, in Government or in the trade unions and other professional bodies, in so far as its validity derives from the amateur tradition, it is a criticism of the outlook and conservation and lack of understanding in society as a whole.

CHARACTERISTICS OF EARLY FALL-OUT FROM THE RUSSIAN NUCLEAR EXPLOSIONS OF 1961

By D. H. PEIRSON and J. R. KEANE

Health Physics and Medical Division, Atomic Energy Research Establishment, Harwell

THE resumption of testing of nuclear weapons by the U.S.S.R. in September 1961 occurred about three years after the last substantial series of explosions in 1958. Since that time, apart from the small French explosions which were confined to the troposphere, the short-lived radioactivity of the debris had disappeared by decay. The atmospheric burden of long-lived fission products had been reduced in mid-1961 by fall-out, at a decreasing rate[1,2], to the equivalent of probably 5–10 megatons. This was a residue of a total stratospheric injection of approximately 60 megatons[3]. Against this comparatively settled background of artificial radioactivity the Russian series during September–November 1961 yielded 25 megatons[4] of fission products. The increment to the stratospheric reservoir of long-lived products may be estimated after the seasonal cycle in 1962. Meanwhile, it is instructive to consider the behaviour of the early fall-out, namely, the tropospheric component consisting mainly of short-lived fission products.

The existing sampling programme[1] was accelerated at several sites to accommodate the rapid decay of the new debris, but analysis of samples was restricted to barium-140 (half-life, 12·8 days) and iodine-131 (half-life, 8·1 days) in addition to the total β-activity and the longer-lived zirconium-95 (65 days). The measurement of barium-140 (by γ-ray spectrometry) and total activity is relatively rapid and straightforward; iodine-131 was included because of the potential hazard to the human thyroid. By this means it is possible to study the inter-relation between these fission product activities measured in samples of atmospheric dust, rain and grass, and further, to compare these results with measurements elsewhere of iodine-131 in milk.

Airborne particulate material is sampled continuously near ground-level, by passing appropriate quantities of air through filters[5,6] at:

Chilton (52° N., 1° W.)	daily period
Milford Haven (52° N., 5° W.)	weekly period
Orfordness (52° N., 2° E.)	weekly period
Eskdalemuir (55° N., 3° W.)	weekly period

A separate sampler[5] is used at Chilton for iodine-131, consisting of a cylindrical filter of esparto grass paper followed by a pack of activated charcoal to trap the non-particulate components of the iodine.

Samples of rainwater are collected daily at Chilton and Milford Haven by polythene funnel and bottle, containing carriers and acid to prevent loss by adsorption[5].

The grass sample is obtained by cutting from about 1 m². Up to 1 kg of grass may be packed into a cylindrical container having a cavity underneath designed to fit over a scintillation crystal of 3 in. × 3 in. diameter.

The total β-activity is measured with Geiger–Muller counters[5,6]. The activities of barium-140, iodine-131 and zirconium-95 are measured by γ-ray spectrometer. The estimate for barium-140 is easily derived from the 1·6-MeV peak due to the daughter activity lanthanum-140; zirconium-95 is obtained at equilibrium from the composite peak at 0·76 MeV, which includes the daughter activity niobium-95. The activity of iodine-131 is obtained only after correcting for interfering activities in the vicinity of the peak at 0·36 MeV.

In addition to the direct sampling of fission products in air, rain and grass a subsidiary experiment was designed to measure the rate of deposition in the absence of rain, by preparing two surfaces consisting of: (*a*) a flat horizontal sheet of cellulose acetate (area 0·30 m²); (*b*) vertical strips of cellulose acetate of height 5 cm shredded to represent grass having a horizontal area 0·23 m².

The exposed sheet and strips were coated with petroleum jelly and the whole was mounted at a height of 165 cm above ground-level, but beneath a roof to shield the surfaces from rain.

The results of these measurements have been reported elsewhere[7,8] in detail. The concentrations of barium-130 and iodine-131 at Chilton are summarized in Fig. 1. The effect of dry deposition may be measured by the deposition velocity (cm/sec) given by:

$$v_g = \frac{\text{rate of deposition (pc./cm}^2\text{/sec)}}{\text{concentration in air (pc./cm}^3\text{)}}$$

The dry deposition velocities measured in terms of total activity, barium-140 and zirconium-95 for each type of collecting surface are shown in Fig. 2. These may be compared with the monthly values (derived from the total activity) for a period before the resumption of weapon testing in September 1961. Since both types of surface are artificial the measured deposition velocities are not necessarily applicable to natural ground or herbage.

Except at levels near the limit of detection the uncertainty in the estimates of barium-140 and zirconium-95 is equivalent to a standard deviation of less than 10 per cent. In the case of iodine-131 the scatter is larger, corresponding to about 20 per cent.

Discussion

Fall-out during the autumn and winter of 1961–62 was largely controlled by the behaviour of the clouds of debris in the troposphere. These clouds circulate zonally subject to removal by rain or dry deposition. The removal process may be considered to be exponential at a rate consistent with a mean residence time in the troposphere of about one month. It is unlikely during such a short period that clouds of debris from individual explosions become well mixed. The radio-

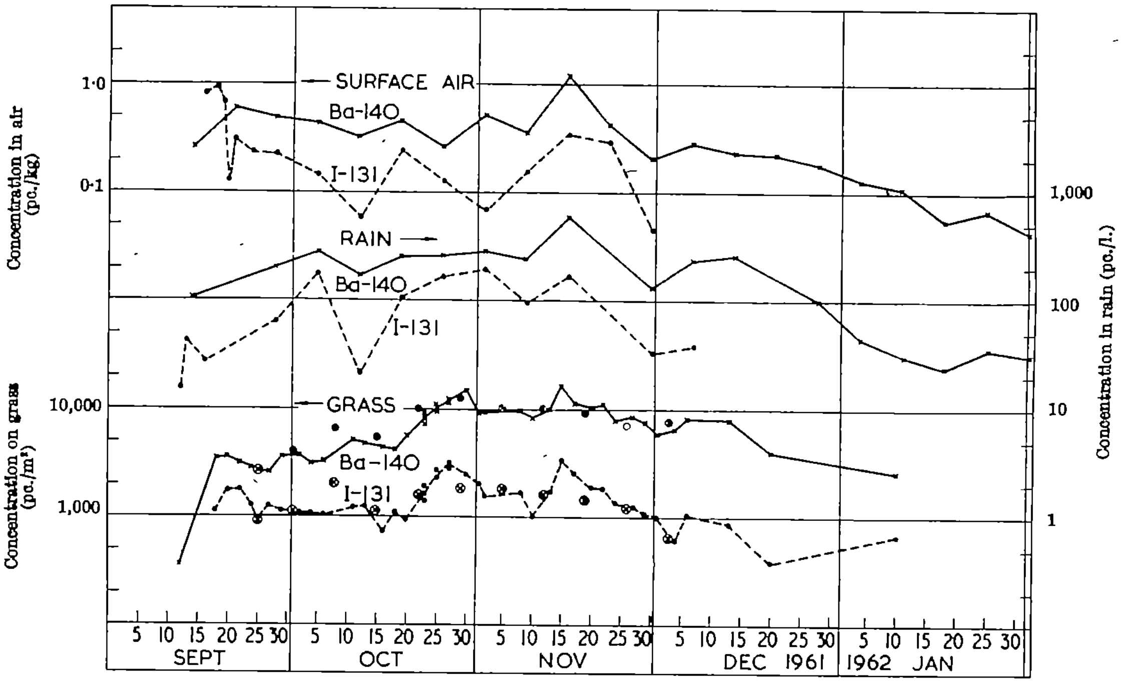

Fig. 1. Barium-140, iodine-131 at Chilton in air, rain and on grass. ○, Barium-140 and ⊗ iodine-131 computed values normalized to September 25, 1961

activity measured at a particular station will fluctuate according to the vagaries of wind and weather and will not necessarily be representative of a large region nor of a latitude band.

Fission products in air. The daily fluctuations in the activity of barium-140 at Chilton which are illustrated in Fig. 3 cannot be related to the calendar[4] of individual test explosions because of the uncertainties in plotting meteorological tracks of clouds which travel, generally eastwards, to the United Kingdom from testing sites in the Arctic (60° E.) and Central Asia (80° E.). However, the peaks observed during September 16–18 may be attributed to the first explosion 16 days earlier on September 1 in Central Asia and the peak on November 18 must be associated with an explosion not later than the last one on November 4, corresponding to a transit time of not less than 14 days. An analysis by Bannon[9] of the winds at several levels shows that the mean time of travel from the Russian testing sites to the United Kingdom at 34,000 ft. varies from 10 days during September to 9 days during November. At 18,000 ft. the corresponding transit times are from 20 days to 15 days. The peak activities of November 15 and 18 may have a double source[9]: (*a*) by flow in the middle troposphere around the world from the explosions on October 30 and 31, while (*b*) flow in the lower troposphere may have brought debris from the test on November 4 into the United Kingdom from the east.

The ratio[7,8] of barium-140 to zirconium-95 (Fig. 3) is a measure of the age of the fission products. Thus assuming a value[10] at formation of 5 then a value of 2·5 at the beginning of October would correspond to a transit time of 16 days. This ratio would represent mainly debris passing Chilton during a first circuit; the steady decrease in ratio, particularly after mid-November, refers to second and later circuits of the ageing clouds of debris, attenuated by fall-out and dispersion. Measured values[7] of the ratio iodine-131 to barium-140 have a mean value of 0·4 but are very variable. This variability is probably due to the earlier volatility of iodine and the general effects of fractionation during explosive fission. Of the iodine-131 sampled from the air by the composite collector, more than 70 per cent appeared as particulate material. This observation suggests that during transit to the United Kingdom the iodine had become mainly attached to dust particles and would afterwards behave as other less volatile fission products. The corresponding ratio of barium-140 to total activity remained in the vicinity of 0·15 until mid-November, but is an insensitive measure of the age of the fission product mixture during the period 10–30 days.

A comparison of the activity of barium-140 in surface air at the four British sampling stations may be made from Table 1. From September to January the activities at Chilton and Orfordness are noticeably greater than those from the two stations in the west and north. In particular, the average concentration[7] reaching Eskdalemuir from the west during the week about November 16 was one-third of that reaching Chilton from the east.

Table 1. COMPARISON OF BARIUM-140 IN AIR AT FOUR SAMPLING STATIONS (PC./KG)

	1961				1962	
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Chilton (S. central England)	0·30	0·40	0·60	0·26	0·077	0·023
Milford Haven (S.W. Wales)	0·13	0·28	0·44	0·19	0·064	0·020
Orfordness (S.E. England)	0·36	0·38	0·58	0·28	0·084	0·027
Eskdalemuir (S. Scotland)	0·16	0·26	0·32	0·20	0·072	0·028

Average weekly concentration: barium-140 activity corrected for decay to mid-week.

Dry deposition. It is clear that the velocities of dry deposition on to the experimental surfaces (Fig. 2) were enhanced by the arrival of the tropospheric debris from the Russian test explosions. Thus, in the case of the horizontal sheet, the velocity increased from 0·1 cm/sec during the time of stratospheric fall-out to ~ 1 cm/sec during the tropospheric period. In fact, acceleration of dry deposition in this case serves as a useful characteristic of tropospheric fall-out with the practical significance that the dry

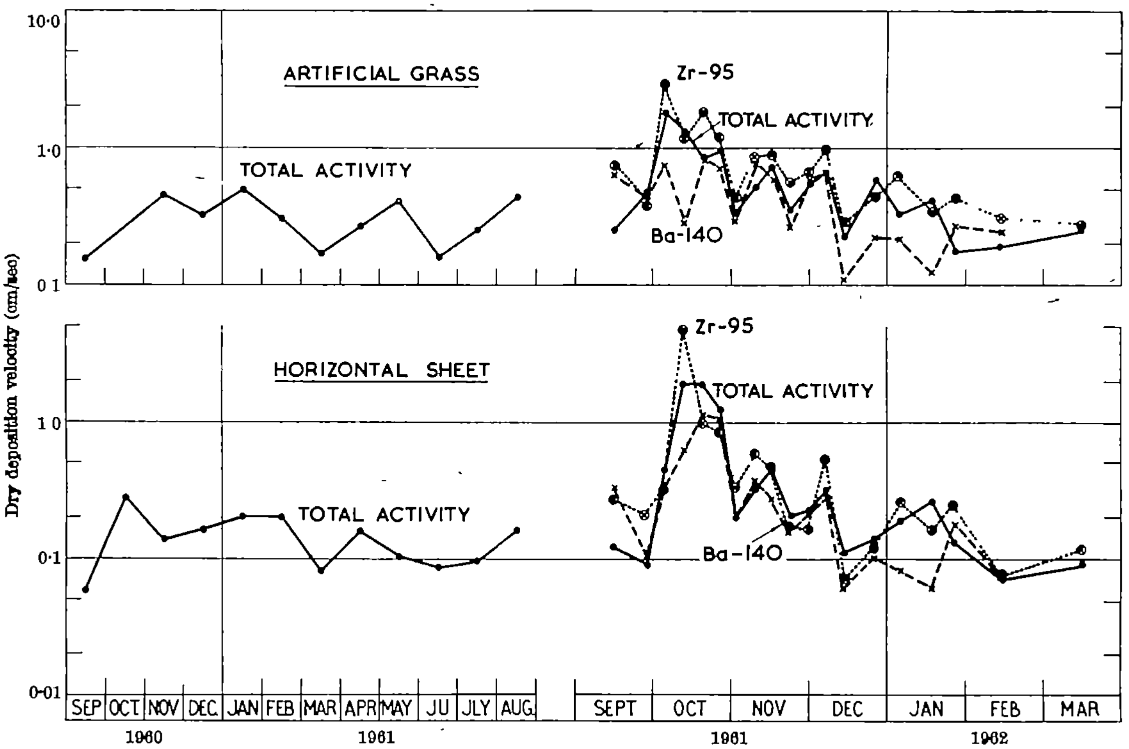

Fig. 2. Dry deposition velocities

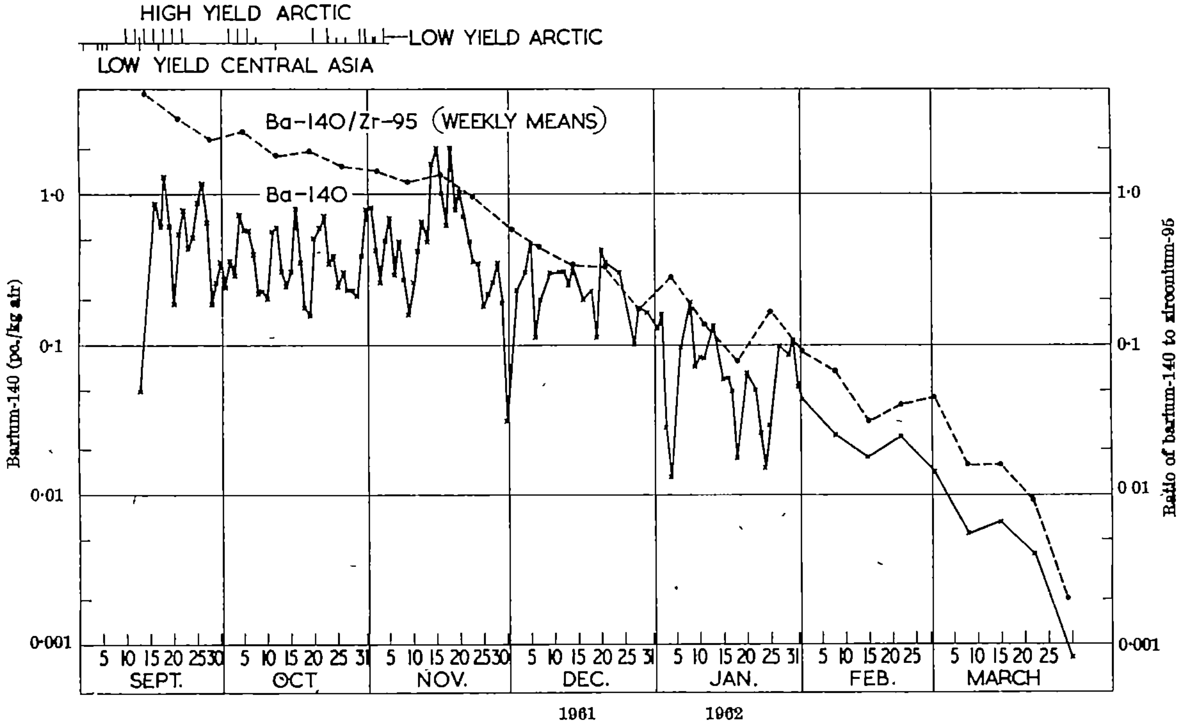

Fig. 3 Barium-140 and zirconium-95 in surface air at Chilton

deposition-rate at Chilton approaches the rate by washout in rain.

The size of particles contained in the Chilton filters were studied by Blythe[11] using autoradiography and microscopic examination. An upper limit to particle diameter was established in the range 1μ–4μ. The collection efficiency of the esparto filter material was measured continuously during the autumn of 1961. The efficiency increased in September to a value corresponding to an effective particle diameter of 0·7μ (assuming unit density). The estimate of the largest particle sizes by microscope is not inconsistent with the effective particle diameter derived from the collection efficiency of the filter. It can be shown by Stokes's Law that the terminal velocity associated with a particle diameter of 0·7μ is much less than the dry deposition velocities observed during the tropospheric period. It is therefore probable that the enhanced tropospheric dry deposition, like that during stratospheric periods, is dependent on processes[12] other than simple sedimentation, such as diffusion or impaction.

The majority of the particles in the range 1μ–4μ diameter, large enough for detachment from the filters for individual inspection, showed[13] an excessive proportion of zirconium-95. This could be explained by extreme fractionation during the fission process or by radioactivation, for example, zirconium-94 (n,γ) zirconium-95. There is no evidence to distinguish between these alternative origins. In addition, the deposition velocity measured for zirconium-95 appears significantly higher than that for barium-140 (Fig. 2).

Fission products in rain. The content of fission products in rain relative to air defines the washout factor[14], thus:

$$W_g = \frac{\text{concentration in rain (pc./kg)}}{\text{concentration in air (pc./kg) near ground-level}}$$

In Table 2 the washout factors for barium-140 and iodine-131 are seen to be similar, but somewhat lower than those for cæsium-137 and cerium-144, of stratospheric origin, sampled[1] at the same season in the preceding year. The concentration of fission products measured in rain includes, of course, a contribution from dry deposition on the collecting funnel. In this case it is remarkable that during a 'tropospheric period' the apparent washout factors, which should include this reinforcement from the enhanced dry deposition rate, are not greater than those during a 'stratospheric period'. Conversely, during the stratospheric period the activity actually washed out by rain is greater than in the tropospheric period, for a given air concentration at ground-level.

Table 2. WASHOUT BY RAIN AT CHILTON

Fission product	^{140}Ba	^{131}I	^{95}Zr	^{137}Cs	^{144}Ce
Period	Sept.–Nov. 1961			Sept.–Nov. 1960	
Mean monthly rainfall (mm)	58 7			123	
Velocity of deposition by rainfall (cm/sec)	0 8	0 7	0 9	2 1	2·0
Washout factor W_g	480	420	500	560	520

Barium-140, iodine-131 and zirconium-95 activities corrected for decay to middle of weekly periods· cæsium-137 and cerium-144 to middle of monthly periods.

Although the washout factor cannot be defined in terms of the fission product concentration in air at the rain-forming altitudes, since these are indeterminant, it serves as a useful parameter. The deposition of fission products in rainfall may also be written as a deposition velocity for comparison with dry deposition (Fig. 2). The values listed in Table 2 emphasize the relative importance of dry deposition during the tropospheric period.

Fission products on grass. The accumulation of fission products on grass is controlled by the rate of dry deposition and of deposition in rainfall. The rate of change of the concentration C_g of a fission product on unit area of grass, corrected for radioactive decay, may be written:

$$\frac{dC_g}{dt} + \lambda C_g = v_g C_a + bRC_r - kRC_g \qquad (1)$$

where C_a, C_r are the concentrations of the fission product per unit volume of air and rain respectively; v_g is the velocity of dry deposition on real grass; R is the rate of rainfall; λ is the radioactive disintegration constant; b is a factor, not greater than unity, that allows for incomplete retention by the grass of the current wet deposition; k is the fraction of deposition on grass washed off by unit rainfall.

Table 3. WEEKLY AVERAGE CONCENTRATIONS OF BARIUM-140 AND IODINE-131

Week	Mid-Date	Fission product	Air concentration C_a (pc./kg)	Rain concentration C_r (pc./kg)	Mean grass concentration C_g (pc./m^2)	Deposition to grass $dC_g/dt + \lambda C_g$ (pc./m^2/week)	Rainfall rate R (mm/week)
1	28.9.61	Ba-140	0 51	172	3,570	3,200	10 6
		I-131	0 23	66	1,080	940	
2	5 10.61	Ba-140	0 45	261	4,420	1,600	14·8
		I-131	0 15	179	1,140	520	
3	12.10.61	Ba-140	0 35	176	4,390	1,700	5·6
		I-131	0·06	22	900	230	
4	19.10.61	Ba-140	0 41	258	6,720	7,800	27 9
		I-131	0 25	112	1,280	1,900	
5	26.10.61	Ba-140	0 28	266	12,060	10,700	28 7
		I-131	0 18	173	2,170	2,100	
6	2.11 61	Ba-140	0 55	271	12,350	−770	6 4
		I-131	0 07	198	2,100	630	
7	9.11.61	Ba-140	0·37	236	9,980	4,600	16·4
		I-131	0·16	99	1,710	1,100	
8	16.11.61	Ba-140	1 22	595	10,450	4,200	1·0
		I-131	0 35	168	1,840	1,300	
9	23 11·61	Ba-140	0 45	—	9,700	2,100	0
		I-131	0 30	—	1,090	280	
10	30 11 61	Ba-140	0 22	125	7,790	760	34 8
		I-131	0 045	34	950	—93	

Barium-140 and iodine-131 activities corrected for decay to middle of week.

It is convenient to divide the measurement period during September–November 1961 into ten consecutive weeks and to apply equation (1) to the measured concentrations[7] of barium-140 and iodine-131 averaged over each week (Table 3). The deposition velocity v_g will vary from week to week as in the case of the artificial surfaces (Fig. 2), the retention factor b will vary with rainfall, approaching unity as the weekly rainfall tends to zero, and k is constant for a given fission product. This provides for each fission product 10 equations with 21 unknowns in v_g, b and k. As a further simplification let v_g be replaced by the mean value $\bar{v}_g$ during the ten weeks; also, it is assumed that the retention factor is dependent on the rainfall rate such that:

$$b = 1 - mR \qquad (2)$$

where the constant m may have different values for barium-140 and iodine-131. This reduces the analytical problem to the solution, for each fission product, of 10 equations with 3 unknowns in $\bar{v}_g$, m and k. The best values with standard errors for these parameters have been computed by multiple regression analysis (Table 4).

Table 4. DRY DEPOSITION, RETENTION AND WASH-OFF PARAMETERS (computed for real grass)

	Dry deposition velocity ($\bar{v}_g$ cm/sec)	Retention parameter (m week/mm)	Wash-off factor (k/mm)
Barium-140	0·26 ± 0·24	−0·010 ± 0·027	0·009 ± 0·018
Iodine-131	0·26 ± 0·18	0·015 ± 0·013	0·020 ± 0·028

± Standard error

The magnitudes of the standard errors in Table 4 are a reflexion of the uncertainties inherent in this type of experiment. The main reasons for these uncertainties are probably: (a) the variability of grass sampling; (b) the assumptions that the retention and wash-off processes depend simply on the weekly rainfall-rate rather than on the intensity or duration of individual rain-showers. Interpretation of the best values of Table 4 will indicate therefore the behaviour of the fission products during the whole period of measurement and not during a separate week.

Values computed from Table 4 for the concentration of barium-140 and iodine-131 on grass have been inserted in Fig. 1 for comparison with directly measured values. The agreement between the computed and measured values is very good, in view of the uncertainties of the experiment.

The dry deposition velocities quoted in Table 4 for barium-140 and iodine-131 are nominally identical. This is consistent with the particulate nature of iodine-131 samples from air, noted previously, and with the observation of Eggleton[15] that the dry deposition velocity on grass of iodine-131, measured in wind tunnel experiments during October–November 1961, was similar to the velocities of other contemporary fission products. The mean value during the 10-week period of the velocity of dry deposition of barium-140 on the artificial grass surface is 0·5 cm/sec, about twice the values computed for real grass. It is clear that agreement between real grass and the chosen form of artificial grass would be fortuitous. In addition, the value of 0·26 cm/sec, for both barium-140 and iodine-131 on real grass is a 'virtual' value. It represents a lower limit because of the contribution to the measured concentrations of fission products in rain, of the dry deposition on the collecting funnel. It has already been noted that this contribution is significantly enhanced during the 'tropospheric period'.

In the case of the retention and wash-off factors it is not possible to check the practical validity of the derived values of Table 4 by comparison with other concurrent measurements. Comparisons are made here with values reported elsewhere, under experimental conditions that may not conform with the present idealized model.

The retention factor b may be derived from the values of m in Table 4 using equation (2). Thus, the retention by grass of barium-140 in current rain in a week having rainfall of 12 mm, the average at Chilton, is 100 (±30) per cent. The corresponding retention of iodine-131 is 80 (±15) per cent. Again, these are 'virtual' values, as in the case of deposition velocity. Estimates made[16] of the retention by grass artificially sprayed with solutions of strontium-89 in a series of field experiments, lie in the range of 16–26 per cent. Garner[17] has assumed a value of 25 per cent. Morgan[18] exposed clean rye-grass to fall-out for 30 days and found that the direct uptake of various fission products lay in the range of 24–100 per cent. In this case the measured uptake corresponds to the present retention factor reduced by wash-off during the 30 days.

The half-lifetimes against wash-off by an average rainfall-rate of 12 mm/week may be derived from the best values of k in Table 4. These are 44 and 20 days for barium-140 and iodine-131 respectively. When combined with radioactive decay the effective half-lives on grass appear as 10 days for barium-140 and 5·8 days for iodine-131. The latter value is consistent, after making allowance for heavier rainfall, with that obtained from a detailed analysis of Booker's[19] measurements during the first 20 days after the Windscale accident in October 1957. In laboratory and field spraying experiments[20] the best estimate of the half-lifetime against wash-off is 14 days. This leads to an effective half-life of iodine-131 on grass of 5 days.

Iodine-131 *in milk*. It is possible to estimate the approximate concentration of barium-140 or iodine-131 on grass by measuring the air and rain-concentrations and by choosing appropriate values for the average dry deposition velocity $\bar{v}_g$, retention factor $b(= 1 - mR)$ and wash-off factor k. It is shown here how this calculation may be extended to produce an approximate estimate of iodine-131 in milk based on the measurement of fission product concentration in air. This estimate is based on the concentration of barium-140 in air since the practical measurement of this quantity is much more rapid and sensitive than that for iodine-131.

Equation (1) may be written in terms of the wash-out factor (section on "Fission Products in Rain"). Thus the fission product deposition on grass:

$$\frac{dC_g}{d_t} + (\lambda + kR)C_g = (\bar{v}_g + bRW_g)C_a \qquad (3)$$

where the washout factor, $W_g = \frac{C_r}{C_a}$, had an average value for barium-140 and iodine-131 during September–November 1961 of 450 (Table 2). Substituting for W_g in equation (3) and using (2):

$$\frac{dC_g}{dt} + (\lambda + kR)C_g =$$

$$[1 \cdot 2.6{,}048\ \bar{v}_g + 450(1 - mR)\bar{R}]C_a \text{ pc./m}^2\text{/week}$$

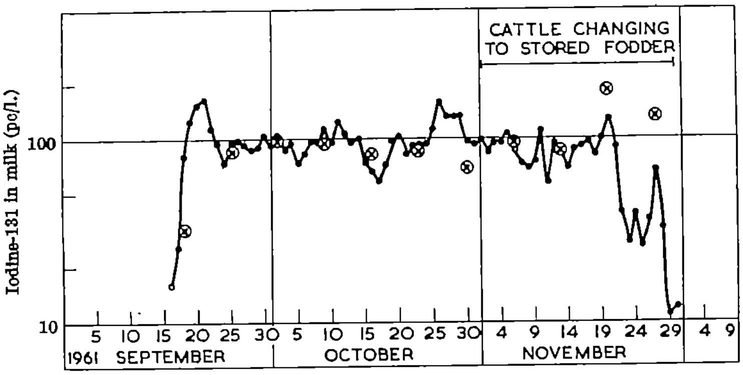

Fig. 4. Concentration of iodine-131 in milk. ⊗, Concentration of iodine-131 in milk computed from barium-140 in air; ●, concentration of iodine-131 in milk measured by the Agricultural Research Council

$\bar{v}_g$ is measured in units of cm/sec, R in mm/week and C_a in pc./kg. Again, in a week having the average rainfall of 12 mm and putting $\bar{v}_g$ equal to its 'virtual' value of 0·26 cm/sec, the increment of iodine on grass would be:

$$\sim 6{,}300\ C_{aI}\ \text{pc./m}^2\text{/week}$$

where C_{aI} is the concentration of iodine-131 in air (pc./kg). About one-third of this increment would be due to the 'virtual' dry deposition.

The ratio of concentrations in air, iodine-131/barium-140 has been shown to be roughly 0·4 (section on "Fission Products in Air"). This value is characteristic of the transit time between the Russian testing sites and the United Kingdom during the autumn of 1961. The increment of iodine-131 on grass at Chilton may be expressed in terms of the concentration C_{aB} of barium-140 in air, assuming average rainfall, to be:

$$\sim 2{,}500\ C_{aB}\ \text{pc./m}^2\text{/week}$$

An estimate may be made of the concentration of iodine-131 in milk from cows. The Radiological Laboratory of the Agricultural Research Council conducted a survey of iodine-131 in milk throughout the United Kingdom at this time[11], and has made available the daily measurements for one herd which grazed within two miles of Chilton. The ratio of concentrations of iodine-131 on grass (pc./m²) (ref. 7) to that in milk (pc./l.) had an average value during September 1961 of 14. Assuming average rainfall the concentration of iodine-131 in milk associated with deposition during the week would be:

$$\sim 180\ C_{aB}\ \text{pc./l.} \qquad (4)$$

where C_{aB} is the concentration of barium-140 in air (pc./kg). The measured grass–milk ratio was 15 during October but increased to 32 in November as the cattle changed to stored fodder. The ratio measured by Booker[12] after the Windscale accident in October 1957 was 11.

The effect of the accumulation of iodine-131 on the grass may be calculated by using expression (4) together with the combined wash-off and radioactive decay of equation (3) which has been shown to be equivalent in the case of iodine-131 to an effective half-life of 5·8 days. In Fig. 4 the calculated values (for open grazing as in September) are compared with the values measured by the Agricultural Research Council. The agreement is good except in November when, as expected, the yield of iodine-131 in milk was reduced as the cattle changed to stored fodder.

Summary

Fall-out of the short-lived fission products from the Russian nuclear explosions of 1961 was controlled by the rapid circulation of clouds of debris in the troposphere in contrast to the slower distribution of long-lived fission products from the stratosphere

Clouds from explosions at the Russian testing sites generally travelled eastwards, reaching the United Kingdom in about 15 days. For clouds in the first circuit the ratio in surface air at Chilton (Berkshire) of barium-140/zirconium-95 ~ 2·5, of iodine-131/barium-140 ~ 0·4 and of barium-140/total fission product activity ~ 0·15. The monthly average concentration of barium-140 in air varied by a factor of two between sampling stations located in southern Scotland and southern England. On arrival over the United Kingdom the iodine-131 activity behaved as particulate material although the variability relative to barium-140 was probably due to the effects of fractionation in an earlier more volatile state.

The rate of dry deposition measured on artificial surfaces increased during the tropospheric period to become comparable at Chilton (annual rainfall 60 cm) with the rate of deposition measured in rainfall. The acceleration of dry deposition (to ~ 1 cm/sec). although accompanied by an increase in size of the dust particles, cannot be attributed to simple sedimentation. Some air filters contained an excessive fraction of zirconium-95 in association with particles having a maximum diameter of 4μ. The velocities of dry deposition of zirconium-95 on the artificial surfaces were significantly larger than those for barium-140.

Barium-140 and iodine-131 were washed out of the atmosphere by rain with equal efficiencies, which were lower than those for long-lived fission products having a stratospheric origin.

The concentrations of barium-140 and iodine-131 in air, rain and on grass measured over a 10-week period have been used to estimate: (*a*) average values for the velocity of dry deposition (on to real grass); (*b*) the effect of incomplete retention by the grass of current wet deposition; (*c*) the effect of rain in washing radioactivity from the grass.

Barium-140 and iodine-131 have the same dry deposition velocity on to real grass of 0·26 cm/sec. This value, which is about one-half the average value for barium-140 on to the artificial grass during the same period, is a 'virtual' value because of the contribution to the measured concentration of fission products in rain of the dry deposition on the collecting funnel.

The retention by grass of barium-140 and iodine-131 in current rain in a week having rainfall of 12 mm, the average at Chilton, was virtually 100 and 80 per cent respectively. Wash-off by this average rainfall-rate produces, after combination with radioactive decay, effective half-lives on grass of 10 days for barium-140 and 5·8 days for iodine-131.

The concentration of iodine-131 on grass and in milk may be roughly estimated from the measured concentrations in air of iodine-131 and barium-140 assuming average rainfall. The ratio of the increment of iodine-131 in milk (pc./l.) to the current barium-140 in air (pc./kg) is particularly useful. For the situation in the United Kingdom during the autumn of 1961 the value of this ratio was about 200.

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STANDARDS OF ENGLISH IN SCIENCE AND TECHNOLOGY

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IT seems to be generally agreed that the standard of self-expression in spoken and written English among sixth-form and undergraduate scientists and technologists is low. Various causes have been blamed, and an imposing array of remedies is being offered; but in all the diagnoses and cures I have seen so far, all the emphasis has been on past failures by English experts and future remedies to be administered by other English experts. It is not my intention to dissociate English teachers from the problem altogether; they must continue to give help where they can; but I want to suggest that scientists and technologists themselves must take most of the responsibility for the low standards of self-expression in their professions, and that a major change of outlook on their part is the only thing that can bring a substantial improvement in the situation.

Justification of this contention starts from an analysis of what is wrong with the way students of science and technology write at the moment. The following examples have been taken from a group of 18 reports of experiments, written for my scientific colleagues:

(*a*) The standard cell, galvanometer and accumulator are connected up as in the diagram. The galvanometer sensitivity switch should be on the 0 01 position. Now the galvanometer was switched on and the selector switch *A* turned to standardise position. Then with the "galvo" key depressed, the fine and coarse resistances were adjusted until zero deflection was obtained on the galvonometer. The potentiometer is now calibrated to read millivolts directly. This standardisation was checked several times during the coarse of the experiment.

(*b*) The matching [of an oscillator and a speaker] was not perfect but is sufficiently good.

(*c*) The dimensions and the weight of the vibrating part of the rod was measured.

(*d*) . . . the value of the e.m.f's of the thermocouples at this point were given by . . .

(*e*) Young's modulus is defined as the stress over the strain. The stress being the applied load per unit area of cross-section and the strain the increase in length per unit length.

(*f*) When these measurements are completed it is possible to obtain a reliable value for λ the wave-length, assuming the frequency, n, is known, then the velocity $v = n\lambda$.

(*g*) After checking the connections, the "test" position on figure 2 was connected to the "test" position on the potentiometer.

(*h*) Also the heat lost from the plate *E* can be found by getting its rate of cooling and multiplying this by the plates water equivelent.

(*i*) This method is an accurate way of determining the Thermal conductivity of a bad conductor in the shape of a thin sheet of large area.

(*j*) [Of a method of measurement] This has some disadvantages, one being that an intense "pulse" and not a note of continuous frequency. It is therefore valuable to have alternative methods.

(*k*) *Procedure.* The diagram was set up as shown . . .

Some spelling: accurracy, peice, consistant, particurlar, bredth, ballastic, milimetre, proceedure, varified (verified), permiability, callibration, feasable, disintergration, staballse, preperation, assummed, critisism.

These examples are typical of the mistakes I have found in the work of sixth-form and undergraduate scientists and technologists. Of the 40 mistakes I noted in this group of reports, 9 were confusions of tenses (as in examples (*a*) and (*b*)), 7 were incorrect agreements of subjects and verbs (as in examples (*c*) and (*d*)), 7 were omissions of some word or words, which made the sentences meaningless (as in example (*j*)), and 6 were confusions caused by mispunctuation (as in examples (*e*) and (*f*)). The frequency of these various errors is also typical.

The errors are not niceties of grammar or style. I did not pick out incorrect uses of shall and will, due to and owing to, different to and different from, or semi-colons where full-stops might have seemed better; such points can be left for debate by grammarians and critics of style. I was concerned only to pick out those errors which led to ambiguity or confusion, which led me to re-read a passage a number of times before I could make out what the writer probably meant, and which often left me doubtful what exactly was intended.

These examples show errors which every student worthy of higher education should have no difficulty in recognizing and avoiding. They reveal carelessness, not lack of knowledge. Most students, when asked to look carefully at what they have written, can correct such confusions at once. In my experience, too, if students are set extra writing practice on a scientific topic, to be marked by an English tutor, the errors occur much less frequently. Perhaps the most significant point is that all the students on higher education courses have at one time obtained a pass in English language at Ordinary Level of the

General Certificate of Education. This means that at one time they were all able to recognize and avoid common errors quite readily, and had reached a sound standard of writing in correct and clear English for their English teachers, on factual as well as imaginative subjects.

So why is slovenly and incoherent writing containing errors of this sort so common in sixth-form and undergraduate writing ?

I suggest it is because students know that their teachers of science and technology will let them get away with it. The teachers do not notice, or do not care about, or have not the time to correct the errors of expression made by their students. Naturally, the students assume that the quality of their writing is of little importance, and that the concern with basic skills of self-expression which they have learned in their English lessons is no longer essential.

I believe that the feeling among students that they have finished with 'English' after Ordinary Level is very strong, and is encouraged by their science teachers. "Don't bother about elegant style; just get down the essentials" is the advice they are given. Sound general advice it is, too; there is no place for imaginative decoration or leisurely periphrasis in scientific writing. But too often this advice is taken—with approval—to mean the cutting out of all connectives, explanations, and indications of relationship; in fact to mean the reduction of continuous prose to note form, so that an experimental report reads like a long telegram. This has a disastrous effect on the students' ability to write coherently and logically, clearly indicating the movement of their thoughts to their readers.

I think it is fair to say that the division between the care English teachers require and the standard of writing other teachers will accept becomes clear early in secondary school. Pupils soon notice that some teachers do not bother about the way work is written so long as the factual details that are down are correct. So even before the Ordinary Level examination an ambivalent attitude has grown up in all but the best students: that they must write 'English' for the English teachers, but that there is another language which is acceptable to other teachers.

Many of the most vehement critics of student scientific writing lay all the blame for low standards on the Ordinary Level English language examination, suggesting that the skills it tests have little or no relevance to the skills required in writing about scientific and technological subjects.

I cannot agree with this criticism. Here is a list of what is required of students by one English language syllabus:

> *Part One* . two tests of composition, one on a general topic, and one on a factual topic, the material for which is supplied. [Account is taken of ideas included, the arrangement of ideas, general expression, and command of English.]
>
> *Part Two* : questions testing ability to make a summary from a given passage, to comprehend a given passage (including the explanation of individual words in relation to the context, and the explanation or re-writing of phrases or sentences), ability to turn direct into indirect speech, to construct sentences (candidates will be expected to be able to distinguish between a phrase, a clause, and a sentence, and to recognize the grammatical function of words, phrases and clauses in their context), to detect and correct errors of expression, to use punctuation correctly, and to recognize, understand and use metaphor and simile.

Apart perhaps from the use of metaphor and simile, these are surely all basic skills required in scientific and technological communication. Indeed, they are the basic skills of all communication through language. So while it must be admitted that the quality of students' writing on scientific subjects is not necessarily reflected by their having passes in the Ordinary Level English Language examination, this is not because the examination has tested the wrong skills; it is because students have decided well before the Ordinary Level examination that the basic skills required in writing 'English' are apparently not important in writing about scientific subjects, and they have therefore adopted another language for scientific writing.

The solution to the problem presented by careless writing in scientific and technological papers does not lie in altering the English language examination; it lies in altering the standard of writing required in scientific and technological examinations, and the persons to do that are the teachers and examiners of science and technology.

Students should be prevented from developing their dualistic outlook on writing standards while they are at school; for once the outlook is developed it is not easily dispelled by sending students to English specialists for two or three hours a week, in time that is taken from study of scientific or technological subjects at sixth-form or undergraduate level. Grafted-on English courses only serve to strengthen the opinion that 'proper English' is something required by a few fanatics in a special department; outside that department it is reasonable to adopt another, scientific language, a common means of communication between scientists, which is quite acceptable to science lecturers.

If there were two different languages this attitude might be acceptable; but there is only one language. It is the common means of communication of ideas and opinions about football pools and focal lengths, about trigonometry and toffee, about æsthetics and aerodynamics; and though the vocabulary used to express these different concepts varies considerably, exactly the same basic patterns of word order, clause arrangement and punctuation are used to express our ideas in meaningful statements. It is confusion in just these basic elements that seems to be the outstanding feature of scientific and technical writing by students.

Why is confused writing accepted or overlooked by teachers who in other respects demand absolute accuracy in scientific work ?

One answer is that there are too many reports to be read, and that reading through so many accounts of familiar work becomes insufferably tedious.

Impatience in the teacher is understandable. All the work is so familiar to him that he knows just how the student ought to be thinking, and what ought to be written down. He has thirty accounts of the same experiment to read; small wonder he gets impatient with the deliberate movements of the inexpert students' thoughts, and urges them to write more rapidly and briefly.

The teacher's superior knowledge also often accounts for his apparent carelessness in overlooking mistakes in the students' writing. He knows what a student means to say in a sentence, and mentally supplies the correct interpretation, even if the wording and punctuation do not make this at all clear (as in example (*f*)). The student, not corrected, thinks that his statement is explicit enough, and accepts that standard of writing for the rest of his work. There is no wilful lowering of standard on the part of teacher or pupil, but the damage is done just the same.

The problem of time is harder to solve in these days of acute teacher shortage. More free time for preparation and marking is the obvious answer; but while there is no possibility of this being obtained, it would be well worth while considering if it is really necessary for so many experiments to be written up. From the point of view of learning and revision there is usually plenty of information in standard text-books which could be amplified by notes of experimental results kept in fairly brief form. Fewer reports written out carefully could be given closer scrutiny by science teachers.

Another answer is that science teachers are not qualified to correct errors of expression; to do so requires specialist knowledge.

But science teachers are not being asked to do work requiring specialist knowledge; they are being asked to correct simple points of inexact expression, where what has been said is wrong, or at best obscure. I do not believe that teachers of science and technology do not know how to put things right. They, too, were able at one time to show a satisfactory standard of self-expression, and understood the basic structure of the English language. It should not take long for all teachers to revive their ability to criticize written and spoken English, once they have determined to make a conscious effort to do so.

Here is the crux of the matter. There is no doubt that most of us have to make a conscious effort to produce clear, accurate expression consistently. Unfortunately, at the moment too few scientists and technologists seem prepared to make the effort to produce good speech and writing in themselves and in their students.

The process of transmitting knowledge through language brings the teacher two grave responsibilities: one is to ensure that his own use of English (and therefore the knowledge and the way of expressing it that he conveys to his students) is accurate and exact; the other is to ensure by checking the accuracy of his students' re-expression of what he has taught them that they have grasped his ideas exactly.

English specialists can help in science sixth forms and undergraduate classes by refreshing memories of basic constructions, selection of vocabulary, rules of spelling and grammar, paragraphing, punctuating and précis writing; they can discuss general problems of communication and, where appropriate, the difficulties of translating science into terms the layman can understand; but periodic courses re-surveying and re-furbishing basic skills will not help if students find that lower standards are acceptable when they return to their scientific studies. Undoubtedly, the main responsibility for keeping high standards in the use of English in science and technology rests with scientists and technologists themselves.

TUNSTALL LABORATORY OF 'SHELL' RESEARCH, LTD.

By HUGH HARVEY

Shell International Petroleum Co., Ltd.

THE derivatives of petroleum are becoming increasingly complex and diversified in their application. A hundred years ago crude oil was wanted for only one purpose—the provision of paraffin oil for lamps, though a few enterprising salesmen touted its medicinal values. To-day petroleum is not only a source of fuels and lubricants, but also a starting point for agricultural and industrial chemicals which enter into virtually every aspect of modern life. Furthermore, although the oil companies have entered the chemical business, they are not necessarily bound to use oil as a starting material. Many of the new lubricants, for example, are synthesized compounds which may or may not have had their genesis in oil.

The manufacture of these products and their use by the consumer gave the oil industry the responsibility of thoroughly investigating their potential for causing injury by the assessment of their toxicity during development and their toxicity and carcinogenicity during manufacture and their ultimate use. Since some of them belong to the category of herbicides and pesticides, it is important that their action be as specific as possible and their toxicity as low as possible for man and animals.

To ensure that the products bearing the 'Shell' brand name have been thoroughly investigated with regard to the hazards from toxicity which could be inherent in their manufacture and use, a research laboratory has been established by 'Shell' Research, Ltd., at Sittingbourne, Kent. This laboratory, known as Tunstall Laboratory, was founded in 1960 with Dr. C. G. Hunter as manager and director of research. Dr. Hunter was previously professor of physiological hygiene and lecturer in occupational medicine and industrial toxicology in the University of Toronto. Until the Tunstall Laboratory was set up, 'Shell' obtained the toxicity information it needed from outside sources, including commercial and academic laboratories. While this procedure was adequate in the past, the growing complexity of products, their diverse applications and their closer than ever contact with man and animals made it desirable that a research centre facilitating the co-ordination of all toxicological problems should be incorporated within the research effort of the company itself.

Sittingbourne, in Kent, some forty miles from London, was chosen as the site of this new laboratory because 'Shell' Research, Ltd., already had a research laboratory there. This is the Woodstock Agricultural Research Centre, the manager and director of research of which is Dr. R. A. E. Galley. Also on this site will be located the new Chemical Enzymology Laboratory of 'Shell' Research, Ltd. The formation of this, under the joint directorship of Dr. J. W. Cornforth and Dr. G. J. Popják, was recently announced. Dr. Cornforth, a member of the scientific staff of the National Institute for Medical Research, was elected a Fellow of the Royal Society in 1953. Dr. Popják, director of the Medical Research Council Radiopathology Research Unit, Hammersmith Hospital, was elected a Fellow in 1961.

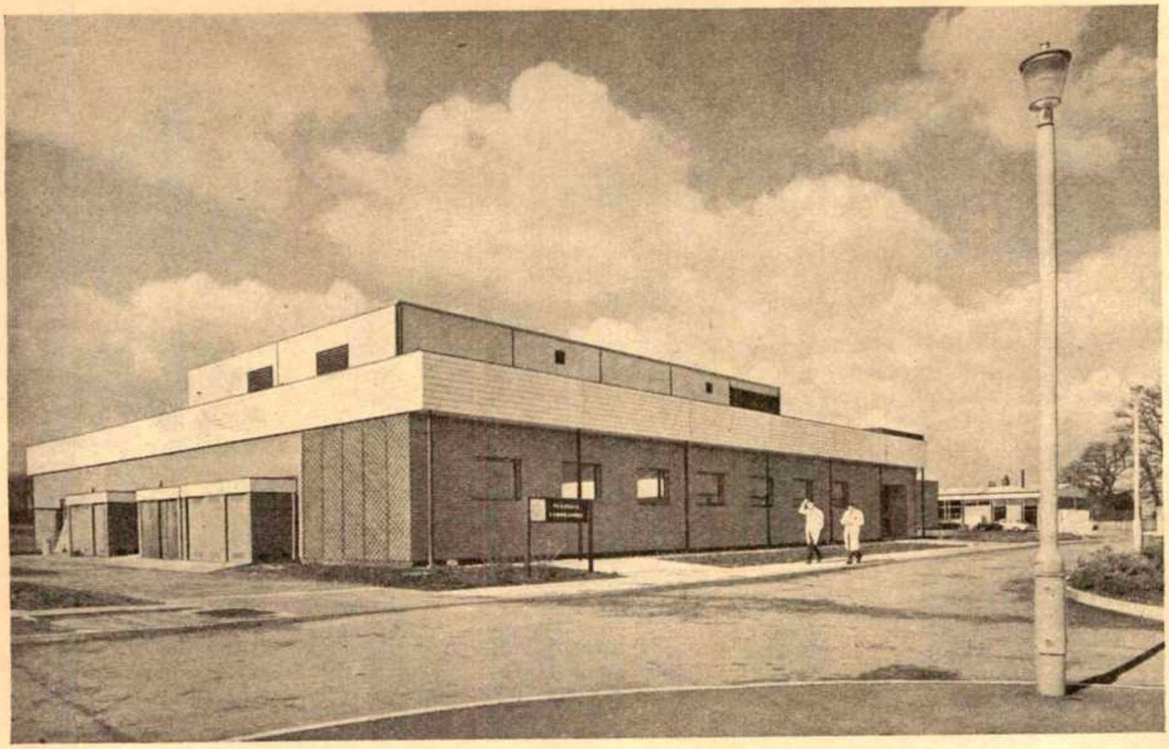

Fig. 1. Front view of the main laboratory of 'Shell' Research, Ltd., Tunstall Laboratory, Sittingbourne

The function of the Chemical Enzymology Laboratory will be to conduct long- and short-term research work into the chemistry of life processes. All these laboratories will share essential services of which administration, canteen and workshops are examples. More important, they will be in a position to work closely with each other. For example, new compounds prepared by Woodstock Agricultural Research Centre as potential insecticides, herbicides, molluscicides or fungicides are examined by Tunstall Laboratory for their mammalian toxicity. At the same time, results from experience in the toxicology laboratory will help the agricultural group in their search for compounds combining low mammalian toxicity with high pesticidal activity.

Tunstall Laboratory consists of an air-conditioned brick building housing administrative, experimental and breeding units. The Laboratory's facilities are expanding according to plan. Within the next few years the total building area will be 27,000 sq. ft. when Tunstall will be one of the most modern and fully equipped units of its type.

The research in the laboratory is organized in four divisions:

(1) *Toxicology and Physiology Division.* This division is concerned with the quantitative toxicity of compounds towards vertebrates. The symptomatology of toxicants and derangements of functions due to them are also studied by pharmacological and hæmatological methods. Attempts are made to develop therapeutic measures for the treatment of over-exposure.

(2) *Biochemistry Division.* Here, the mode of action of toxic doses of substances is evaluated by study of their metabolism, distribution, absorption and excretion.

(3) *Chemistry Division.* This division prepares formulations for application, makes analyses for toxic substances and their metabolic products, and determines the composition of respired atmospheres.

(4) *Pathology Division.* Structural alteration is estimated in this division by anatomical and histological techniques.

Much of the initial work of the Laboratory has been concerned with the development of techniques, as, for example, those for evaluating the effects of contact of oil products and additives with the skin of mammals. Again, in the field of industrial chemicals, the laboratory has been active in developing techniques to be used in the examination of the inhalation of vapours and this in turn involves the design of methods of generating atmospheres of known composition.

A large part of the research programme is concerned with the toxicity of agricultural chemicals. The wide usage of the cyclodiene insecticides aldrin, dieldrin and endrin, and the recent introduction by 'Shell' of the related 'Telodrin', necessitated extensive examination of their biological effects, including rates of absorption and excretion by mammals. The significance of regular intake of small quantities of these substances is also being assessed by research into the mechanisms of their toxic action and possible threshold levels of activity.

Tunstall has also investigated enzyme inhibition in the mammal following exposure to organo-phosphate compounds, particularly the insecticide 'Phosdrin'; the dermal and chronic oral toxicity of synthetic detergents; the chronic oral toxicity of potential food contaminants such as solvents used in extraction of edible oils; and the toxicity or otherwise of additives in plastics.

Information derived from such studies is used to recommend procedures for the handling and application of chemicals, to ensure that the danger to man and wild life is kept to a minimum.

OBITUARIES

Prof. T. R. C. Fox

Terence Robert Corelli Fox, the first Shell professor of chemical engineering in the University of Cambridge, died in London on October 5 at the age of fifty. He was born on May 2, 1912, the only son of the late C. Fox, and was educated at the Regent Street Polytechnic Secondary School from where he won a minor scholarship to Jesus College, Cambridge, which he entered in 1930. At Cambridge Fox read for the Mechanical Sciences Tripos and throughout the course swept all before him. He obtained first-class honours in the Tripos of 1933 with the Rex Moir Prize, which is awarded to the candidate placed top of the list, and distinctions in theory of structures and in aeronautics. He received in addition three University prizes for the best performance in thermodynamics, in aeronautics and in structures.

On going down from Cambridge he joined Imperial Chemical Industries, Ltd., as a technical assistant and had four years of practical experience at Billingham before returning to Cambridge as a University demonstrator in engineering in 1937. He was elected a Fellow of King's College in 1941 and was promoted University lecturer in 1945. As one of his grateful pupils stated in *The Times* of October 9, Fox, while being a good and clear lecturer, was probably at his best as a college supervisor; in this capacity he gave invaluable service to King's. In the years immediately after the War he played a prominent part in the Department of Engineering in the re-organization of the courses which led to the division of the Tripos into two parts in 1947.

In 1946, Cambridge received a great benefaction from the Shell Group which enabled the University to establish a department of chemical engineering and Fox was elected to the new Shell professorship. At this time chemical engineering was a somewhat neglected subject in British universities. The number of students was small, and scientifically the subject was probably well behind its American counterpart. Fox spent a year visiting American universities to see how the subject was treated there, and on his return drafted a syllabus which combined the best of what he had seen with certain additional features which were the product of the Cambridge engineering tradition and of his own meticulous and penetrating mind. When he turned his attention for the first time to chemical thermodynamics, for example, his scrutiny went far deeper than that of most physical chemists. The staff, which he assembled from various disciplines, after recovering from the realization that they still had much to learn, came to share Fox's rigorous approach to the subject. The result has been that chemical engineering enjoys the undisputed status of an academic discipline not only at Cambridge but also at a dozen other universities; for Fox's doctrines have spread widely, and five members of the Cambridge staff have left to become professors elsewhere. The fact that the subject has shaken off its dubious associations with the old-fashioned chemical technology and that it has become the antithesis of an 'easy option' is due as much to Fox as to anyone.

Fox also carried the main burden of housing and equipping the new department. He first secured a temporary site where he built a small but efficient laboratory in huts. No sooner was this completed than he began to plan a more permanent laboratory. On this he lavished every care and, though his meticulous attention to the master-plan and to every detail was probably a trial to both the architect and contractor, the building in Pembroke Street to which the Department moved in 1959 stands as a memorial to his far-sightedness.

The burden of administering a university science department to-day can be heavy, but it is small compared with the labour and anxiety of creating one. Fox, who was a gentle and modest man, exhausted himself during twelve years of effort and was forced by ill-health to resign his chair in 1959. He returned as a University lecturer to the Department of Engineering to which he had earlier given devoted service.

Fox, in spite of his outstanding ability, was devoid of any personal ambition to make a name for himself as a public figure, a writer or research worker. He devoted himself single-mindedly to the well-being and efficiency of his department, and many of his former colleagues and pupils will remember gratefully the humanity with which he did so. John Baker

Mr. Walter Buddin

Walter Buddin died on August 14 at Reading, after a short illness. His death must have come as a considerable shock to many friends who had talked with him less than a month earlier at a meeting of the British Mycological Society in Harpenden. On that occasion, he had appeared in good health, and was certainly in his usual cheerful spirits. For many years, no meeting of the Society had seemed complete without him, and there was no member whose loss could have caused a greater gap.

Born on November 5, 1890, Walter Buddin eventually proceeded to Sidney Sussex College, Cambridge, and studied in the School of Agriculture. After taking his B.A. degree (he later took the M.A.), he was awarded a Board of Agriculture Research Scholarship in 1912, and went with it to Rothamsted, where he took part in research on partial sterilization of soil under the direction of Sir John Russell, and later visited the United States. During the First World War, he served with the R.A.M.C., spending part of his time in Egypt, where he contracted paratyphoid fever—a misfortune that may have been responsible for impaired health in later life. In 1919, he became a Ministry of Agriculture research exhibitioner at the Cheshunt Experimental Station, where he collaborated with the director, Dr. W. F. Bewley, in studying contamination of glasshouse water supplies by the spores of plant-pathogenic fungi; their joint paper on this subject attracted wide attention and is still quoted. During 1921–46, Buddin held the post of economic mycologist to the Southern Advisory Province and was attached to the University of Reading. In 1946 he transferred to the analogous position in the newly formed National Agricultural Advisory Service and continued in the new laboratory at Reading until his retirement in 1956.

During his thirty-five years in the agricultural advisory service, Buddin somehow made time to carry through and publish a not inconsiderable volume of fundamental research on fungal diseases of crops and ornamental plants, in addition to the more *ad hoc* investigations on disease control that arose out of his work as an adviser. Most of his more important work was published in collaboration with Miss E. M. Wakefield. Best known is their joint work on the violet root rot disease, in the course of which they established that *Helicobasidium purpureum* was the perfect state of the pathogen, until then known only as a sterile mycelium (*Rhizoctonia crocorum*). Although this discovery attracted widespread attention, equal credit is due to Buddin and Wakefield for their preliminary study of the biology of *R. crocorum* in culture. They were among the first mycologists to isolate this fungus in pure culture—at that time a considerable technical feat. Twenty years later, when I followed in their footsteps, I was able to confirm their observations and to admire the thoroughness and mycological finesse of their work.

Gifted as he was in his capacity as a research worker, yet as an agricultural adviser Buddin had, in my opinion, no equal. He combined an immense zest for his job with an uncanny knack of being able to enter into the minds of farmer and research worker simultaneously, so that he was the ideal interpreter of one to the other. Above all this, he had an extraordinary flair for seeing how research findings could be put into practice, though few people can have realized just how great was his contribution in this respect, because Buddin was a most generous man and seemed to care little about recognition for himself. Many young men besides me had this generous help from him, were made to feel that their research work was worth doing and were taught by example rather than by precept that one had to become an expert in general crop husbandry before one could become useful as a plant pathologist. A large part of Buddin's success with farmers was due to his kindly and never-flagging interest in the affairs of other people and to a most retentive memory. In the houses of his hosts, he was able to ask the most direct and personal questions without causing the slightest offence; a few years later, this information would be deployed to great effect in inquiries about the well-being of the family concerned.

Buddin's outstanding services to science and agriculture were widely recognized. He was elected an honorary member of the British Mycological Society in 1954 and of the Association of Applied Biologists in 1959, and was the first British biologist to hold both those distinctions. His association with the British Mycological Society was a particularly close one; at one time or another he held most of the offices of the Society, though he characteristically refused to be nominated for the presidency. His final service to the Society was as treasurer during 1947–61, during which time the Council received much wise advice, and the most tedious business was invariably enlivened by Buddin's pithy comments. Wit and philosopher though he was, it was perhaps Buddin's infectious enjoyment of his life and work that most endeared him to his friends and helped them to do the same. To have been helped by, and to have enjoyed the friendship of, such a man will be the treasured recollection of many of us.

S. D. GARRETT

Prof. V. N. Beklemishev

PROF. VLADIMIR NIKOLAIEVITCH BEKLEMISHEV, head of the Division of Entomology of the Martsinovsky Institute of Medical Parasitology and Tropical Medicine of the U.S.S.R. Ministry of Health, member of the U.S.S.R. Academy of Medical Sciences, died in Moscow on September 4 at the age of seventy-two years.

Prof. Beklemishev's death will leave in the field of medical entomology a gap that will not be easy to fill. His contribution to basic science was immense, linked with his interest in applied research and particularly related to problems of public health and to malaria eradication. Thirty years ago, while still on the staff of the University of Perm, Prof. Beklemishev became interested in the biology of impounded waters giving rise to large-scale breeding of *Anopheles*. This work led to the study of epidemiology of malaria, to which Prof. Beklemishev had devoted most of his work since 1932, when he was appointed head of the Entomology Division of the Malaria Institute in Moscow (now the Martsinovsky Institute of Medical Parasitology and Tropical Medicine).

In his new post, Prof. Beklemishev devoted all his knowledge, energy and experience to the organization of country-wide control of malaria, tick encephalitis and other arthropod-borne diseases.

Beklemishev's outstanding book on the comparative anatomy of invertebrates, first published in 1944, saw a second edition in 1952 and was translated into many languages. His book on the ecology of *A. maculipennis*, published in 1948, was equally successful. Beklemishev's main achievement was the thorough planning and organization of collective research on the bionomics of arthropod vectors of communicable diseases in the U.S.S.R. One of the results of this work was a recent book on seasonal changes in the biology of *A. maculipennis* over the whole vast territory of the U.S.S.R.—a unique achievement in the field of entomological science. He also wrote a foreword to the monograph on age-grouping methods in Diptera of medical importance (prepared by one of his pupils, Dr. T. S. Detinova), which was recently published by the World Health Organization.

Beklemishev greatly increased our understanding of ecological and phenological factors in the distribution of malaria and introduced new notions of 'landscape malariology' as a basis for planning malaria control and eradication. The study of the physiology of malaria vectors led Beklemishev and his collaborators to the introduction of new methods of age-grading of female *Anopheles*. The importance of this in the epidemiological follow-up of malaria eradication was fully realized in the U.S.S.R. and is now increasingly appreciated in other parts of the world. The age-grading methods developed by the school created by Beklemishev are now being used for the study of the biology of house flies, tsetse flies, black flies and midges in other countries. Beklemishev's wide interests and keen intellect contributed greatly to the study of population biology of arthropods, to the study of the evaluation of parasitism in blood-sucking vectors of human and animal diseases, to the development of nosogeography of communicable diseases and to the analysis of general and specific aspects of natural foci of infection.

He was the author of a series of handbooks on medical entomology and taught this subject to thousands of students at the Lomonosov University, at the Martsinovsky Institute and at the Central Postgraduate Medical School in Moscow. Beklemishev's brilliant teaching always emphasized the link between basic research and practical problems of public health.

The highest U.S.S.R. State prize for scientific work was given to Beklemishev twice: the first time for his study of comparative anatomy of invertebrates; the second time for his entomological work in the field of malaria eradication in the U.S.S.R., so ably led by Prof. Sergiev, his friend and colleague. He was a consultant on entomology and vector control to the Ministry of Health, U.S.S.R., and co-editor of the journal *Meditsinskaya Parasitologia i Parazitarnye Bolezni*. He was also a member of the Expert Advisory Panel on Malaria of the World Health Organization.

Beklemishev had an immense fund of knowledge in the field of entomology, hydrobiology, geomorphology and biostatistics and was always ready and eager to place his experience at the disposal of those who consulted him. His lectures were a model of clarity in presentation and often enlivened by delightful humorous asides. His main outside interest was the history of art and he was a keen student of painting and sculpture. He was a kind and a wise man and his absence will be sadly felt by his many friends and admirers everywhere. L. J. Bruce-Chwatt

NEWS and VIEWS

The Royal Society of Edinburgh

The following have been elected officers and members of Council of the Royal Society of Edinburgh for the Session 1962–63: *President*, Prof. E. L. Hirst; *Vice-Presidents*, Dr. A. W. Greenwood, Prof. R. A. Rankin, Dr. C. E. Lucas, Prof. J. A. Macdonald, Dr. H. R. Fletcher and Prof. G. L. Montgomery; *General Secretary*, Prof. Norman Feather; *Secretaries to ordinary meetings*, Dr. Mowbray Ritchie and Prof. A. E. Ritchie; *Treasurer*, Dr. J. R. Peddie; *Curator of Library and Museum*, Dr. R. Schlapp; *Councillors*, Prof. J. H. Burnett, Prof. C. F. Davidson, Prof. D. C. Pack, Dr. A. B. Stewart, Prof. Jack Allen, Dr. S. C. Curran, Dr. G. H. Mitchell, Dr. Magnus Pyke, Prof. R. B. Fisher, The Rev. Dr. A. M. Gillespie, Prof. A. Robertson and Prof. P. A. Sweet.

The Smithsonian Institution, Washington: New Appointments

On October 31, Dr. Remington Kellogg retired from the posts of assistant secretary of the Smithsonian Institution and director of the United States National Museum, Washington, after forty-two years in Government service. Dr. Kellogg, one of America's leading biologists, will continue his research at the Smithsonian as an honorary research associate of the Institution.

Dr. Albert C. Smith, who has been director of the Smithsonian's Museum of Natural History since 1958, becomes an assistant secretary of the Institution. Dr. Smith, well known for his work in phanerogamic botany, is a native of Springfield, Mass. He received his Ph.D. in 1933 from Columbia University. Thereafter he was associated with the New York Botanical Garden, the Arnold Arboretum of Harvard University, and the National Herbarium of the Smithsonian. In addition to study and research in England, France, Switzerland, Germany, Holland and the Scandinavian countries, he has participated in several botanical expeditions to Colombia, Peru, Brazil, Fiji, British Guiana, and the Lesser Antilles.

Mr. Frank A. Taylor has been appointed director of the United States National Museum (composed of two Smithsonian museums—the Museum of Natural History and the Museum of History and Technology). He has served in various curatorial and administrative positions at the Smithsonian Institution since 1922. Since 1958 he has been director of the Museum of History and Technology, the new building of which is now nearing completion on Constitution Avenue. He will continue to hold the latter position in addition to his new post. Mr. Taylor is a native of Washington, D.C. He holds an engineering degree from the Massachusetts Institute of Technology and a degree of law from Georgetown University.

Dr. T. Dale Stewart has been appointed director of the Museum of Natural History. Dr. Stewart's service at the Smithsonian dates from 1927. In 1942 he succeeded the late Dr. Aleš Hrdlička as curator of the Museum's Division of Physical Anthropology, a position he held until early 1961, when he was made head curator of the Department of Anthropology. He will also continue in the latter position. Dr. Stewart, a native Pennsylvanian, is a graduate of George Washington University and holds an M.D. degree from Johns Hopkins Medical School. His research work has been principally concerned with bone structure and pathology, dental caries, anthropometry of Eskimos and Indians, and cranial deformities. He is frequently called on by the Federal Bureau of Investigation as an expert on the identification of skeletal remains. He is the author of many scientific papers, has been on numerous scientific expeditions in many parts of the world, and is the recipient of many professional honours, including the Viking Medal and award for outstanding anthropological research. He was recently elected to membership in the National Academy of Sciences.

Botany at the University of the Witwatersrand: Prof. E. R. Roux

Dr. E. R. Roux, senior lecturer in the Department of Botany at the University of the Witwatersrand, has been appointed to the chair of botany in succession to Prof. N. P. Badenhuizen, who resigned some time ago to go to Canada. After matriculating at Jeppe High School, Dr. Roux went to the University of the Witwatersrand. He obtained his M.Sc. degree in 1926 and was then appointed junior lecturer in the Department of Botany. Soon afterwards he was awarded a scholarship of the Exhibition of 1851 and went to the University of Cambridge, where he obtained his Ph.D. degree for a thesis on plant physiology in 1929. On his return to South Africa he was employed for a time in the Government Low Temperature Research Laboratory. Later, as the holder of a Carnegie grant and the Thomas Smartt scholarship, he conducted investigations on fruit storage problems in the Department of Chemistry at the University of Cape Town. During the Second World War he conducted investigations on fish oils and various marine biology problems, and played an active part in helping to establish the vitamin-oil industry in Angola. In 1946 he returned to Johannesburg on his appointment to the staff of the University of the Witwatersrand as senior lecturer in botany

The Meteorological Research Committee

NOVEMBER 1962 sees the twenty-first anniversary of the Meteorological Research Committee, set up during the Second World War "to advise the Secretary of State for Air as to the general lines along which meteorological research should be developed". Official meteorology in Britain is virtually a monopoly of the Meteorological Office, a sub-department within the Air Ministry, and the Meteorological Research Committee has been continuously effective in bringing the advice of leading university workers, in meteorology and related fields, to bear on the research activity of the Office. During these years, under the successive chairmanships of Profs. Sydney Chapman, G. M. B. Dobson, Sir David Brunt, Sir Charles Normand and Prof. P. A. Sheppard, the Committee has seen research in the Meteorological Office develop from a fringe activity into a full directorate employing some sixty staff in the scientific officer class alone. Well-known pioneering research includes that of the Meteorological Research Flight with its quite unique records of data on cloud physics, and on ozone and water vapour, the application of advanced electronic computing to weather forecasting by dynamical calculation, and more recently the preparation of experiments for forthcoming flights of rockets and Earth satellites. In these twenty-one years, meteorological research has come of age and the guidance and encouragement afforded by the Meteorological Research Committee are appropriately acknowledged in the November 1962 number of the *Meteorological Magazine*. with historical surveys and accounts of present work.

The Registrar General's Quarterly Return for England and Wales

IN the second quarter of 1962 the birth-rate continued to run at the high level of 18·8, compared with 18·0 in the second quarter of 1961. But, whereas the birth-rate for the second quarter is normally higher than in the first, in 1962 it dropped by 0·6 per thousand, but it is still likely that the total number of births in 1962 will be approximately 850,000. Deaths in the second quarter continued at the same level as in previous years, as did infant mortality The Registrar General's *Quarterly Return*, which contains these data, also includes an abridged life table for 1959–61, showing a life expectancy at birth of 68·1 years for males, and 73·9 for females, and a median length of life of about 73 years for males and 77 years for females (*The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates;* quarter ended 30th June, 1962. No. 454, 2nd Quarter 1962. Pp. 24. London: H.M.S O., 1962. 2*s*. 6*d*. net). Another table shows that cancer and accidents are now responsible for the largest number of years of life lost by the male working population. 115 and 98 per 10,000 population respectively.

The Geological Survey of Great Britain

THE *Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the Year 1961* reports continued progress in various field surveys and records that rather less than one-quarter of Great Britain has not yet been mapped on the 6-in. scale (Pp. iii+83. London: H.M.S.O., 1962. 5*s* 6*d*. net). In addition to this primary survey work. several other areas are being re-surveyed. The main part of this *Summary of Progress* deals with the usual reports of the geologists working in the various districts. Also included are the sectional reports covering palæontology, petrology, chemical and spectrographic examination, water, geophysical and atomic energy discussions. In the Museum, normal curatorial work has proceeded, and advantage is taken to illustrate topical subjects. A display illustrating Tristan da Cunha was on exhibition a few days after the volcanic eruption.

The Soil Survey of Great Britain

REPORT No. 14 of the Soil Survey of Great Britain describes new areas surveyed in 1961 amounting to about 2,100 square miles (Pp. viii+46. London: H.M.S.O., 1962. 5*s*. net). Reconnaissance surveys in several counties of England and Wales were continued and detailed surveys of the Nuffield Farm Project in the Teign valley of Devonshire and of the lowland mosses of Lancashire were completed. The latter enabled the Agricultural Land Service to prepare land utilization maps of this area. In Scotland mapping is proceeding satisfactorily and much of the agricultural lowland is now covered. In addition to the routine work, surveys were undertaken for special purposes, and other work included experiments on the influence of added nitrogen on the increase of organic matter and the development of a biological nitrogen-cycle on an initially inert material. The report includes a map showing the districts where work is proceeding.

Soils and Fertilizers

THE October issue of *Soils and Fertilizers* follows the usual pattern of abstracts, summaries of research reports, a list of bibliographies, two book reviews and a review article. One review is of a symposium on "Nitrogen in the Tropics with Particular Reference to Pastures", held in Brisbane in 1960 and published as Bulletin 46 of the Commonwealth Bureau of Pastures and Field Crops; the other is on "Sol. Microflore et Végétation: équilibres biochimiques et concurrence biologique", by B. Boullard and R. Moreau. The review article is by B. W. Avery, of the Soil Survey of England and Wales, on "Soil Type and Crop Performance". This is a topic that is assuming great importance with progress in the classification and mapping of soils on one hand and the accumulation of experimental evidence of soil fertility on the other. The productive capacity of a soil depends on the interaction of a great many factors coming under such headings as climatic conditions, management and the more permanent characteristics of the soil, and numerous workers in countries of north-west Europe and in the United States have analysed combinations of these factors in attempts to make yield predictions. This is obviously of particular importance in the selection of suitable sites for horticultural crops and perennial crops such as fruit trees. Since it is clearly out of the question to have long-term field experiments on all the thousands of different soil types, it is essential to decide what criteria in classification are of most significance in the soil–plant relationship and to classify soils so that the soil map may be reliably used in a wide application of the results of fertility studies.

Concrete Research

The Magazine of Concrete Research is published periodically by the Cement and Concrete Association at Wexham Springs, Stoke Poges, Bucks., and the March issue (14, No. 40; 1962) contains, besides seven technical papers, an editorial on the future of prestressed concrete based on a lecture given by R. E. Rowe (in charge of research into structural design at the Cement and Concrete Association), earlier this year. Among the significant observations made by that author were the possibility of achieving concrete cube strengths of 12,000–14,000 lb./in.^{2} under closely controlled conditions of compaction and curing; the importance of specifying design stresses in concrete appropriate to the stress field in which it was required to function; consideration of possible biaxial or triaxial stresses and not simply uniaxial compressive strength as sole criterion of ultimate strength; the practical importance of the strength/density ratio in prestressed concrete in future; the production of expansive cements and their scope in factory manufacture of precast units. Rowe also emphasized the fact that ". . . knowledge of the true failure criteria of concrete was still very limited. Future research would undoubtedly increase our knowledge, and lead to a revision of our ideas of permissible and ultimate load stresses. The information we already had suggested that this would mean higher values for these stresses". The other subjects traversed in this issue are reinforced concrete under thermal gradients; behaviour and ultimate strength of prestressed concrete hollow beams under combined bending and torsion; dynamic loading of prestressed concrete beams; deformation of a rectangular slab with free edges under transverse loads; triaxial testing of fresh concrete; studies of pore size distribution in mortars; and strength tests on scaled-down concretes suitable for models with a note on mix design. It may not be generally realized that the Cement and Concrete Association provides, on request, a free service of technical information and advice on all aspects of concrete construction by a highly experienced advisory staff. The Research and Development Services of the Association includes in its activities the making and testing of structural models, for example, the Hammersmith Flyover, the Medway Bridge, etc. It has available a *Sirius* electronic computer for structural calculations and data processing; it also specializes in production of small specimen surface finishes to assist architects, engineers and others in developing methods of improving the appearance of concrete.

United Kingdom Postgraduate Awards, 1962–64

THE Association of Universities of the British Commonwealth has now issued its 1962–64 list, *United Kingdom Postgraduate Awards*. Primarily the purpose of this booklet is to set out in a form suitable for quick reference summarized information about fellowships, scholarships, grants, etc., tenable at universities in the United Kingdom. There is also appended a short list of awards tenable overseas by graduates from the United Kingdom. In the first section, information is supplied under the headings of the universities concerned—details being given of the subject of the award, its value, the length of time for which it is tenable, eligibility, number, closing date for application and details of where further particulars can be obtained. Similar treatment is given in the following sections, which deal with agriculture and veterinary science, applied science and technology, arts, dentistry, education, law, medicine and surgery, and science (specific subjects); the list is completed with a useful index of names. Copies can be obtained from the Association of Universities of the British Commonwealth. 36 Gordon Square, London, W.C.1 (5*s*., plus 6*d*. postage).

The Hospital Library in Sweden

IN *Library Association Pamphlet* No. 23 are collected translations by Mr. and Mrs. Frykman of a symposium of five papers on the hospital library in Sweden originally issued by the Swedish Library Association in 1958 (*The Hospital Library in Sweden* Pp. 40. London: Library Association, 1962. 10*s*.; L.A. members, 7*s*. 6*d*.). The papers deal with the administration and organization of the hospital library; the principles of book selection for hospital patients; technical aids for the hospital library and patients; reading therapy in the service of the sick; and some personal reflexions on patients, doctors and books. The new technical aids are illustrated and some indication is given of the cost of the services recommended. The pamphlet gives a valuable indication of the possibilities and of the standards that could reasonably be expected, but it is also a notable contribution to constructive thinking on this general theme.

The American Society for Metals

DR. ROBERT J. RAUDEBAUGH, of Plainfield, New Jersey, was elected president of the American Society for Metals at the annual meeting of the Society held recently at the Hotel Biltmore. Dr. Raudebaugh, who is supervisor of Iron Nickel Alloys, Product Development of the Development and Research Department of the International Nickel Co., Inc., New York, succeeds Dr. Carl E. Swartz. Other officers and trustees of the Society who were elected at the same time, and who with Dr. Raudebaugh will take office, are: *Vice-President*, Merrill A. Scheil, director of Metallurgical Research, A. O. Smith Corp., Milwaukee, Wisconsin; *Secretary-Trustee*, 1962–64. Dr. Stewart G. Fletcher, vice-president–technical director, Latrobe Steel Co., Latrobe, Pennsylvania; *Trustees*, 1962–64, Dr. Robert F. Thomson, technical director, Engineering Research, Research Laboratories, General Motors Corporation, Detroit, Michigan; Dr. Alexander R. Troiano, professor and head of Department of Metallurgical Engineering, Case Institute of Technology, Cleveland, Ohio.

Applications of Engineering in Medicine and Biology

THE Leverhulme Trust has made available, each year for five years as from September 1963, four Leverhulme Bursaries, each of value £1,000 plus Federated Superannuation Scheme for Universities and National Health Insurance allowances, to enable persons engaged in medical or biological research to attend the postgraduate course in "Applications of Engineering in Medicine and Biology" which is in operation in the Department of Electrical Engineering, Imperial College of Science and Technology. The first annual course commenced in October 1962, and these bursaries will therefore be available for the course commencing in October 1963 and for subsequent courses. The courses are planned to meet the needs of present or intending research workers, qualified in medicine or biology, who require for their research a basic understanding of and familiarity with modern electronic or other physical apparatus and the principles and applications of engineering and mathematical techniques. Each annual course comprises lectures, tutorials and laboratory instruction, occupying ten months of full-time study. A specialized laboratory has been established that is well equipped for training in the electrical and mechanical principles that are involved in physiological research and instrumentation. Instruction is provided in the use of electronic circuits and semiconductor devices, in automatic data-handling, in the use of analogue and digital computers, and in other aspects of physics and engineering having application in medicine and biology. Further information concerning these bursaries can be obtained from the Registrar, Imperial College of Science and Technology, London, S.W.7.

Award for Biomagnetic Research

THE Biomagnetic Research Foundation is offering an award of 1,000 U.S. dollars for the development of a classic experiment demonstrating with simple means a biological effect of a static magnetic field. The best experiment will be chosen at the second Biomagnetic Symposium in November 1963 and repeated within one year by J. M. Barnothy and M. F. Barnothy on behalf of the Biomagnetic Research Foundation. To qualify for the competition, the experiment has to meet the following conditions: the treatment of the live animals or specimens *in vivo* should not necessitate a more extended, stronger or more inhomogeneous magnetic field than that which can be produced with pole pieces with a Varian 4-in. electromagnet. The treatment time should not exceed 200 h in the field. The biological effect should be observable after treatment, but within a time interval of 6–100 h after removal of the specimen from the field. The effect must be demonstrated on an adequately large number of specimens, to ensure a probability-level better than 1 : 10,000. The experiment should be of such nature that the influence of uncontrollable environmental factors, such as variation of the Earth's magnetic elements, sunspot activities, barometric pressure, cosmic radiation, Moon phases, etc., can be considered as negligible. Fuller details of the conditions of the award can be obtained from the Biomagnetic Research Foundation, 833 Lincoln Street, Evanston, Illinois.

Southern Pennines Geological Conference

DURING recent years there has been a great revival of interest in the geology of the Southern Pennines, more especially in the Carboniferous rocks of the region. This has been due in no small measure to a large increase in the number of research students, and to the evolution of new techniques both in the field and in the laboratory. The renewed activity has led inevitably to a certain amount of overlap, and there have been several instances in which a partial or even complete overlap of research interests has been discovered only at a late stage of the investigations. In order to co-ordinate research effort, and to facilitate contact between all those actively interested in the area, whether amateur or professional, a conference was held at the University of Keele during September 24–26. Prof. F. W. Cope (Keele) welcomed the delegates and gave a short introductory address on the first evening of the meeting, and, on the following two days, papers with reports of work in progress, or recently completed, were read by Prof. F. W. Shotton, Dr. F. M. Broadhurst, Dr. T. D. Ford, Dr. H. G. Reading, Dr. H. E. Sadler, Dr. A. A. Wilson, Mr. R. H. Johnson and Mr. B. K. Holdsworth. Following the success of the conference, it was decided that a second one should be held at Keele during September 1963. Dr. M. H. Mason, of the Geology Department, Keele, who acted as honorary secretary, will continue in that capacity for the 1963 meeting.

Announcements

SIR WILLIS JACKSON, professor of electrical engineering, Imperial College of Science and Technology, has been appointed chairman of the Television Advisory Committee. As such, Sir Willis succeeds Sir Charles Daniel, who has resigned after serving as chairman for ten years. The Committee was set up by the Postmaster-General to advise him on the development of television broadcasting and very-high-frequency sound broadcasting.

THE Biochemical Society of France has awarded the 1962 Charles Leopold Mayer Prize to M. François Chapeville for his work leading to the demonstration of the importance of certain ribonucleic acids in passing on genetic information. M. Chapeville has been working under Dr. P. Fromageot at Saclay and has also carried out research in the United States with Dr. F. A. Lipmann on the reactions that enable the living cell to condense amino-acids into protein macromolecules. The prize, which is awarded annually for notable research work on the nucleic acids and nucleoproteins, is valued at 5,000 N.F.

A COLLOQUIUM on clinical and diagnostic aspects of enzyme multiplicity is to be held at the University of Ghent on April 27. The colloquium is being organized by the University in collaboration with the Belgian Society for Clinical Chemistry. Further information can be obtained from Prof. R. J. Wieme, Laboratory of the Medical Clinic, University of Ghent, Pasteurdreef 2, Ghent.

A SPECIAL meeting of the Iron and Steel Institute will be held in India during the early spring, at the invitation of the Indian Institute of Metals and the National Metallurgical Laboratory, Jamshedpur. The programme will include a symposium on "Recent Developments in Iron and Steelmaking with Special Reference to Indian Conditions", and visits to various Indian steelworks. Further information can be obtained from K. Headlam-Morley, The Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1.

A TWO-DAY symposium, entitled "The Nature, Synthesis and Activity of Enzymes", will be held at the Luton College of Technology during April 18–19. The subjects under consideration include: isolation of pure enzymes; studies on the amino-acid sequence and the active site; following the progress of enzyme reactions; organization of the cell; mechanism of protein biosynthesis; control of enzyme synthesis in cells. Further information can be obtained from the Registrar, Luton College of Technology, Luton, Bedfordshire.

IT is regretted that the announcement on p. 324 of *Nature* of October 27 relating to Dr. R. A. Pattle was in error. This should have referred to Dr. R. E. Pattle, who is on the staff of the Chemical Defence Experimental Establishment. He is best known for his identification of the lining film which lowers the surface tension of the lung alveoli. He has also carried out work on the toxicology of atmospheric pollution.

CALORIMETRY

THE seventeenth annual Calorimetry Conference was held during August 22–24 at the University of California, Berkeley. Hosts for the occasion were the Inorganic Materials Research Division of the Lawrence Radiation Laboratory and the College of Chemistry. Local arrangements were made by a committee comprised of Prof. N. E. Phillips (chairman), Prof. R. Hultgren, Dr. D. N. Lyon and Mr. I. Pratt.

In keeping with the traditions of previous conferences, a wide variety of topics pertaining to calorimetry was discussed, including experimental techniques, results and their interpretation. Thirty-seven papers were presented, the principal one of which was that given as the Huffman Memorial Lecture by Prof. E. F. Westrum, jun. (University of Michigan). Prof. Westrum discussed the "Thermodynamics of Globular Molecules" and set out lucidly the problems of understanding the behaviour of the so-called plastic crystals. Thermodynamic measurements on these substances can yield valuable information about transitions, and about rotation of molecules and molecular groups in the solid. Much of the available experimental information has been obtained by Prof. Westrum and his students.

Invited papers were given by M. L. McGlashan (University of Reading), D. Patterson (University of Montreal), A. M. Karo and A. W. Searcy (University of California). Each of these papers served to emphasize a particular part of the programme. For example, McGlashan's discussion of the calorimetric determination of the change of enthalpy of vapours with pressure and Patterson's application of the Prigogine theory to the explanation of heats of mixing of polymer solutions introduced a series of contributions on measurements of heats of mixing, solution and dilution.

Karo described the information about lattice vibrations which is obtainable from thermodynamic properties of crystalline solids and illustrated his theme by taking examples of alkali halide crystals. In particular, he showed how accurate experimental heat capacities are sufficient to distinguish between several possible ionic models. Papers contributed in related fields dealt with thermodynamic properties of solid yttrium hydrides, transition metal and technetium hexafluorides, palladium hydride, lithium metal, helium and methanes.

High-temperature thermodynamic properties were introduced by Searcey's contribution on the interpretation of the properties of inorganic solid solutions (uranium–oxygen and cerium–oxygen systems), which was coupled with a plea for more experimental measurements. The particular kinds desired were not represented at the Conference; high-temperature studies which were reported had more to do with the measurement of stored energy.

Many other specific topics were touched on, for example, precision bomb calorimetry, which provides accurate and fundamental information on heats of formation and is the very basis of thermo-chemistry; techniques of micro-calorimetry for measuring very small energy changes; improvement of standards of measuring energy and temperature.

Again, in keeping with the practices of previous conferences, no proceedings will be published. The work discussed will in due course appear in regular journals. A report on the Conference would not be complete, however, without mentioning the annual dinner held at Spenger's Fish Grotto in Berkeley. Dr. Nicholas Kurti of the Clarendon Laboratory, Oxford, was the after-dinner speaker and chose as his topic "What's Cooking", a deceptively calorimetric title. Although he did not, as advertised, prove his text, which was the aphorism, "The discovery of a new dish contributes more to the happiness of mankind than the discovery of a star", he kept his audience thoroughly entertained and no one worried unduly.

GAS CHROMATOGRAPHY

AN informal symposium of members of the Gas Chromatography Discussion Group of the Institute of Petroleum was held on September 7 at the Royal College of Advanced Technology, Salford, with Mr. C. S. G. Phillips in the chair. Dr. Ramage, head of the Chemistry Department, welcomed the Group at the College. The first paper was a repeat by Dr. A. J. P. Martin (Abbotsbury Laboratories, Elstree) of his opening address* to the fourth International Symposium on Gas Chromatography (Hamburg, June 1962) entitled "Future Possibilities in Microanalysis". Dr. Martin stated that much could be gained, particularly in the sciences where the amounts of available materials are severely restricted, if the scale of analytical operations were reduced by a factor of 10^6 or greater. It should be possible to design apparatus to weigh 10^{-9} g at room temperature and 10^{-11} g at a few degrees absolute. In gas chromatography he envisaged the possibility of the column effluent being passed over a directly weighed leaf of adsorbent to give a mass integral chromatogram and a system of multiple leaves providing fraction collecting facilities. Time-of-flight mass spectrometry and X-ray crystallography could provide the means of identification. As far back as 1946, Engström (*Acta Radiologica*, Stockholm, Suppl. 63; 1946) had used the characteristic absorption of very long X-rays to carry out an analysis on samples of the order 10^{-9}–10^{-12} g. The main difficulty in the type of picochemistry envisaged by Dr. Martin lay in the limitation imposed by the size of our hands. This was exemplified in the 'stone-age' character of the instruments available for handling sizes observable under a simple microscope. Essentially then, smaller hands would be required and ideally these should have similar translatory and sensory facilities as the hands made by nature. Some form of mechanical–pneumatic system used in conjunction with the human hand was the approach suggested. Although at first sight a reduction of about ten to one might not seem difficult to achieve, it should be borne in mind that the aim would be to design a system capable of reproducing itself by the same reduction factor without modification of the system until, ultimately, a stage was reached when, possibly, single molecules could be examined. At this final stage the energy of the electron microscope would be a disturbing factor as would Brownian movement, hence the need for the final stage to be able to relay back the sense of touch and be able to operate near the absolute zero of temperature. As the scale of operation was reduced the increased surface to volume ratio of the apparatus would give rise to problems associated with the activity of the surface and the grain structure of metals might pose problems in fabrication. The much-reduced size would, however, allow the use of materials prohibitively expensive on present size scales. Surface tension and viscosity effects would also need to be considered.

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* To be published in full in the *Proceedings of the Hamburg Symposium*.

In concept, if not as yet in size, the adaptation of conventional laboratory balances as detectors to give mass integral chromatograms described by Mr. S. Thorburn (co-authored by Dr. S. C. Bevan, Brunel College, London, W.3) was a natural follow-on to the paper by Dr. Martin. Initially a standard balance had been used at room temperature with the column effluent passing into a liquid absorber, due correction being made for vapour losses. The present version makes use of a commercially available micro-analytical thermo-balance (the oven of which can be used as a column thermostat with a range far in excess of that required by present high-temperature operation) coupled to a recorder. The absorber is glass cloth impregnated with concentrated sulphuric acid, and experience so far shows it to be quite efficient for a wide range of organic compounds. With the present set-up, a 6–10 component sample of 4–5 mg total weight can be quantitatively determined to an accuracy of ± 2 per cent. One limitation of the present design is the susceptibility to variation in gas flow-rate, but with a dynamically symmetrical design of absorber as suggested in the discussion by Dr. Martin a considerable improvement in sensitivity and stability could undoubtedly be obtained.

The desirability of using a sensitive detector giving results directly in terms of mass rather than a property which, by the time it is traced on to a recorder chart, may be some undefined variable function of mass was made apparent in the third paper of the day, "Studies on the Accuracy of Gas–Liquid Chromatography Analysis", presented by Mr. A. F. Williams (Imperial Chemical Industries, Ltd., Nobel Division, Ayrshire). Despite such difficulties, Mr. Williams showed that by concentrating on two types of detector (thermal conductivity cell and flame-ionization) and two types of injection (capillary pipette and micro-syringe) it was possible to decide on conditions to give the accuracy called for on each type of sample and to provide the service required of an analytical department. All the results reported had been obtained using packed columns. With the thermal conductivity cell, sample sizes less than 1 µl., and mixtures of benzene, toluene and xylenes of widely differing composition it had been found that with nitrogen as carrier gas it was essential to control closely the sample size in order to obtain accurate results; to a lesser degree this also applied to helium. With other types of sample the control of column loading might not be so critical. Thus in contrast to the benzene–toluene–xylene mixture where a micro-syringe was essential, in the case of the methylchlorosilanes the sample pipette originally used by James and Martin was satisfactory for sample addition. Calibration on peak height was preferred to peak areas obtained with an integrator of the servo-tachometer type. With a flame-ionization detector, results had been obtained for various benzene–toluene–xylene mixtures using splitter technique and direct injection in dilution in carbon bisulphide. Within themselves the repeatability was generally better than ± 2 per cent of the mean, but agreement between results by the two methods was not quite so good. There was an indication that different calibration factors were required for different sample compositions when the splitter technique was used. Dr. J. N. A. Ridyard queried the need to use a splitter or dilution technique with the flame ionization detector. It had been his experience that subject to suitable design of probe and relative position and size of jet, direct injection of samples of weight well in excess of what Mr. Williams had used was possible without loss of linearity. Mr. I. V. Mortimer reported that the results of a study on the quantitative aspects of a thermal conductivity cell were disappointing. A similar study with the flame ionization detector had shown that for concentrations used with capillary columns there was a minimum value for hydrogen flow-rate to the detector below which the response was non-linear and above which it was linear. The exact form of the response curves was variable dependent on the geometry of the jet and collecting electrode to within quite fine limits. The points of view put by the author and contributors to the discussion might well be summarized by saying that quantitative results can best be obtained by knowing both your sample and your detector.

The afternoon session was devoted to the problem of tabulating and reporting retention data. Dr. A. B. Littlewood (University of Durham) had reviewed the literature and attempted to correlate data in terms of specificity of liquid phase. In the simplest case of alkanes in liquid phases of any type, he found that the latter could be put into one order generally independent of whether the Vg value or the slope of the log Vg–carbon number plot (hereafter referred to as a log-plot) was used as the criterion. In both cases values were greatest for an alkane solute–alkane liquid phase interaction. Values obtained for the relative retentions of successive homologues could also be used to put liquid phases into an order, but generally the differences between values for different liquid phases were only significant for the most polar liquids. The Vg value or slope of the log-plot was recommended for giving an order to the weakly selective phases. The results obtained for relative retentions of successive homologues also applied when a polar grouping was introduced into the solute molecule. For the retentions of a variety of solutes with a non-polar liquid phase an approximate correlation could be obtained by plotting log Vg against $\frac{n^2 - 1}{n^2 + 2} \cdot M$ where n is the refractive index and M the molecular weight of the solute. From the available data and making use of the relative values of successive homologues there was every indication that the retention of solutes in a given non-polar liquid phase could be predicted to within a factor of two. For gradation of polar and non-polar liquid phases to include diverse types of solutes Dr. Littlewood had attempted to construct plots of the type used by Rohrschneider (*Zeitschrift fur Analytische Chemie*, 170, 256; 1959; see also *Journal of Chromatography*, 8, 308; 1962) for classifying liquid phases according to selectivity to alkanes, alkenes and alkynes. With such plots it was found possible to predict the chromatographic behaviour of a liquid–phase sequence such as dibutyl-, diethyl- and dimethyl-formamides. Deviations from a general liquid phase 'polarity order', for example:

paraffin < dinonyl phthalate < dibutyl phthalate < tricresyl phosphate < methoxy polyethylene glycol 350 < 1,2,3-trans (2-cyano ethoxy propane)

could in some cases be ascribed to solute–solvent hydrogen bonding, but there appeared to be no indications of specific aromatic–aromatic interactions.

Dr. J. F. Smith (Natural Rubber Producers' Research Association, Welwyn Garden City) put the case for presenting retentions relative to the *n*-alkanes; either his own R_{x9} (relative to *n*-nonane) or the retention index system of Kovats was suitable. The relative merits of bracketing the compound to be characterized with *n*-alkanes in one run, or determining the retention of the compound either on its own, or with one *n*-alkane, or bracketed with two *n*-alkanes and then comparing with a separate run of four *n*-alkanes were discussed as was the use of non-linear log-plots when retentions were measured from the point of injection instead of the air-peak. For detectors not responding to air, methane could be used to give the hold-up if the column temperature were greater than 70° C. Dr. P. A. T. Swoboda presented evidence that the same retention index values were obtained by interpolation from retentions measured from the point of injection as were obtained from retentions measured from the air peak. With the former procedure, however, as Dr. Smith had mentioned, the slope of the log-plots had to be determined from the asymptote. Published V_g values for alkyl nitro- and nitrate compounds (Evered and Pollard, *Journal of Chromatography*, 4, 451; 1960) were used by Dr. Swoboda to illustrate an example of the temperature independence of values of retention index. A written contribution by H. van den Dool and P. de C. Kratz showed that there was good agreement between values of retention index obtained under isothermal and temperature programmed conditions.

Mr. B. A. Knights suggested that in steroid chemistry the gas–liquid analogue (*Nature*, 194, 833; 1962) of the R_M value $\left[\log.\left(\frac{1}{R_F} - 1\right)\right]$ of liquid–liquid systems which could be broken down into contributions from the parent molecule and functional groups should be used to keep the nomenclature in this field as uniform as possible.

In summary, it would appear from the papers and discussion during the afternoon session that once a liquid phase has been characterized in terms of the slope of the log-plot for *n*-alkanes at several temperatures, workers need only report their results relative to one or more *n*-alkanes and the devotees to each system would soon provide a wealth of derived data.

C. G. SCOTT

COASTAL PROCESSES

A SEMINAR on "Coastal Processes" was held at the Department of Geography, University of Cambridge, during June 29–30. Thirty scientists attended the seminar, including geographers, oceanographers, engineers and geologists, most of whom were from the United Kingdom. Those attending from overseas were four engineers from the Dutch Rijkswaterstaat, including J. B. Schijf, director of research, Prof. A. J. Guilcher of Paris and three Australian engineers. Prof. J. A. Steers of Cambridge acted as chairman for the five sessions into which the discussions were divided, namely: (*a*) wave generation as it affects sediment movement; (*b*) beach processes, in the surf zone and beyond; (*c*) beach cycles, annual and long term; (*d*) equilibrium shapes of coastline in plan; (*e*) world-wide sediment movement.

Wave generation. For the purposes of studying movement of sediments it was pointed out that only two categories, storm waves and swell, need be considered. The former are still within the fetch while the latter have moved outside it and travel in ever-enlarging circles across the oceans of the world. Waves are seen to be an efficient means of spreading the energy of the wind in given storm areas over the surface of the Earth. Seas, partially or fully enclosed, experience less swell action than coasts bordering the major oceans and, therefore, the beach profiles are significantly different.

Beach processes. The breaking of waves on the beach creates two distinct zones in which sediment is transported: the surf zone and the offshore area beyond the breaker line. Although great turbulence and high velocities exist in the surf zone, more sediment can be transported in the offshore area because of the greater width of action. This width can be miles in some cases, out to depths of 40 fathoms for 10-sec waves and 100 fathoms for 15-sec waves. The difficulties of verifying the theory on this movement were outlined by many speakers.

The action of swell accreting a beach was discussed with emphasis placed on the mass transport[1] of the waves in moving the sediment towards the shore. It was noted how the ripple formation on the sea-bed changes with variations in wave height, period and direction—this disturbed state producing larger rates of sediment transport. The action of storm waves on the steep swell beach profile was seen to be a combination of a hydraulic jump from the backwash occurring at a point on the beach face where the sand is in quasi-suspension due to the excessive ground-water returning to the sea at this point[2,3]. However, after the material has been forced into an offshore bar the erosion ceases since waves are then dissipated on the bar.

Beach cycles. Storm waves, due to their short duration, are not significant in moving sediment along the coast. Swell, on the other hand, being produced consistently from wind systems, which are repetitive year in and year out, moves material in one particular direction when returning it from the offshore bar to the beach. Any interference with the longshore drift can result in erosion of the offshore area, which then requires more sediment during the next storm sequence before the bar is sufficiently high to protect the beach. Thus, storm waves display the previous erosive action of the swell.

Although most of the discussion related to wave action at a constant sea-level it was noted how tidal fluctuations spread the energy of waves over a greater width of beach profile. The combined action of (*a*) tidal streams, (*b*) the salt water wedge and (*c*) wave propagation into an estuary, causes continuous siltation.

Coastlines in plan. The arrival of the most persistent swell from a specific direction causes a net movement of sediment along the shore. It is important to know its direction in the design of maritime structures. A model study of the action of oblique swell on a straight sedimentary coastline with headlands, reported elsewhere[4], indicated that half-heart-shaped bays were formed. The depth of indentation depends on the supply of material from upcoast, but for a condition of complete non-replenishment an equilibrium is reached for any given wave direction, in which event sediment movement ceases altogether. Examples from actual coastlines were cited in which a predominant swell direction was known to exist and where the bay formation was well developed.

The most efficacious location of groynes within such bays was discussed. The use of artificial headlands to stabilize coastal transport by promoting bay formation was submitted as a better solution than groynes, which in general influence only sand within the surf zone and are subject to rip formation.

Coastlines of the world. The half-heart shape of bay can be used as a tool to determine the direction of sediment movement on any coastline. This has been done by examination of Admiralty charts covering all oceans of the world and is reported elsewhere[5]. Where the equilibrium shape is not available other factors such as sand spits behind islands, as reported by Prof. Guilcher, can be used. The final world-wide pattern of sediment movement so obtained has correlated well with the wind systems of the globe. The work of J. L. Davies on similar lines[6] was reported.

The meeting was convened in order to bring together research workers from various fields who were interested in the particular study of sedimentary coastlines. It also served the purpose of indicating the different conditions experienced on coasts open to the ocean swell and those where the majority of the waves are generated locally. Emphasis was placed on wave action in the transport of material as distinct from tidal or other currents, which are of minor importance unless they are concentrated in narrow or shallow channels.

R. SILVESTER

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COMPARATIVE ENDOCRINOLOGY IN EUROPE

A CONFERENCE of European Comparative Endocrinologists was held in London in the rooms of the Zoological Society, by courtesy of the Council of the Society, during September 19–21, and was attended by about 100 representatives of twelve countries. It was opened by Prof. G. J. van Oordt, who had been invited, on the occasion of his retirement from his chair of general zoology and endocrinology in the University of Utrecht, to review aspects of European comparative endocrinology with which he and his colleagues had been closely connected.

He chose to deal particularly with developments in the field of sexual endocrinology, a subject which he introduced forty years ago into the biological laboratories of the Netherlands. The far-reaching influence that he has exerted through his own investigations, as well as through those of his pupils, was clearly brought out by the variety of topics on which he was able to touch, the major ones including the control of the reproductive processes of fishes and birds, and the factors influencing spermiation in amphibians. In declaring the Conference open, he expressed his confidence in the bright future of comparative endocrinological studies in Europe, and this optimism was certainly justified by the interest and variety of the forty-eight papers which made up the rest of the scientific programme. It must be sufficient here to mention the main trends that emerged, for his address will be published in full, together with abstracts of the other communications, in the current volume of *General and Comparative Endocrinology*.

A welcome feature was the prominence of invertebrate endocrinology, which was the subject of fourteen of the papers, for there is sometimes a tendency for such conferences to concentrate on the higher vertebrates, and thereby to lose the great advantage of observing the working out of common principles in widely different groups of the animal kingdom. Studies of insects were naturally conspicuous here, and the widening range of application of endocrinological principles was seen in an account of the role of sex ectohormones (pheromones) in the termite super-organism, the analysis of which can be pursued by methods fully analogous with those used in endocrine studies within the bodies of individual animals.

The control of colour change in crustaceans and the growth and moulting of both insects and crustaceans were discussed by several speakers, and it was gratifying to find that the endocrinological basis of these latter processes is now being studied in the difficult but important group of mites. The increasing attention that is being attracted by the lower invertebrates was also apparent, for several papers dealt with the annelid worms, with particular reference to the influence of neurosecretory centres on their reproduction and life-cycles, including the induction and inhibition of the heteronereid phase.

The pituitary gland inevitably predominated in several sessions, in one or other of its many functional aspects. Prof. van Oordt's address was followed by several papers dealing with the control of colour change in Amphibia, and the younger workers present may well have been encouraged to find how vigorously research and controversy are still being maintained in a subject that has for so long been a classical field of comparative vertebrate endocrinology. The pars distalis and its hormones also received attention, and papers on both electron microscopy and the more traditional polychromatic staining methods showed that while the complexity of this region is in full accord with the known variety of its functions, we are still in no position to systematize our knowledge of its histological differentiation.

The neurohypophysial hormones were dealt with by a number of workers, and rightly so, for in few fields has the application of the comparative method yielded more stimulating results in recent years. Problems of the phyletic distribution of these hormones and of their modes of action were prominent in these papers, and attention was also given to the fine structure of the hypothalamic neurosecretory cells from which they arise. The modes of action of hormones received further attention in other contexts also, the relationships between hormones and the metabolism and structure of their target cells being the theme of several papers, particularly with reference to thyroxine and the adrenocortical hormones. The sex hormones, prolactin, and the parathyroid gland were examined from the point of view of their influence on metabolism and differentiation in fishes, birds and mammals, while discussion of the function of melatonin, and of the histophysiology of the epiphysis, reminded the audience of familiar features of vertebrate organization that are only now beginning to receive searching analysis.

Time had been set aside at the end of the Conference dinner to enable members to discuss whether or not similar meetings should be planned in the future; but in the event this proved to be largely unnecessary, for it was made apparent throughout the three days that, despite the continued growth and proliferation of conferences and reunions, this meeting had met a real need. Partly this was so because representatives from many countries had been able to meet together at relatively small cost, but another reason was that this particular gathering had been planned to give younger workers the opportunity to report concisely on investigations that were still in progress, and to exchange ideas with those working on related problems in other laboratories. A contributory factor, too, was the congenial environment provided in the Zoological Society's rooms, and at Beit Hall (Imperial College of Science and Technology) and Bentham Hall (University College, London), for this made it possible to provide ample opportunity for informal discussion throughout the period of the meeting.

As a result, the participants came rapidly and unanimously to the view that they wished to have more of such conferences, and an invitation from Madame Herlant-Meewis to meet next autumn in Brussels was accepted with acclamation. The Brussels reunion, the second Conference of European Comparative Endocrinologists, will be held during September 16–18, 1963. Further announcements relating to it will appear in due course in *General and Comparative Endocrinology*, which will also publish its proceedings in abstract form.

E. J. W. BARRINGTON

EDUCATION AND TRAINING IN NUTRITION

IT is becoming increasingly recognized that the provision of food adequate both in quantity and in nutrient composition will not automatically abolish hunger and malnutrition. A book, published in connexion with the Freedom from Hunger Campaign[*], is based on the thesis that it is "ignorance of the relationship between food and health" which would remain as the major bar to good nutrition. The book develops this thesis in a most readable and orderly way. It begins by giving several examples of the ways in which ignorance or misinformation can lead to malnutrition, even when nutritious foods are available. One is the practice in Indonesia where some of the children suffering from protein deficiency are not allowed to eat dried fish, because it is believed to cause worms. A second is the tendency for people moving from the countryside into the towns to buy new foods with low nutritional value because they are easy to prepare, or because they are considered to be "prestige foods".

That increased knowledge of nutrition may improve nutritional health is shown by several examples. Only one of these, in Israel, has, however, been adequately evaluated in terms of better dietary patterns in the families of those children who took part in a co-ordinated plan. In this, the children themselves were involved in growing foods in school gardens and in cooking and serving meals, as well as receiving instruction in food values.

This book is an excellent brief account of the whole problem of nutrition education for the people, of the training of professional nutritionists, and of the need to include study of the social aspects of nutrition as well as the physiological. However, one is a little sceptical of the belief on which the book is founded, that nutritional knowledge in itself is an assurance of sound nutritional habits where food supplies are adequate. A recent survey in Britain has shown that most people believe that sugar is bad for teeth and that brown bread is more nutritious than white. This does not prevent the enormous consumption in Britain of sugar, nor does it stop people eating far more white bread than brown.

There is a still a widespread belief that people can get used to poor diets without any great impairment of health or of working efficiency. *Nutrition and Working Efficiency*[†] makes it quite clear that the chief reason for apparent lethargy and sluggishness of the inhabitants of many tropical countries is not laziness or racial characteristics or an enviable philosophy of life, but undernutrition and malnutrition. It cites many examples of the relationship between work output and adequate availability of food, and points out that improved nutrition can, in many parts of the world, break the vicious circle of poor nutrition—physical inefficiency—low food production—poor nutrition. The need is mostly for calories, but there is often a need also for more protein and vitamins. Moreover, heavy muscular exertion, needing increased muscular development and training, may itself require increased protein supplies, so that increased calories alone may not result in improved working efficiency in those who have lived much of their lives on inadequate diets.

The main purpose of this book is to direct the attention of international and national agencies and private employers to the improvements which can be obtained through improved nutrition, and to outline the measures by which these can be achieved. These include stores where cheap food is available and subsidized food canteens, and provision should be made for the families as well as the workers. Through the stores and the canteens, the opportunity should be taken also to improve food habits and nutrition education.

JOHN YUDKIN

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[*] FFHC Basic Study No. 6: *Education and Training in Nutrition.* Pp. vi+56. (Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1962.) 2*s*. 6*d*.; 50 cents.

[†] FFHC Basic Study No. 5: *Nutrition and Working Efficiency.* Pp. 47. (Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1962.) 2*s*. 6*d*.; 50 cents.

TEXTILES, COLOUR CHEMISTRY AND DYEING AT LEEDS

THE report to the Worshipful Company of Clothworkers of the City of London of the Advisory Committee on the Departments of Textile Industries and Colour Chemistry and Dyeing in the University of Leeds for the session 1960–61 covers the eighty-seventh session of the former*. During this period the number of students fell slightly to 377, and 56 students obtained higher degrees, postgraduate diplomas and degrees, and 26 were awarded diplomas. In textile physics, work on the αβ-transformation in keratin continued, a method of evaluating the 'crystallinity' of keratin by quantitative assessment of X-ray scatter, was evolved which provides a useful standard of reference for other methods, and interesting differences in fine structure between wools of different quality were recorded. In textile chemistry studies of the by-products of the fleece continued, and work on the constitution of the resin acids of wool has indicated that they are, for the most part, not acids but polar auto-oxidation products of the alcohols. Filaments with properties very similar to ordinary viscose rayon have been produced by a wet-spinning process from a derivative of pectin. Investigations of the polymerization of methacrylic acid within nylon have shown that the polymer is cross-linked, and following studies of the sorption of methacrylic acid from aqueous fibres by nylon 66 fibres, work has been started on the general acid-combining properties of nylon and related materials. In textile engineering the theory explaining the transmission of sound among the looms in a weaving shed has been elaborated, confirmed by measurement and applied to predict the diffuse sound-levels to be found and the broad variations to be expected in industrial weaving sheds. In textile technology, the performance and design of knitting machine mechanisms, relations between structure and properties of woven fabrics, and fundamental principles underlying textile finishing operations have been investigated, as well as the effects of chemical treatments on the surface characteristics of fibres. It has been demonstrated that oxidized wool fibres can be set in a solution of tetrakis (hydroxymethyl) phosphonium chloride but not in water.

In the Department of Colour Chemistry and Dyeing, which is in its eighty-second session, there were 46 undergraduate students and 15 research workers. Work was continued on the kinetic and thermodynamic aspects of dyeing from the vapour phase, on the mechanism of formation of *di*oxazine dyes, the chemistry of 1 : 1′-dinaphthyl and perylene, the reactions of 9 : 10 benzo*meso*benzanthrone and the measurement of changes of hue of azo pigments resulting from closely controlled changes in the conditions of coupling, as well as the study of solvent-assisted dyeing and printing processes. An investigation of the isomers of indanthrone was completed and also work on the kinetic and thermodynamic aspects of dyeing cellulose acetate rayon with sulphated dyes, and on the effect of chemical structure of a group of related 2 : 1 metal-complex dyes on their dyeing behaviour in presence and absence of solvents. Lists of publications during the year are included in both reports.

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* Report to the Worshipful Company of Clothworkers of the City of London of the Advisory Committee on the Departments of Textile Industries and Colour Chemistry and Dyeing in the University of Leeds for the Session 1960–61. Pp. 48. (Leeds: The University, 1962.)

GEOMAGNETIC DISTURBANCES ASSOCIATED WITH THE NUCLEAR EXPLOSION OF JULY 9

By Dr. P. R. PISHAROTY

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THE geomagnetic observatories at Annamalainagar (geographically 11° 24′ N.; 79° 41′ E.; geomagnetically 1·8° N.; 149·4° E.) and Trivandrum (geographically 8° 29′ N.; 76° 57′ E.; geomagnetically 0·9° S.; 146·3° E.) have recorded marked changes in the Earth's field around 09 h U.T. on July 9, 1962. These are reproduced in Fig. 1. A megaton nuclear charge was exploded at this time at a height of 320 km over Johnston Island (geographically 16° 42′ N.; 169° 24′ W.; geomagnetically 14·3° N.; 265·5° E.). The changes at the two stations are similar in type and somewhat resemble a magnetic storm on a much smaller scale. The disturbance in H consists of a positive sudden commencement (detectable in the originals), an extremely short initial phase if any, and a main phase in which the H value is depressed below the normal—the total duration being about 12 min at both the stations. The details of the changes are given in Table 1. Those in the magnetograms at Alibag (geographically 18° 38′ N.; 72° 52′ E.; geomagnetically 9·5° N.; 143·6° E.) are very much less, the most noteworthy feature at this place being an oscillation motion in the D-trace, similar to what happens when lightning flashes occur near the station. All these changes are apparently a direct consequence of the injection into the exosphere of a large amount of protons and helium nuclei produced by the explosion.

Atomic blasts at ground-level have been found to have no effect on normal-run magnetograms[1]. The atomic explosion ten miles or so above ground, of the

Table 1. GEOMAGNETIC FIELD CHANGES COMMENCING 09 H U.T. OF JULY 9, 1962

Stations	Duration	Maximum change in H	Maximum change in V	Maximum change in D
Annamalainagar 1·8, 149·4	12 min	-30γ	-3γ	0 60′ (with superposed feeble oscillations)
Trivandrum − 0 9, 146 3	12 min	-38γ	-40γ	Feeble oscillations
Alibag 9·5, 143 6	12 min	-8γ	$+2\gamma$	Oscillations well marked

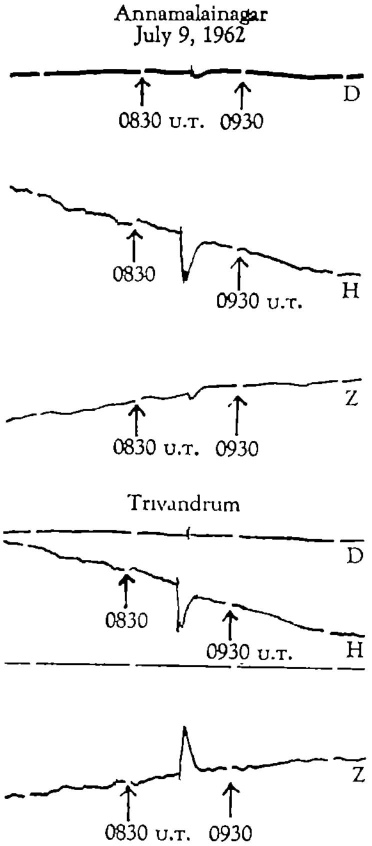

Fig. 1

type carried out over Christmas Island in April 1958, had no noticeable effect even at a distance of 2,000 km from the site of explosion[2]. Atomic explosions carried out as the *Argus* experiments[3] did not produce any geomagnetic effects at Trivandrum or Annamalainagar or Alibag similar to those observed with the explosion of July 9, 1962. The great altitudes of the *Argus* shells (7,000 km) over the equatorial stations, and the low yield (kiloton range) of the explosions, were perhaps the causes. The explosions[4] of August 1 and 12, 1958, over Johnston Island also did not produce noticeable changes in the magnetograms located far from the Pacific Island, except in a few induction-type magnetograms of very high sensitivity. The heights of these explosions were less than 100 km, so that most of the injected particles would have had characteristic life-times of less than 0·2 sec and therefore would have had no chance of gyration and drift around the globe. The geomagnetic effects observed in the Pacific Islands must therefore be attributed to the dynamo effects of the expanding plasma cloud on the vertical components of the Earth's field as explained by Lawrie *et al.*[6].

The newspaper report of the explosions of July 9 stated that the nuclear charge was exploded at a height of 320 km (somewhat less than the 480 km of the *Argus* explosions). The particles generated and injected by the explosion would be trapped by the geomagnetic lines at the point of injection. The maximum height, h_m, of the corresponding line of force over the magnetic equator at that latitude would be $(R + h) \sec^2 \theta - R$, where R is the radius of the Earth, h the height and θ the geomagnetic latitude of the point of injection. For the July 9 explosion, h_m works out to be nearly 800 km. From the integral-invariant traces given by Dessler[7], the altitude of the integral-invariant equator for 0·2 Γ, corresponding to the latitude of Johnston Island, lowers through a height of about 600 km as it comes over central South America—an effect principally due to displacement of the geomagnetic dipole from the centre of the Earth towards the Pacific. To a first degree of approximation, we can therefore assume that the eastward drifting electrons mirroring at the geomagnetic equator itself and attaining a maximum height of 800 km at the longitude of Johnston Island would be totally absorbed by the atmosphere at 200 km as they pass over South America in the course of their eastward drift. (The characteristic life-time of one MeV electron at 200 km is scarcely 6 sec.) Hence the effects observed at the South Indian stations must be attributable to positively charged particles drifting westwards (from 169° E.) and arriving over south India (80° E.) after traversing only a quarter of the global circuit.

The two minima in H reckoned from the instant of the explosion (marked by the initial sharp increase presumably caused by the hydromagnetic shock wave) are 2·0 and 3·3 min at both the stations. They correspond to around-the-world drift-times of nearly 8 and 13 min respectively. As the disturbance did not last beyond 12 min the slowest particles had a drift-time of about 48 min. There is no question of any particle completing even a single drift around the world due to the low height of the corresponding integral-invariant equator over central South America. The drift time, T, in min[8], modified for helium nuclei and protons, is given by:

$$T = 87/E \text{ for helium nuclei}$$
$$\text{and } T = 44/E \text{ for protons}$$

where E is the energy of the non-relativistic heavy particles expressed in MeV units. If the geomagnetic effects at the South Indian stations are taken to be due to the drift of ^{1}H particles, their energy spectrum has peaks at 5·5 MeV and 3·3 MeV, and extends down to about 1 MeV. On the other hand, if they are due to the drift of ^{4}He or ^{3}He particles, their energy spectrum has peaks at 11 MeV and 7 MeV, and extends to 2 MeV. For thermonuclear fusion-bombs particles of this energy appear to be quite feasible[9].

It is possible to estimate the total number of positively charged particles (^{1}H or ^{4}He) which would have passed over Annamalainagar or Trivandrum causing the geomagnetic disturbance, through the drift of a sheet of charged particles gyrating round the magnetic lines at about 700 km above the stations. The H component of the magnetic disturbance would be given by $2\pi nev$, where n is the number of particles per sq cm, e the charge on each and v the westward drift velocity. Integrating this over the time of the disturbance and the latitudinal extent of the gyrations ($\precsim$ 30°), the total number of protons, which passes over a station near the dip equator, is $2 \times 10^{22} \int \Delta H \, dt$, where ΔH is expressed in γ and t in min. $\int \Delta H \, dt$ for both Trivandrum and Annamalainagar works out to be nearly 150γ min. Hence the number of particles of unit charge which passed over these stations can be estimated as 3×10^{24}; this number would be halved if all the particles were helium nuclei instead of protons.

The northward and southward transits of these particles are apparently responsible for the ultra-short period oscillations in the D trace at Alibag. Perhaps the equatorial electrojet has suppressed to a large extent the transmission of the magnetic effects of these transits to the equatorial stations of Annamalainagar and Trivandrum.

A detailed paper on this subject will be published shortly in the *Indian Journal of Meteorology and Geophysics*.

I thank my colleagues at Colaba for useful discussions and at Annamalaingar and Trivandrum for collecting the data.

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PHYSICAL HYDROGEN EMBRITTLEMENT OF STEEL AT ELEVATED TEMPERATURES

By G. H. BROOMFIELD

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THE effect of hydrogen in ferritic steels with strengths below 100,000 lb./in.^{2} is usually observed in laboratory tests as a reduction in the true breaking stress. In production and service the effects are manifested as hairline cracks in large sections subject to transformation and cooling stresses, and reduced fatigue life. These effects have been attributed to the embrittling influence of hydrogen in solid solution in steel at temperatures below 100° C (refs. 1 and 2).

At higher temperatures hydrogen has a chemical effect. A reaction with carbon in the steel to form methane results in sub-surface porosity, predominantly at grain boundaries, with consequent permanent embrittlement. The conditions of temperature and pressure under which chemical embrittlement occurs have been defined by Nelson and Effinger[3]: 1 per cent chromium, 0·5 per cent molybdenum steel is not embrittled below 330° C.

There is little or no information on the effects of solid solution hydrogen on the physical properties of steel at temperatures above 100° C.

It has been suggested that a high proportion of the hydrogen evolved by corrosion reactions at steel surfaces in high-pressure water systems, at temperatures in the range 250°–300° C, enters the steel. If this is assumed to be the case it can be shown that possibly significant amounts of hydrogen may accumulate in thick pressure vessels[4]. Consequently tests have been commenced to examine the temperature dependence of hydrogen embrittlement in a low alloy pressure vessel steel.

The steel used in the tests had nominally 1 per cent chromium, 0·5 per cent molybdenum and 0·1 per cent carbon. Tensile test pieces with diameters of 0·252 in. and parallel lengths of 0·894 in. were obtained and tested as follows.

(*a*) A batch of specimens was machined from a 2-in. diameter rolled bar, with their axes parallel to the direction of working. They were austenitized and slow cooled and then heated at 640° C for 16 h and slow cooled (a treatment corresponding to normalizing and stress-relieving of a large forging).

The specimens were tested after holding for 4 h at the test temperature in air at atmospheric pressure or in hydrogen at 1,750 lb./sq. in.^{2} g (except for a test in hydrogen at 20° C, in which the specimen was first held for 3 h at 150° C, at 1,750 lb./in.^{2} g).

(*b*) A second batch of specimens was machined from the centre 2 in. of a 6-in. thick forging, with their axes at right angles to the direction of working. The forging had been normalized and stress-relieved, and the specimens were heated for 2 h at 640° C and slowly cooled.

Table 1 RESULTS OF TENSILE TESTS IN HIGH-PRESSURE HYDROGEN, HIGH-PRESSURE ARGON, AND AIR

Test temperature	Atmosphere	Material	U.T.S. 10^3 lb./in.^{2}	Breaking stress 10^3 lb./in.^{2}	Elong. (per cent)	R of A (per cent)
20	Air	Bar	68·3	149	38	71
20*	Hydrogen	Bar	69 2	131	41	69
150	Air	Bar	67·0	147	31	70
150	Hydrogen	Bar	67·5	132	29	65
200	Air	Bar	68·8	147	31	70
200	Hydrogen	Bar	68·7	128	30	63
200†	Hydrogen	Bar	67·6	128	29	63
30	Argon	Forging	63·2	122	38	61
20	Hydrogen	Forging	62·4	80·6	18	35
100	Hydrogen	Forging	58·0	95 2	31	50
200	Hydrogen	Forging	62·2	108	31	50
250	Argon	Forging	59 8	128	31	60
250	Hydrogen	Forging	63·6	112 8	25	50

* Held in hydrogen at 150° C for 3 h, cooled in 10 min, tested immediately.

† Held in hydrogen at 200° C for 20 h, at a stress of about 20,000 lb./in.^{2} test completed at the normal speed.

These specimens were held for 4 h at 250° C in hydrogen or argon at 1,750 lb./in.^{2} g before testing under pressure at 250° C or below. The cooling-rate to 150° C was approximately linear and took 10 min, the time taken to reach 30° was 40 min.

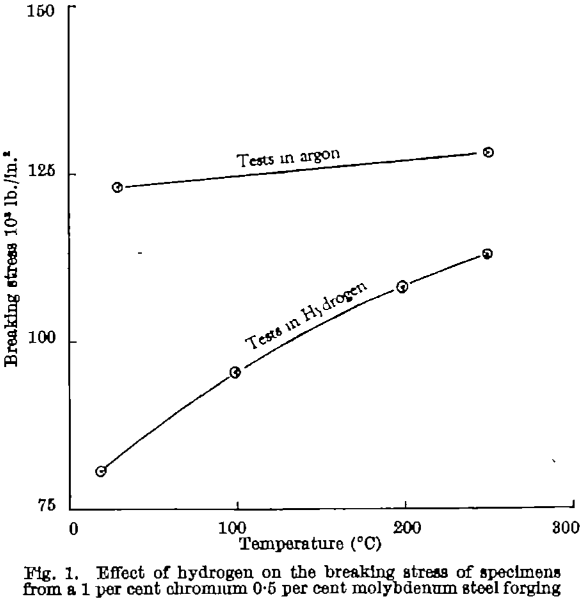

Fig. 1. Effect of hydrogen on the breaking stress of specimens from a 1 per cent chromium 0·5 per cent molybdenum steel forging

The test machine cross-head speed was approximately 0·010 in./min during all the tests.

The tensile test results are given in Table 1 and the effect of test temperature on the breaking stress of specimens from the forging is plotted in Fig. 1.

The 'diffusible' hydrogen (hydrogen evolved on heating at 100° C in vacuum) taken up by the forged steel specimens at 1,750 lb./in.^{2} g and 250° C was approximately 1·4 c.c. (N.T.P.) per 100 g steel (70 p.p.m. atomic).

These preliminary results show that hydrogen has a significant effect on the true breaking stress of this steel at temperatures up to 250° C. Because the type of steel is not prone to chemical attack by hydrogen at the temperature of these tests, it is concluded that the embrittlement observed at elevated temperatures is due to effects of hydrogen in solution in the steel.

It is planned to extend this work to investigate the effect of varying the strain-rate to see whether the strain-rate-dependent temperature of minimum ductility observed by Yagunova and Popov[5] and also by Baldwin and Adenis[6] is transferred to high temperatures at high-strain rates, and also to investigate the effect of hydrogen on neutron irradiated steel, in the form of parallel and notched test pieces. It is also proposed to test irradiated steel for the possibility of delayed fracture.

I thank Dr. D. R. Harries and other colleagues at this Establishment for helpful discussions, and the U.K. Atomic Energy Authority for permission to publish these results.

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DETERMINATION OF SULPHUR IN PETROLEUM BY X-RAY ANALYSIS

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NON-DISPERSIVE X-ray fluorescence analysis using X-rays generated by the β-rays of tritium (hydrogen-3) was used for the determination of sulphur in petroleum in the concentration-range of 0·1–5 per cent with a relative accuracy of 5 per cent or better.

The aim of this work was to provide the physical basis for the development of a rapid automatic sulphur-detecting device which can be incorporated in the refinery stream, avoiding the delay usually introduced by laboratory analysis. A continuous sulphur-detecting device based on the absorption of tritium-excited X-rays is already commercially available[1]; but the fluorescence method can be made more sensitive and is less influenced by the C/H ratio and the presence of interfering elements in the sample.

In the non-dispersive X-ray analysis which has been investigated the fluorescence spectrum is transformed into a pulse height spectrum by means of a proportional counter. The pulse height spectrum is then analysed by electronic means. With this method intensity losses can be kept much lower than with dispersive methods, because much more efficient geometric arrangements can be realized. This economy in X-ray intensity makes the use of low-intensity X-ray sources possible, as they can be made using radio-active isotopes. This application has been described before[2]. These X-ray sources have the great advantage of being small and working without the cumbersome power supply of conventional X-ray tubes. Thus a compact, relatively small measuring head can be built comprising X-ray source, sample holder and counting tube in a so-called 'reflexion-geometry' with an incorporated pre-amplifier. The electronics, essentially a single-channel pulse height spectrometer combined with a pulse counting device, may be several metres distant from the measuring head.

For exciting the characteristic X-rays of sulphur, a minimum energy of the incident radiation of 2·47 keV is necessary. Tritium absorbed in a layer of titanium deposited on a copper sheet was used as X-ray source. The radiation spectrum of this source consists mainly of characteristic titanium radiation at 4·5 keV (ref. 3), which is well suited for exciting the sulphur radiation.

Proportional counting tubes with thin windows (6μ thick polyester foil) were used. Since these counting tubes cannot be made entirely gas-tight, a continuous gas-flow was necessary in order to maintain a sufficient purity-level of the counting gas (commercial propane).

Fig. 1 shows the superimposed pulse spectra obtained with several concentrations of sulphur in n-heptane. The peak due to the K-radiation of sulphur is sufficiently resolved from the peak due to the back-scattered titanium radiation. For the laboratory measurements the pulse height channel of the analyser was set on the sulphur peak of the pulse spectrum and locked to this position by an electronic stabilizing device compensating variations of the gas amplification in the proportional counter tube. The relationship between sulphur content in percentage weight (S) and the net count-rates in 10^3 counts per second (N) is not quite linear, but can be sufficiently approximated by a square correction:

$$S = aN(1 + bN)$$

The calibration curve has been determined for solutions of thiophene in both n-heptane and benzene. The following values for the parameters a and b, which will change according to the construction, were obtained:

	a	b
n-Heptane	0·54	0·04
Benzene	0·60	0·04

The experimental points fitted these calibration curves with errors not exceeding ± 2 per cent relative or ± 0·003 per cent absolute, whichever is the larger. The parameter a varies with changes in ambient pressure and temperature (+0·065 per cent/mm

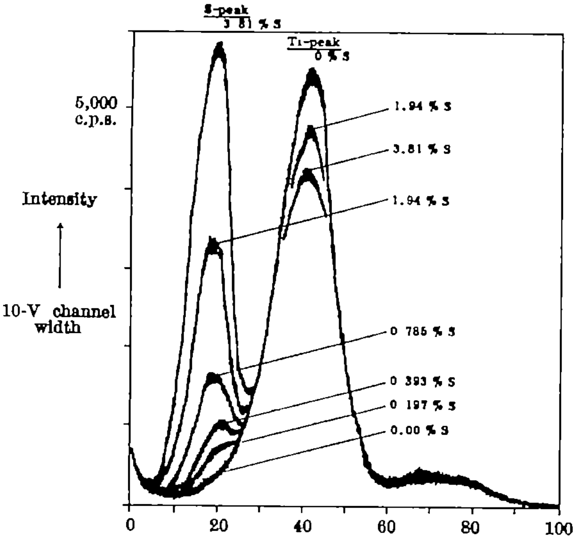

Fig. 1. Pulse spectra obtained with different sulphur contents in *n*-heptane

mercury and −0·16 per cent/°C). The readings are not affected by changes in sample thickness so long as it exceeds 0·25 mm.

The influence of elements other than sulphur contained in the sample on the reading has been investigated. Thus a change in the C/H ratio from 5·3 (*n*-heptane) to 12 (benzene) causes a variation in the readings of roughly 10 per cent. Other elements may influence the reading by varying the absorption of the sulphur radiation or by emitting characteristic X-rays (*K*-, *L*- or *M*-radiation) in the energy region of the *K*-radiation of sulphur: for example, for chlorine the sensitivity of the apparatus is about twice that for sulphur, and for mercury about half.

Six samples obtained from an oil refinery have been analysed for their sulphur content using the calibration curve obtained in *n*-heptane. Table 1 gives the chemical composition of the samples as given by the supplier and the sulphur content as determined with the method described here (last column). The measuring times were 1 min or less. depending on sulphur content.

Table 1

Sample	C/H ratio	Elements other than C, H and S (per cent)	S suppliers indication (per cent)	S measurements (per cent)
Light gas oil	6·67	0 54	0·10	0·098
Light gas oil	6·47	0·49	0 79	0·785
Light gas oil	6·30	0·30	0·82	0·830; 0·825
Atmospheric residue	7·22	0 38	2·09	2·05
Atmospheric residue	7·34	0·96	3 66	3·68
Atmospheric residue	7·21	1·41	2 41	2·40

Table 1 shows the good accuracy achieved with this method and short measuring times. Furthermore, differences in the composition of the six samples with respect to elements other than sulphur were sufficiently small not to influence the reading.

This work was undertaken under the sponsorship of Messrs. Sperry Gyroscope Co., Ltd., Brentford, England, whom we thank for permission to publish this account.

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ROLE OF ION-MOLECULE REACTIONS IN THE RADIOLYSIS OF HYDROCARBONS

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THERE is continued interest in the application of ion-molecule reactions to the mechanisms of radiation-induced reactions. Libby[1] has recently presented equations (presumably empirically derived) for the heats of formation (ΔH_f) of hydrocarbon ions and molecules and formulated, for $n > 3$, a general equation for the heat of the ion-molecule reaction (ΔH_r).

$$C_nH_{2n+1}^+ + C_mH_{2m+2} \rightarrow C_{n+m}H_{2(n+m)+1}^+ + H_2 \qquad \text{(i)}$$

This equation predicts that for increasing m, ΔH_r will go from positive (endothermic reaction) to increasingly negative values (exothermic reaction) indicating that this type of ion-molecule reaction should be of greater importance for higher molecular weight compounds.

I have calculated ΔH_r values for reactions of this type, up to a C_6 product ion, which is the extent of the experimental results available. The results are shown in Fig. 1, in which ΔH_r values for the reaction of a particular ion are plotted against the reactant molecule for a series of reactant ions. The dashed curve represents ΔH_r values for reactions of the general type:

$$C_nH_{2n+1}^+ + C_nH_{2n+2} \rightarrow C_{2n}H_{4n+1}^+ + H_2$$

All the data are taken from Field and Franklin[2]. In cases where there is more than one value for the heat of formation of an ion (depending on its configuration) I have taken the lowest value (that is, the most stable ion) for both the reactant and the product ion. If one of the other values is used the curve shifts in a vertical way but the trend of the plot remains the same. The graph illustrates that all the reactions indicated of the CH_3^+, $C_2H_5^+$ and $C_3H_7^+$ ions are exothermic, and reactions of $C_4H_9^+$ and $C_5H_{11}^+$ are endothermic. Considering reactions of different ions with one substrate, the reactions of CH_3^+, $C_2H_5^+$ and $C_3H_7^+$ with methane are exothermic, though decreasingly so in that order, and the reactions of $C_4H_9^+$ and $C_5H_{11}^+$ are endothermic.

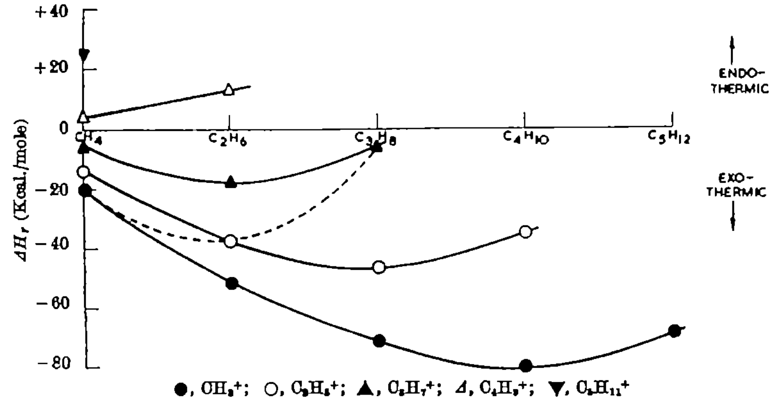

Fig. 1. ΔH_r of reactions versus reactant molecule for different ions

The general trend of these calculations indicates, therefore, that while ion-molecule reactions of this type are of importance for low molecular weight alkanes, they are less likely to occur with high molecular weight ones.

Experimental evidence for ion-molecule reactions has been obtained using conventional mass spectrometers at slightly elevated pressures of gas in the ionization chamber, that is, 10^{-3} mm mercury. Under these conditions secondary ions resulting from the reaction of an ion with one molecule are obtained. Ion-molecule reactions involving carbon–carbon bond synthesis have been found for methane[3], *cyclo*propane[4], ethylene[5], propylene[6], butene[6], 1–3 butadiene[7], acetylene[8], benzene[9] and toluene[9]. Apart from methane no *n*-alkanes appear to give rise to ion-molecule reactions of this type under these conditions, and for the *cyclo*alkanes, although *cyclo*propane does give secondary ions, *cyclo*hexane does not. (This statement is based on preliminary observations carried out by me several years ago[7]. Stevenson has reported the reaction[3] $C_2H_3^+ + C_2H_6 \rightarrow C_3H_5^+ + CH_4$ in ethane, although it was not included in a similar table in a later paper[10]. Field and Lampe[11] obtained unambiguous evidence for the hydride ion transfer reaction $C_3H_5^+$ + neo $C_5H_{12} \rightarrow C_5H_{11}^+ + C_3H_6$ for neopentane and somewhat more inferential evidence for this type of reaction in other alkanes. Some workers consider that the hydride ion transfer reaction is an important general reaction in the radiolysis of gaseous alkanes[12].) In contrast ion-molecule reactions of unsaturated hydrocarbons are very common and it seems likely that all such compounds exhibit them.

Recently, Rudolph[17] and Melton[13], Field[14] and Wexler and Jesse[15] have developed mass spectrometers which utilize ionization chamber pressures of ≃ 0·1 mm mercury to study ion-molecule reactions. For various reasons appearance potentials of product ions cannot be measured and reaction schemes have to be characterized by more indirect means, for example, the so-called 'mixture' method[14]. Methane[15,16], ethylene[14] and acetylene[17] have been examined in this manner and although the results are in some cases extremely complex (for example, ethylene) it seems likely that higher molecular weight ions are formed by further reactions of secondary, tertiary, etc., ions with substrate molecules. There is evidence for 'sticky' collisions in some cases.

An important question of great pertinence to radiation chemistry is whether those compounds which show no evidence of ion-molecule reactions at the lower pressures (10^{-3} mm mercury) will do so at the higher ones available in the foregoing instruments and under the conditions of gas phase radiolysis.

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THERMAL DECOMPOSITION ALONG DISLOCATIONS

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DURING the course of some recent solid-state thermal decomposition investigations, a number of instances occurred when the reaction-rate under certain conditions remained constant over long periods. In the reaction:

$$A_{\text{solid}} \rightarrow B_{\text{solid}} + C_{\text{gas}}$$

this behaviour is represented by the equation $\alpha = at$, where α = the fraction of the total decomposition = P/P_∞. Here P = pressure of gas at time t and P_∞ = pressure of gas when all the solid has decomposed. The quantity a is a constant at any given temperature. Processes such as nucleation and movement of an interface control the topochemical behaviour of decomposition in the solid state. These generally lead to a $d\alpha/dt$ which is not constant but time-dependent[1]. This is a natural consequence of mechanisms proceeding in more than one dimension. The 'linear' behaviour is rare, and its topochemical interpretation generally presents a perplexing problem. Two conditions appear to favour the occurrence of

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'linear' kinetics. They are: (1) relatively low temperature, (2) prolonged irradiation or cold working. Some examples are presented now.

In the thermal decomposition of polycrystalline NaBrO$_3$ (ref. 2) the surface nucleates instantly and follows a contracting envelope equation, that is, the rate begins at its maximum value and decreases continuously to zero as the crystal becomes 'used up'. The inset in Fig 1 is a typical α/t curve (373° C) for NaBrO$_3$. At lower temperatures (330° C), the shape changes and is shown as curve *A* in Fig. 1. The initial portion again fits a contracting envelope equation but the rate soon settles to a constant value. For clarity a slight break is inserted at the start of the 'linear' region. Curves *B* and *C* represent reactor irradiated ($1{\cdot}4 \times 10^{17}$ n.v.t.) and ground material respectively, taken at approximately the same temperature (330° C). They behave in the same fashion except that the beginning of the 'linear' region is shifted to lower values of α. Note: at the higher temperature the product of the reaction, sodium bromide, forms a eutectic at the surface with undecomposed sodium bromate and a thin liquid layer appears, but this does not materially affect the topochemical argument.

The decomposition of colloidal α-PbN$_6$ is shown in Fig. 2. At 'normal' temperatures, shown as curve *A*, the shape of the α/t curve is sigmoidal. This results from simultaneous nucleation and growth followed by decay[3,4]. On reducing the temperature to the lowest practical value, a curve such as *C* is observed. An unusually large linear region occurs which spans about a 17 per cent range of α. With prolonged neutron irradiation ($9{\cdot}5 \times 10^{17}$ n.v.t.), curves such as *B* are observed at approximately the same temperature as *A*. These are characterized by a long initial linear region extending to about $\alpha = 35$ per cent.

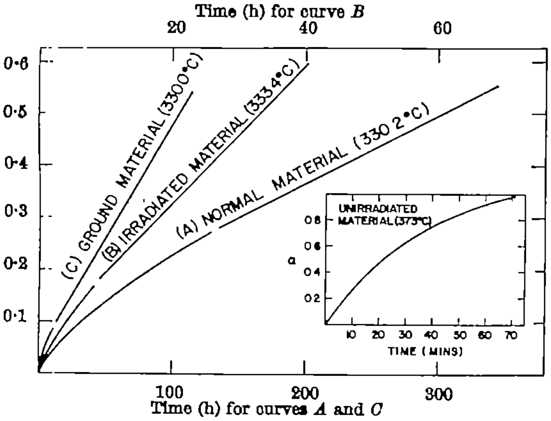

Fig. 1. α/t curves for the decomposition of NaBrO$_3$

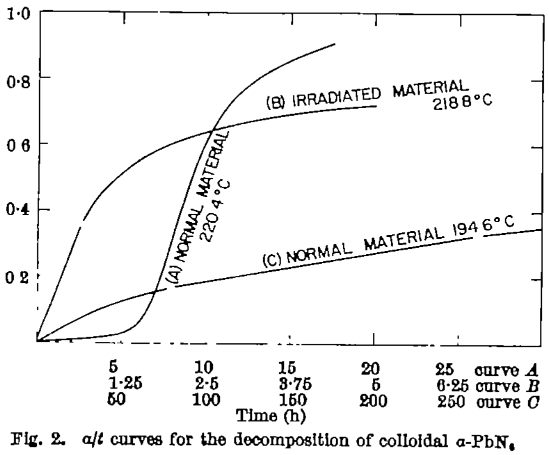

Fig. 2. α/t curves for the decomposition of colloidal α-PbN$_6$

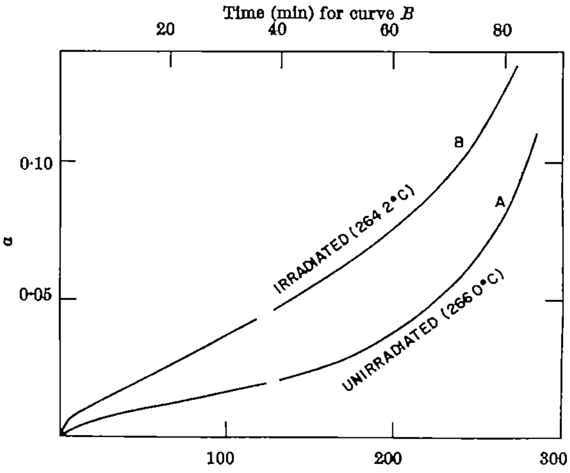

Fig. 3. α/t curve for the decomposition of dehydrated nickel oxalate

In the decomposition of dehydrated nickel oxalate[5] neutron irradiation ($1{\cdot}4 \times 10^{16}$ n.v.t.) causes an extension of an initial linear period. This is shown in Fig. 3, where the initial acceleratory region of the reaction is plotted. The two experiments were at approximately the same temperature, but the irradiation has the effect of extending the linear region to higher values of α. A lowering of temperature apparently has the same effect but its magnitude is not sufficiently large for a definite conclusion to be drawn.

Note that with all the irradiations, an enhancement in overall rate occurs besides the effects specifically mentioned here. For a discussion of these effects the reader is referred to the original papers.

I believe that the 'linear' kinetics result from the preferential decomposition along dislocation lines and that such a mode of decomposition is energetically more favourable than at regular reactant-product interfaces. Having postulated a lower activation energy, it is true to say that these reactions gain prominence at lower temperatures. The volume decomposed by this mechanism is a cylinder with the dislocation line as its axis. If one considers that the approximate cross-section of this cylinder extends only to the region where the excess strain due to dislocation ends, then there are normally insufficient dislocations to account for the size of the effect mentioned here. However, it is emphasized that decomposition might occur preferentially at the surface step which appears when a screw dislocation meets a surface. The crystal may be described as one atomic plane in the form of a spiral ramp. Decomposition at a step results in successive layers being peeled off without ever destroying the step. This mechanism is akin to Frank's for crystal growth from slightly supersaturated solutions[6,8,10,11], except that the process is in a reverse direction.

Preferential activity at dislocations have been cited recently. Singh[12] inferred preferential thermal decomposition (especially at low temperatures) around the sites of dislocations where these meet surfaces. He does not quote any kinetic measurements but presumably the phenomenon he describes is confined to the very initial stage of the thermal decomposition. Gevers[13] finds that the mechanism of etching at dislocations (silicon carbide) is formally analogous to the Frank growth mechanism. He finds that deep polygonized etch-pits are generated at the emergence of screw dislocations as compared with shallow rounded etch-pits elsewhere on the same face. Horn[14] reported preferential chemical attack by acids on AlB_2 at screw dislocations. The dissolution had to be rapid, otherwise a general disintegration occurred, but it is interesting that under special conditions preferential chemical reactivity is observed at the dislocation. Horn also finds that prolonged rapid dissolution of a crystal with a screw dislocation extending between crystal faces results in a hole through the crystal at the site of the screw dislocation.

Unfortunately, none of these investigations included kinetic measurements which would give the relative amount of reaction at dislocations compared with the generalized reaction. The present work indicates that the dislocation effect can be quite large (anything up to 50 per cent reaction) under appropriate conditions.

The enhanced linear region on grinding is easily understood since a large number of extra dislocations are introduced in this way. With irradiation, decomposition may occur preferentially along damage tracks. Such a mechanism may result in linear kinetics in the first instance. Young[7] observed decomposition along fission fragment tracks but there was also a radial decomposition from the track resulting in a t^2 dependence for the pressure. While neutron irradiation may not yield tracks as linear as fission fragments, it is possible that they do yield a type of damage which strongly favours a unidirectional decomposition process. Irradiation may also introduce mechanical defects resulting from large strains imposed on the crystal by decomposition products[9]. Radiation-induced defects may initiate quite directly the formation of pits, which afterwards grow preferentially in the neighbourhood of surface discontinuities. This is the interpretation advanced by Hennig, Dienes and Kosiba[15] for the neutron irradiation-induced oxidation of graphite.

I thank Dr. G. J. Dienes for helpful discussions.

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A TRITIUM ISOTOPE EFFECT ON ION EXCHANGE CHROMATOGRAPHY

By HUBERT GOTTSCHLING and PROF. ERNST FREESE*

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IN experiments designed to measure the incorporation of tritiated 2-aminopurine (AP) into deoxyribonucleic acid[1] we often had to separate AP from other compounds by ion exchange column chromatography. To our surprise we always found that the radioactivity ran slightly ahead of the optical density in the otherwise well-separated AP peak. A more detailed examination of this isotope effect with purified AP is described here.

Analytical grade anion exchange resin 'Dowex 1–*X*8', 200–400 mesh, from Bio-Rad Laboratories, Richmond, California, and 2-aminopurine nitrate from Sigma Chemical Co., St. Louis, was tritiated by Schwarz BioResearch, Inc., Mount Vernon, New York, using the Wilzbach method[2,3]. We purified 32·5 mg of this preparation on a 'Dowex 1' formate column (100 mm length, 20 mm diameter), using 0·1 M ammonium formate at *p*H 9·2 as eluant. The fractions containing the bulk of the 2-aminopurine were evaporated to dryness and redissolved in 50 ml. water. The activity of this sample was $1{\cdot}2 \times 10^9$ c.p.m./ml. and the specific activity about 4×10^{14} c.p.m./mole.

The scintillation liquid was: xylene 468 ml., dioxane 468 ml., ethanol 280 ml., naphthalene 98·4 g, 2,5-diphenyloxazole 6·13 g, and α-naphthylphenyloxazole 61·3 mg.

(1) *Column.* For most experiments a 'Dowex 1' column of 200 mm length and 12 mm diameter was used in the formate form. In each experiment a solution containing 5 ml. non-radioactive 2-aminopurine nitrate (2 mg/ml.) and 0·1 ml. of the tritiated 2-aminopurine solution were applied to the column at *p*H 11 (using dilute ammonia). The elution solvent was always 0·1 M ammonium formate at the *p*H values indicated. After every experiment the column was regenerated by 2 l. of 5 M formic acid and subsequent washing with water to neutrality. Before each experiment the *p*H of the column was adjusted to *p*H 11 with 0·1 N ammonia. The flow-rate was about 0·95 ml./min and fractions of 7·6 ml. were collected. (2) *Quantization.* The AP concentration in the eluted fractions was calculated from the extinction E at λ_{max} (= 302–303 mμ for *p*H 9–12) and the molar extinction coefficient ε_{max} which we determined to be $5{\cdot}86 \times 10^6$ cm^2/mole. (3) *Paper chromatography.* In order to identify the fractions further, descending paper chromatography was used with Whatman No. 1 paper and the solvent concentrated hydrochloric acid/isopropanol/water, 65 : 210 : 65. The nitrogenous bases show a characteristic ultra-violet absorption or fluorescence on the

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paper. (4) *Radioactivity*. Tritium was determined with the 'Tricarb' liquid scintillation spectrometer (Packard). From each chromatographic fraction 0·1 ml. was added to 9·9 ml. of scintillation liquid. The radioactivity on paper chromatograms was determined by cutting out small sections, eluting them with 0·5 ml. water, adding to the whole 9·5 ml. of scintillation liquid and then counting. This procedure gives reproducible results.

Fig. 1 demonstrates the isotope effect. Here the sample of AP ($11 \cdot 18 \times 10^{-6}$ moles) had a higher specific activity ($3 \cdot 28 \times 10^{14}$ c.p.m./mole) than the material used throughout the following experiments. The column was 400 mm long and 9 mm wide, and the elution buffer 0·1 M ammonium formate at *p*H 9·05.

One could use this chromatographic method in order to obtain samples of higher specific activity. The result of such an enrichment procedure, using two fractionations at *p*H 9·7, is summarized in Table 1. The first fractionation is represented as a part of Fig. 2. In both column runs, all fractions with a specific activity larger than the original were collected and re-chromatographed on the same column. Table 1 shows that the specific activity of AP has been increased by a factor of 2·1 with a recovery of about 20 per cent of the original counts. A better separation should be obtainable by a longer column.

If both the radioactivity and the concentration distribution were gaussian and had the same standard deviation a semi-logarithmic plot of the specific activity versus fraction number would result in a straight line. But our plot shows a rapid increase of the specific activity with decreasing fraction number, making it difficult to extrapolate the specific activity of earlier fractions, for which the extinction cannot be measured. For the repeated run at *p*H 9·7 (Table 1), the earliest fraction of which the extinction could still be measured with sufficient accuracy ($E = 0 \cdot 012$), had a specific activity of $14 \cdot 07 \times 10^{12}$ c.p.m./mole. Using the half-life of tritium (12·26 years) one can calculate the number of tritium atoms per AP molecule to be 30×10^{-4}. In contrast, the original material (specific activity $2 \cdot 46 \times 10^{12}$ c.p.m./mole) had 5×10^{-4} tritium atoms per AP molecule. Earlier fractions would have a much higher specific activity.

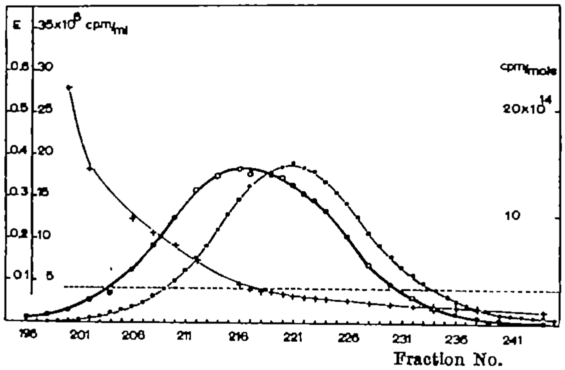

Fig. 1. Distributions of extinction E at 303 mμ (●), radioactivity in c.p.m./ml. (○), and specific activity in c.p.m./mole (+) of AP fractionated on 'Dowex 1' formate at *p*H 9·05. The horizontal broken line indicates the specific activity of the original AP sample

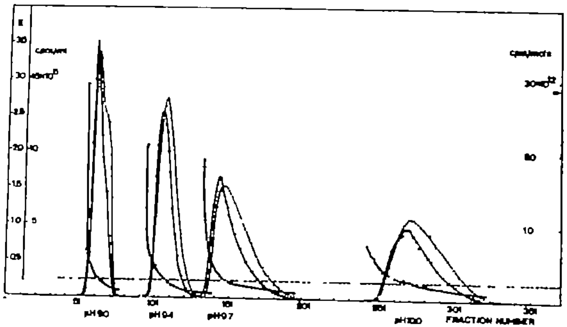

Fig. 2. Distributions of extinction E at 303 mμ (●), radioactivity in c.p.m./ml. (○), and specific activity in c.p.m. mole (+) of four AP samples fractionated at different *p*H values on 'Dowex 1' formate. The horizontal broken line indicates the specific activity of the original AP samples

Table 1. ENRICHMENT IN SPECIFIC ACTIVITY OF TRITIATED AP BY REPEATED CHROMATOGRAPHY ON 'DOWEX 1' FORMATE AT *p*H 9 7

	Amount (moles $\times 10^{6}$)	Radioactivity (c.p.m. $\times 10^{-7}$)	Specific activity (c.p.m./mole $\times 10^{-12}$)
(*A*) Material placed on column	50·5	12·4	2·46
(*B*) Material collected after 1st run and again placed on column	10·7	4·16	3·89
(*C*) Material collected after 2nd run	4·82	2·51	5·21
C/*A*	0·095	0·202	2·1

The question is now which isotope effect is responsible for this partial resolution of the tritiated from the non-tritiated AP. Three possibilities come to mind: (1) The increase in mass by the tritium label. (2) The steric difference. (3) A difference of electrical charge, that is, of the *p*K value. The first two possibilities are unlikely, already for theoretical reasons, since the stable replacement of (one or two) hydrogen atoms by tritium causes only a small change of the mass (1·5 or 3 per cent) and of the steric configuration. This change would not seem to be sufficient to cause the observed difference in chromatographic behaviour. If the effect reflects a difference in the *p*K values, the degree of separation should depend on the *p*H of the eluting buffer. In order to test this, four samples of tritiated AP equal in amount and specific radioactivity were fractionated on the same column (regenerated after each run) but at four different *p*H-values: 9·0, 9·4, 9·7 and 10·0. In Fig. 2, the observed AP concentration and radioactivity distributions are plotted versus the fraction number. (The fraction number is counted from the moment at which the eluted material first showed the *p*H of the eluting buffer; this took 3 fractions at *p*H 9 and 6 fractions at *p*H 10.) The figure also contains the corresponding values of the specific radioactivity.

To evaluate the distributions we define: N = fraction number, r_N = distribution of radioactivity, e_N = distribution of the optical density. The total content of each curve is then:

$$R = \sum_{N=1}^{\infty} r_N \text{ and } E = \sum_{N=1}^{\infty} e_N$$

By calculating these and the following values from the experimental results the results can be easily summarized (Table 2).

(1) The respective mean values:

$$\bar{N}_R = (1/R) \sum_{N=1}^{\infty} N \cdot r_N \text{ and } \bar{N}_E = (1/E) \sum_{N=1}^{\infty} N \cdot e_N$$

increase exponentially with increasing *p*H. As shown in Fig. 3, their logarithm increases linearly with the *p*H so long as it is below the *p*K value which for non-tritiated 2-aminopurine is 9·93. For higher *p*H values the column should become rapidly impenetrable as is indicated by the highest point in Fig. 3.

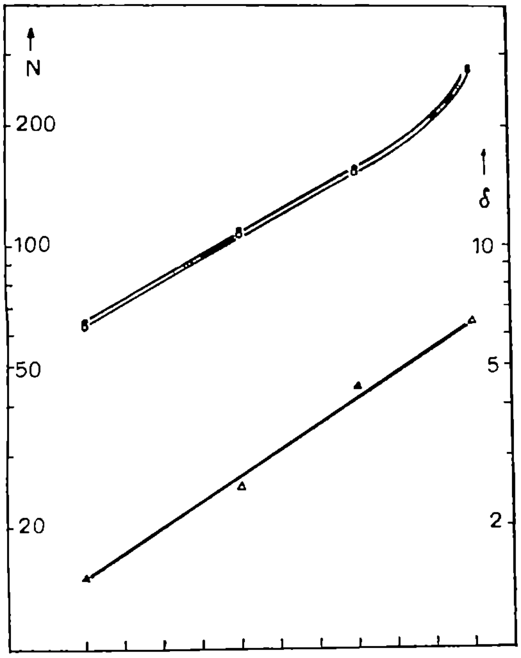

Fig. 3. Mean values $\bar{N}_E$ (●) and $\bar{N}_R$ (○) of the extinction and radioactivity distributions of Fig. 2 and the difference δ (Δ) between them; as function of the pH of the eluting buffer

(2) The difference of the two mean values:

$$\delta = \bar{N}_E - \bar{N}_R$$

also increases exponentially with the *p*H of elution in the range examined as shown again in Fig. 3.

(3) A measure of the separation is the amount of overlap between the normalized radioactivity and extinction distributions:

$$\Delta = \frac{1}{2}\sum_{N=1}^{\infty}\left\{\left(\frac{r_N}{R}+\frac{e_N}{E}\right)-\left|\frac{r_N}{R}-\frac{e_N}{E}\right|\right\} = 1-\frac{1}{2}\sum_{N=1}^{\infty}\left|\frac{r_N}{R}-\frac{e_N}{E}\right|$$

This overlap value can vary between zero (complete separation) and 1 (complete overlap). The calculated values Δ (see Table 2) show that there is a slight decrease in overlap with increasing *p*H but at *p*H 10 (>*p*K) the overlap-value is again larger.

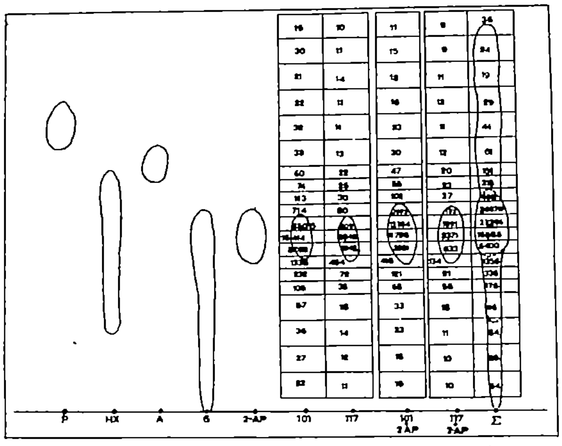

Fig. 4. Paper chromatographic analysis of an early (101) and a late (117) fraction of a 'Dowex 1' formate fractionation of tritiated AP at *p*H 9 4

Table 2. CHARACTERISTIC VALUES OF THE DISTRIBUTIONS IN FIG. 2 AS DEFINED IN THE TEXT

*p*H	*R* (c.p.m. $\times 10^{-6}$)	*E* (moles $\times 10^{6}$)	$\bar{N}_R$	$\bar{N}_E$	δ (fractions)	δ (ml.)	Δ
9·0	1·186	48·26	62·77	64·27	1·5	11·4	0·8408
9·4	1·199	50·40	106·81	109·31	2·5	19·0	0·8393
9·7	1·240	53·30	151·40	155·83	4·43	33·7	0·8308
10·0	1·192	54·20	272·94	279·78	6·84	52·0	0·8781

In order to convince ourselves that the observed isotope effect is not due to some radioactive contamination we concentrated fractions 101 and 117 of the column run at *p*H 9·4 and tested them by paper chromatography separately and together with purin (P), hypoxanthine (HX), adenine (A), guanine (G), and standard AP. Fig. 4 gives an outline of the chromatogram. The numbers in the areas give the radioactivity in c.p.m./cm^2 (not corrected for background). It can be seen that the radioactivity from both fractions 101 and 117 coincides with the 2-aminopurine spots. Any hypothetical radioactive contaminant should especially show up in fraction 101. Since this is not observed and since all other purines used in this chromatogram are well distinguished from AP, which also showed the expected fluorescence, any significant contamination of the AP sample by another radioactive compound seems excluded.

When 2-aminopurine is eluted from an anion exchange column the tritiated molecules move slightly faster than the non-tritiated ones. This effect is apparently caused by a charge difference between the molecular species since their steric difference is extremely small and since the column mainly distinguishes molecules of different average charge. The anionic *p*K of tritiated 2-aminopurine should then be slightly higher than that of non-tritiated one. The explanation agrees with the finding that the separation of the two peaks increases with increasing *p*H. For the separation of radioactive from non-radioactive 2-aminopurine, however, it is better to elute at a *p*H lower than the *p*K value and rather to use long columns under pressure, since the elution is faster than at a *p*H close to the *p*K value.

The effect of hydrogen isotopes on the biological and chemical properties of molecules has been studied much more extensively for deuterated than for tritiated compounds. Of interest in this connexion is, for example, the observation that the *p*K of ND_4OD is 4·96 and of NH_4OH 4·75 (ref. 4). A tritium isotope effect has been observed for the partition chromatography of organic acids[5]. For ion exchange chromatography the partial separation of carbon-14 labelled from unlabelled amino-acids has been reported[6,7]; it was most effective when the labelled carbon occurred next to the dissociable group (carboxyl or amino).

For 2-aminopurine the tritium atom could exist in a stable form at either the 6 or the 8 position (or both). This must in some way affect the resonance of the ring and hence the electronegativity of that nitrogen from which the proton separates at high *p*H. Details of this electronic effect are not known.

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ORGANOMETALLIC COMPOUNDS AS POSSIBLE LASER MATERIALS

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IN this article we propose the use of organo-metallic rare-earth compounds for laser action. The feasibility of the proposed approach is estimated numerically, using europium salicylaldehyde as an example.

The output power attainable in laser action is limited in practice by the energy transfer from the pump to the emitting system. Optically pumped systems in existence to-day are characterized by narrow absorption bands, resulting in poor utilization of the energy content of the spectra emitted by the pumping light source. A molecular system capable of absorption over a broad spectral band, in conjunction with sharp emission lines, is sought in order to obtain laser action at higher power outputs than are at present accessible.

Various covalent organic compounds of europium, samarium and terbium have been reported[1,2] to show the desired behaviour. The organic constituents of these compounds contain one or two substituted benzene rings, such as salicylaldehyde, for each of the three covalent bonds formed with the rare-earth atom.

The process of optical absorption and emission in these compounds can be visualized in the following manner, involving the three basic steps indicated in Fig. 1. The peculiarities of this three-step process are as follows. Absorption (step 1) is dominated by the organic constituent of the complex molecule, and is only slightly influenced by the nature of the rare-earth atom; it occurs in a broad, quasi-continuous ultra-violet band in the region between 3000 and 4000 Å. The intramolecular energy transfer (step 2) is radiation-less and very dependent on temperature; at liquid nitrogen temperatures, transfer efficiencies as high as 85 per cent[1] have been observed. The re-emission of excitation energy (step 3) is fully radiative; it shows the sharp fluorescence spectra characteristic of the rare-earth atom, but modified somewhat by the nature of the organic constituent. When the complex compounds are dissolved in an organic liquid, all three steps are further modified to some degree by the nature of the solvent.

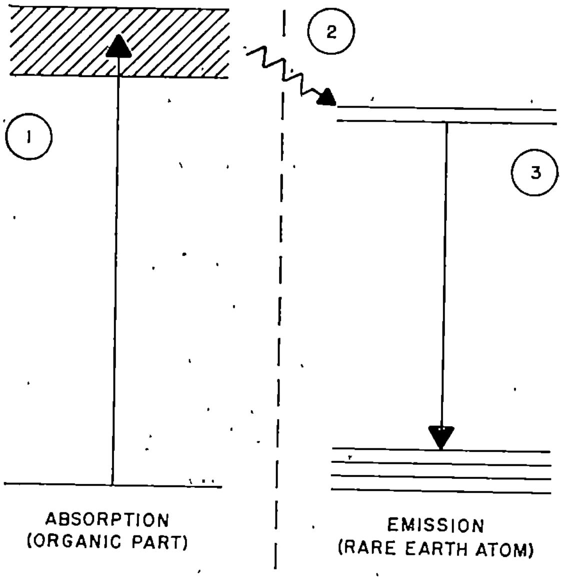

Fig. 1. *Step* 1. Near ultra-violet absorption caused by a strong, quasi-continuous band is due to the organic constituent. *Step* 2. Part of the energy absorbed reaches the rare-earth atom by way of radiationless intramolecular transfer, exciting it to higher electronic states. *Step* 3. Fluorescent emission of the entire excitation energy in sharp lines characteristic of the rare-earth atom

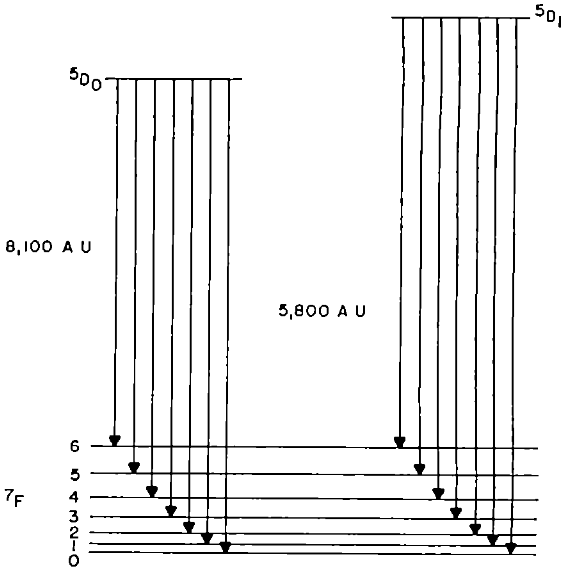

Fig. 2. Ground-state and first excited states of europium in organic compounds. Most of the levels are split by the molecular electric field into additional components

The optical properties of the organometallic compounds discussed here render them very attractive as potential laser materials. Experimental verification could be attempted using either liquid or solid solutions in organic liquids, or in organic plastics, respectively. Many transparent plastics are known to take on dyestuff in their monomer state; these could be machined after polymerization to optical tolerances required by the conventional designs of optical cavities used in ruby lasers.

In the following discussion, the pump power required to start laser oscillation is estimated, using the complex compound Eu(5-NO$_2$–C$_6$H$_3$OCHO)$_3$ as an example. Experimental observation of fluorescence spectra of complex europium compounds[2] can be explained by transitions between states $^5D_0$, $^5D_1$ and ^7F (Fig. 2).

Since the molecular field around europium is basically of three-fold symmetry, it can split the ^7F septet into as many as 33 states[3]), and the D-levels into 3 states at the most, resulting in a total of 99 lines. For the purpose of the following estimate, 100 lines of equal transition probability will be assumed.

The onset of laser oscillation at wave-length λ in an optical cavity with end-plate areas A of reflexion coefficient α is determined by the inequality:

$$n \geqq (8\pi 2/3)(\Delta\nu\,.\,\tau/\lambda^2)(1 - \alpha)A \qquad (1)$$

where n is the population of the upper state, and the population of the lower state is assumed to be negligible. $\Delta\nu$ is the line-width of the transition at wave-length λ, and τ is the life-time of the upper state. Assuming, furthermore, that the energy transfer process from the organic constituent to the rare-earth atom excites all the upper D-levels with equal probability, the required rate of intramolecular energy transfer becomes:

$$dN/dt \geqq 100(8\pi 2/3)(\Delta\nu/\lambda^2)(1 - \alpha)A \qquad (2)$$

For a transfer process of quantum yield 0·85, the corresponding flux of ultra-violet quanta needed is:

$$dN_{\text{uv}}/dt \geqq (100/0{\cdot}85)(8\pi 2/3)(\Delta\nu/\lambda^2)(1 - \alpha)A \qquad (3)$$

Inserting an estimated line width[4] of 0·1 cm^{-1}, as well as $\lambda = 8 \times 10^{-5}$ cm, $A = 1$ cm^2, and $\alpha = 0{\cdot}99$, the inequality (3) yields a flux of $1{\cdot}4 \times 10^{19}$/sec. The average energy of ultra-violet quanta in the interval between 3000 and 4000 Å is approximately $(h\bar{\nu}) = 6 \times 10^{-12}$ erg. Consequently, the minimum required pump power turns out to be:

$$P_{\text{uv}} = (dN_{\text{uv}}/dt)(h\bar{\nu}) = 8{\cdot}5 \text{ watts} \qquad (4)$$

It is not too hard to obtain ultra-violet sources of such an intensity in the laboratory, especially for pulsed laser experiments. Moreover, the line structure of the emission spectra assumed in the foregoing estimate is more complicated than one expects to encounter; since certain transitions will be suppressed or eliminated by selection rules on account of the symmetry of the molecular field, the required pumping power will be diminished accordingly. Thus the preceding estimate indicates that organometallic lasers are feasible in principle. Experimental realization, however, will depend on overcoming various problems, the most prominent of which might be the photodecomposition under intense ultra-violet irradiation frequently observed in organic compounds.

We thank Prof. G. Feher of the University of California at San Diego for critically reviewing this article, and Prof. Feher and Dr. T. G. Pavlopoulos for discussions.

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CONFIRMATION OF THE ORIGIN OF THE LUMINOSITY BEHIND THE SHOCK WAVE IN AN ELECTROMAGNETICALLY DRIVEN SHOCK TUBE

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IT has been postulated recently that the luminous front observed behind shock waves in electromagnetically driven shock tubes, known as 'tee' or 'Kolb' tubes, arises from excitation produced in the spark discharge that launches the wave along the tube[1-3]. If this hypothesis were correct, then relaxation times, measured from the instant a particle is subjected to shock heating to the instant when it arrives at the luminous front, are meaningless if this time interval is interpreted in terms of the shock processes at the wave front.

A tee-tube has been used in this laboratory for measuring relaxation times for shock waves in the Mach number range 28–6, propagating into air or argon at room temperatures and initial pressures of 1 mm mercury. The method used was essentially that adopted by other workers[4,5], with improvements in the analysis of particle paths. Part of this work has been devoted to confirming that the origin of the luminous front, observed at distances of more than nine diameters from the discharge, lies in the process of shock heating at the wave front and not in the excitation at the discharge electrodes. Two experiments which lead to this conclusion are described below.

The relaxation-time measurements were carried out in a 'Pyrex' tee-tube of 3·5 cm internal diameter, with an overall length of 200 cm. The back-strap and spark gap in the tube formed part of the discharge circuit for a condenser bank of 94 μF., which normally stored 2,300 joules at 7 kV. The circuit was closed by a subsidiary triggered spark gap, and the discharge, which was not crowbarred, had a ringing frequency of 330 kc/sec. A drum camera, with a relative aperture of *f*2·5 and a writing speed of 0·18 mm/μsec at the film for 33,600 r.p.m., was used for obtaining streak photographs of the passage of the luminous gas behind a slit of width 1 mm along the length of the tube. Kodak 'Royal-*X* Pan' film was used in the camera and developed to an ASA value of about 1,600 with Kodak time standard developer. A typical photograph illustrating the shock front, the luminosity of which is ascribed largely to impurities, and the luminous front is shown in Fig. 1, labelled according to the normally accepted interpretation of such photographs. Records taken with the incident shock reflected off a probe fixed in the tube have confirmed that the luminosity identified with the shock wave is immeasurably close to the trajectory of the shock. It should be particularly noted that the relaxation time to the luminosity front, in Fig. 1, is measured along the particle path; it is not equal to the time measured between the passage of the shock wave and the arrival of the luminous front at a fixed station.

This tube was then modified by including a diaphragm (0·006 mm aluminium), about 17 cm from the spark gap to establish the origin of the light in the luminous front. RCA.931*A* photomultipliers were used for recording the radiation from the tube on a 'Tektronix 555' oscilloscope. The entry slit of one photomultiplier was covered with a thin film

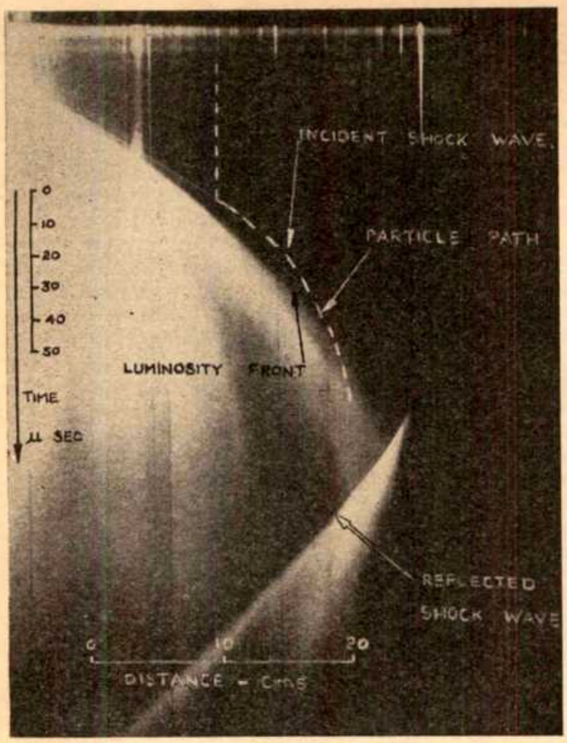

Fig. 1

interference filter, with a band width of 26 Å at half-peak transmission centred on the H_β line at 4861 Å. The other photomultiplier was fitted with a filter of band width 20 Å, centred on 5000 Å. The first photomultiplier then recorded H_β emission, with the continuum in the pass-band, and the second recorded the continuum only in a region selected as free of line radiation.

Experiments were carried out with this arrangement in which all permutations of hydrogen and argon on either side of the diaphragm were used and the response of the photomultipliers at various stations downstream of the diaphragm obtained for successive discharges of the same energy content. The initial pressures were the same on both sides of the diaphragm, namely, 1 mm mercury. The pressure in the tube, before filling with the appropriate gas, was reduced to less than 1 μm mercury, but no particular effort was made to reduce the level of impurities in the tube or in the commercial quality gases. Throughout these measurements, it was found that the oscillograph traces were governed by whichever gas was downstream of the diaphragm, and were independent of the upstream gas. The combinations, hydrogen/argon and argon/argon, gave the same luminosity pattern in both shape and magnitude to within experimental reproducibility; similarly, the records for hydrogen/hydrogen and argon/hydrogen were characteristic of hydrogen and markedly different from those of argon. These effects were found to persist from a point only 14 cm downstream of the diaphragm up to the end of the tube.

This result furnishes strong evidence that the origin of the luminous front lies in shock heating rather than spark heating.

The second set of experiments was carried out in an attempt to reconcile this conclusion with the experimental evidence found by Cloupeau[1] that the light emanating from the region between two normally reflected shock waves of equal strengths (region *A*, Fig. 3) was less than that from the region behind the incident luminous front just before the point of reflexion (region *B*, Fig. 3). Cloupeau interpreted this result as evidence that spark heating was responsible for the observed luminosity behind the incident shock waves because simple shock-wave theory shows that the increased density and temperature behind reflected waves should give rise to enhanced radiation compared with conditions behind the incident wave.

Our experiments were carried out in an '*H*' type tube, formed by two opposed sets of electrodes and back straps with a common centre tube (Fig. 2). The electrodes were connected in series to the same condenser bank and spark-gap system as before. The distance between the electrodes could be varied from 40 cm to 170 cm, and by doing this, it is possible to study the collision of two shock waves with Mach numbers in the range 22–8 without altering the power input. A typical photograph, obtained with the drum camera when the '*H*' tube was filled with argon to an initial pressure of 1 mm mercury, is shown in Fig. 3, in which the incident waves have a speed of Mach 12 at the point of reflexion.

Examination of a series of such photographs showed that the luminosity in region *A* was less than that in region *B* (confirming Cloupeau's observations) when the incident waves had speeds of less than about Mach 10. For higher speeds, the luminosity in region *A* equalled or exceeded that in region *B*.

To explain these observations on the basis of heating at the shock front, it is necessary to take into account the severe attenuation of the speed of the incident shock waves, and the finite relaxation time of the process giving rise to the luminosity. These effects imply that luminous particles in region *B* (Fig. 3) have suffered a larger change in enthalpy than those traversed by the incident shock near the

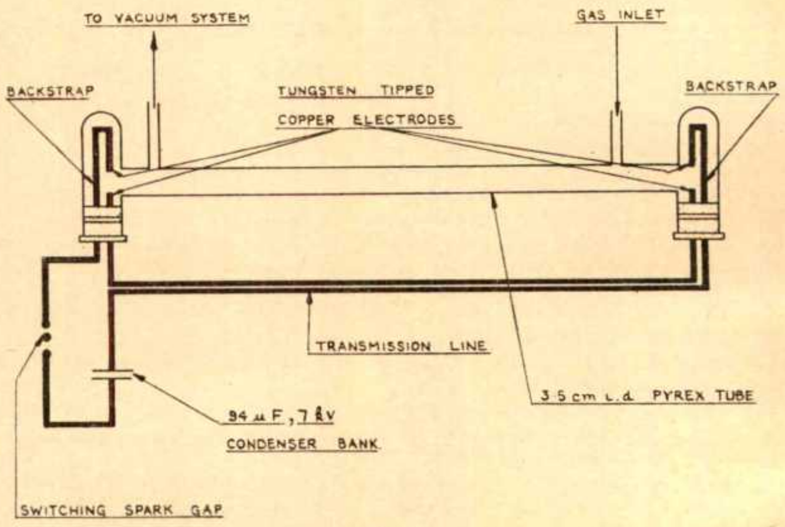

Fig. 2

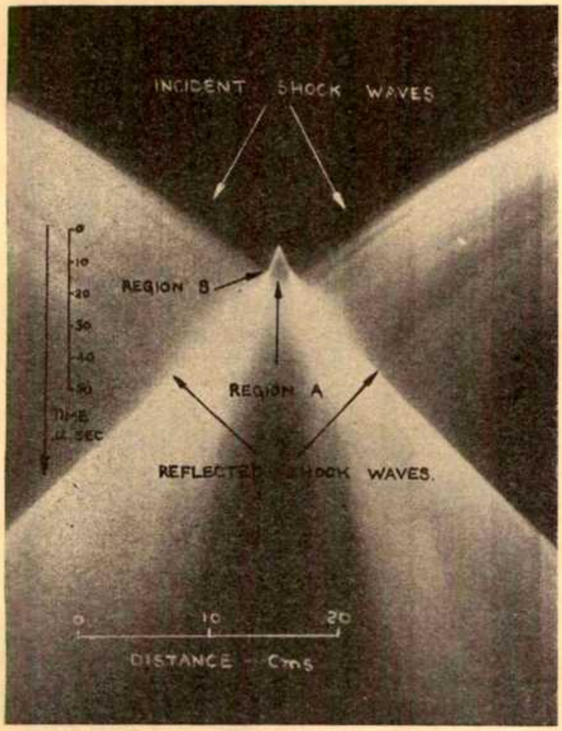

Fig. 3

point of reflexion. The light which these latter particles would produce, if the reflected shock did not destroy their directed motion, is only recorded on these photographs when the relaxation time is infinitesimally small.

Additional information on the luminosity which would be present if the shocks were not reflected but allowed to proceed down the tube was obtained by splitting the H-tube into two tee-tubes, so preserving the electrical characteristics of the discharge and photographing the movement of the gas in only one of the tubes. In this way, photographs, similar to those shown in Fig. 1, were obtained. The method of characteristics was used to trace particle paths between the point on the shock trajectory with a selected Mach number and the luminous front. Ideal gas behaviour was assumed.

Three regions of luminosity were obtained from these observations for each selected Mach number of the incident waves at the point of reflexion in the colliding shock experiment. They are specifically: (A) that lying between two reflected shock waves produced by colliding shock waves of the chosen Mach number; (B) that occurring behind the part of the incident luminous front nearest to the cusp of the reflected shocks; (C) that found at the luminous front when particles traversed by the incident shock of the selected Mach number have relaxed to a condition of quasi equilibrium without further heating.

A comparison of the regions, using a Joyce Loebl microdensitometer, type *Mk*.III*b*, showed conclusively that region A is more luminous than region C, throughout the range of Mach numbers examined here, and that region A is more or less luminous than region B, when the Mach number of the incident shock is more or less than about Mach 10. In terms of relaxation times, the time taken for the luminosity to reach a peak was found to be less for region A than for region C.

These observations, and previous ones using two gases and a diaphragm, are consistent with the hypothesis that the luminous front observed at a distance of more than nine diameters from the electrodes is brought about by shock heating. They also support the use of the tee-tube to obtain relaxation times for shock heating, apart from limitations due to impurities and precursor radiation.

I wish to thank my colleagues here for many valuable discussions and, in particular, Dr. I. Macpherson, under whose guidance this work was carried out.

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SOIL ORGANIC RÉGIME OF COASTAL SAND DUNES

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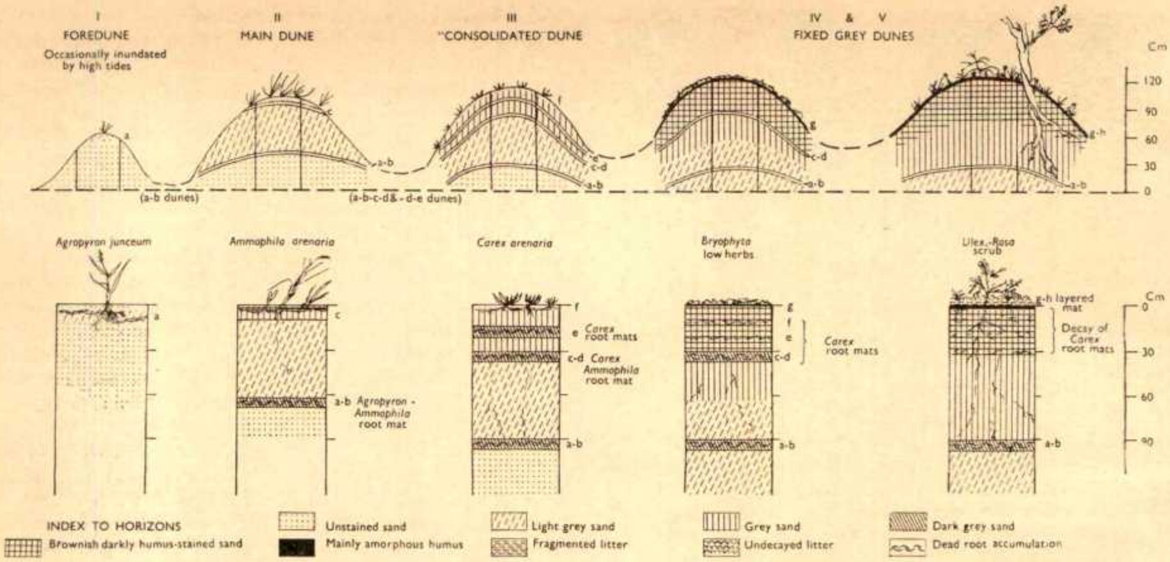
ALTHOUGH the colonization of sand dunes by vegetation has been intensively studied, the soil organic régime of coastal sand dunes and its role in soil development and dune stabilization are little known. It has been investigated in the field and by microscopic techniques at Seaton Sluice, Northumberland. The soil organic régime has five main stages of development (Fig. 1).

(1) In the saline foredunes which are building around tufts of *Agropyron junceum*, humic material (decaying organic matter) is almost confined to pale grey patchy stains in the horizon of maximum root development a few cm below the freshly accumulating sand. These grey patches contain rotting leaves attacked by white and brown fungal hyphæ which are restricted to the leaf fragments. They contain also a small amount of dark humic material some of which has the ovoid form of fæcal pellets resembling those produced by Acarina and Collembola[1].

(2) In the main accumulating dunes above the level of the highest tides, where marram grass (*Ammophila arenaria*) has rooted in successive deposits of wind-blown sand, dark-grey root-rich horizons, 5–10 cm thick, occur at vertical intervals of about 60 cm and are separated by pale grey sand. Near the surface they contain mycelia attached mostly to loose humic material but with a few hyphæ adhering to adjacent mineral grains. Fragmental remains of chitinous fauna also occur.

(3) In the 'consolidated'[2] dunes where *Carex arenaria* is dominant, sand is accumulating less rapidly than on the main dunes nearer the shore. These changes are reflected in the occurrence of root-rich horizons at vertical intervals of only 15 cm



The letters a-h indicate the relative positions of "marker" horizons at successive stages of soil development in the dunes.

Fig. 1

in the grey topsoil which is 30 cm deep. Beneath a 2 cm thick layer of freshly deposited sand, they contain leaf and faunal remains, definite fæcal pellets, dark humic matter and white and brown hyphæ. Growing from the dark humic material, many brown hyphæ surround the grains of sand, holding them together in clusters (Fig. 2) showing that fungi play an important supplementary part in stabilizing the root-rich horizons.

(4) On the fixed dunes where the sand is protected from erosion by a carpet of mosses, lichens and low herbs, a dark grey-brown almost black top-soil, 2 cm thick containing a mat of fine roots and rhizoids, is very high in humic matter and overlies brownish-grey-stained sand to a depth of about 30 cm. In places a vertical sequence of alternating light grey and dark grey horizons 1–2 cm thick extends below the surface to a depth of about 8 cm and rests on the brownish-grey sand. Near the surface discoloured rust-brown and blackened leaf residues occur, and mycelia are plentiful. The thin top-soil, however, is characterized by its abundant blackish-brown humic material, some having the form of sub-spherical pellets 0·1 mm in diameter and almost certainly of fæcal origin, some binding the grains together in clusters to form aggregates (Fig. 3).

(5) On the oldest of the fixed dunes which support a scrub of hawthorn (gorse, furze) (*Ulex europaeus*) and *Rosa* sp. with an undergrowth of mosses and grasses, distinct *L*, *F* and *H* horizons occur in a blackish-brown sandy mat 8 cm thick overlying a lighter brown horizon which in turn at a depth of 30 cm overlies alternating horizons similar to those associated with marram, extending to a depth of more than a metre. The *L* horizon consists mainly of loose, recently added residues and the *F* horizon is dominated by roots, the leafy residues having been reduced to fibres. In these horizons brown fungal hyphæ are abundant but in the *H* and lower *F*, fæcal pellets similar to those produced by mites are plenti-

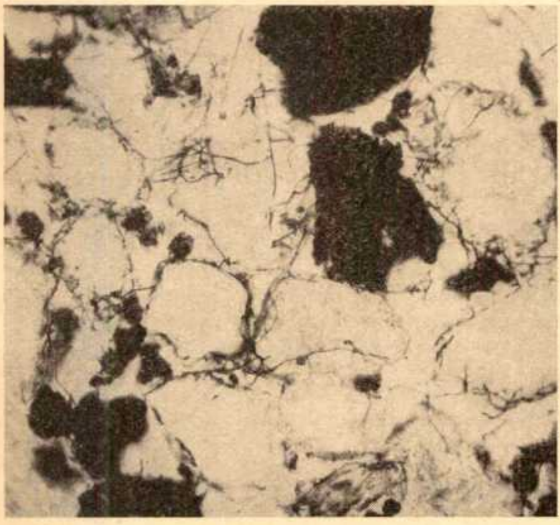


Fig. 2. Photomicrograph of a thin section showing sand grains held together by fungal mycelium (× *c*. 48)

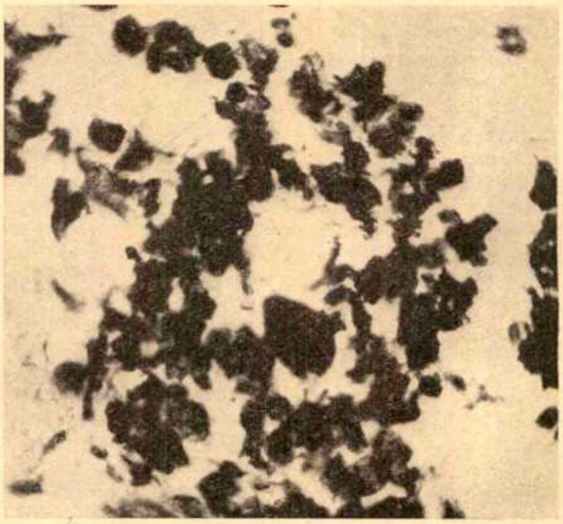


Fig. 3. Photomicrograph of a thin section showing humic material surrounding sand grains (× *c*. 48)

ful. These pellets have partly collapsed in the A_1 and the lower part of the H horizons.

In essentials these sequential changes in the soil organic régime of dune soils with increasing age are strikingly similar to those of soils in similar stages of development on limestones, dolerite or andesite.

This work was undertaken[3] under the supervision of the late E. Crompton and was supported by the award of a Nature Conservancy Research studentship.

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CHANGES IN THE PROTEIN CONTENT OF THE SPINAL MOTOR NEURONES OF THE MOUSE AFTER SEVERANCE OF THE AXON

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IN the ventral horn motor cells of the mouse there is no true chromatolysis after severance of the axon, and the so-called 'regressive phase' is lacking[1]. In the cytoplasm, the concentration of the gallocyanin-stainable material increases by about 35 per cent between the first and the third day after cutting the nerve, and does not vary significantly until the eighteenth day afterwards. In the nucleoplasm, the concentration of the material drops sharply until a minimum of about 50 per cent of the normal value is attained at the ninth day after operation, and slowly rises during the following days. In the nucleolus no change was detected[1].

The investigation reported here was undertaken to examine, by quantitative histochemical methods, the protein content of the neurones after severance of the axon.

Table 1 shows the results of cytophotometric determinations of the concentration of the material stainable by the alloxan–Schiff reaction[2]. This technique is known to reveal the ε amino-groups of the lysine residues of the polypeptide chains[3].

Table 1. MATERIAL STAINABLE BY THE ALLOXAN–SCHIFF TECHNIQUE IN THE VENTRAL HORN MOTOR NEURONES OF THE MOUSE

Days after cutting the axon	Cytoplasm	Nucleoplasm	Nucleolus
2	118 ± 3·8	81 ± 5·4	101 ± 3·0
3	133 ± 5·1	85 ± 8·0	99 ± 5·1
6	147 ± 5·7	105 ± 8·8	109 ± 3·4
10	193 ± 8·8	98 ± 8·8	112 ± 3·4
13	167 ± 6·3	88 ± 5·3	107 ± 3·1
15	146 ± 5·1	97 ± 6·1	108 ± 3·0
18	160 ± 7·7	104 ± 4·5	105 ± 3·5

Values are the means and *S.E.* for concentration in neurones of the operated (right) side, expressed as percentages of concentrations in neurones of the control (left) side. For each animal, 15 cells from both sides were measured. For each cell, values for cytoplasm were obtained from 8–14 measurements on different circular areas 1·5μ in diameter taken at random, values for nucleoplasm from 6–11 measurements, values for nucleolus from one measurement. Standard errors were calculated as:

$$s = \frac{\bar{x}}{\bar{y}}\left(\frac{v(\bar{x})}{\bar{x}^2} + \frac{v(\bar{y})}{\bar{y}^2}\right)^{1/2}$$

where $\bar{x}$ and $\bar{y}$ are the means of the operated and of the controls, and $v(\bar{x})$ and $v(\bar{y})$ their respective variances (see ref. 4).

It may be seen that the concentration of the protein amino-groups in the cytoplasm increases sharply during the first days after operation, while no significant change can be detected in the nucleoplasm. In the nucleolus the protein concentration increases slightly, but significantly.

In alloxan–Schiff preparations, the neurones from the operated side contrast sharply with normal ones (Fig. 1), and are much more easily detected than in preparations stained by other methods.

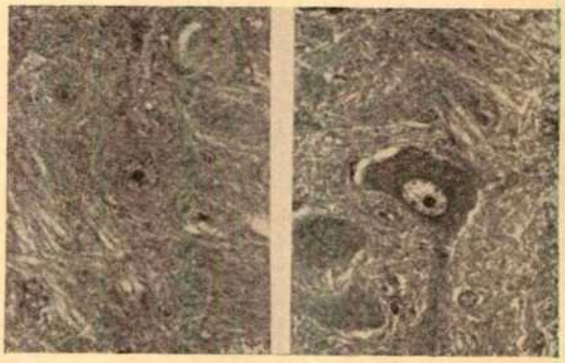

Fig. 1. Spinal cord of a mouse, lumbar segment, six days after unilateral section of the right sciatic nerve. Fixed in alcohol–formalin–acetic acid, 85 : 10 : 5; alloxan–Schiff reaction. Left, control side: The motor neurones are barely visible, since their cytoplasm and nucleoplasm are stained with the same intensity as the surrounding structures. Right, operated side: The cytoplasm of the motor neurones is heavily stained; the nucleoplasm, by contrast, seems lighter than in the controls, but cytophotometric measurements show that the intensity of staining is about the same

The ferric–ferricyanide reaction for SH groups[5] gave substantially the same results. A few cytophotometric measurements showed approximately the same increases in concentration of the reacting material as in alloxan–Schiff preparations. However, since the ferric–ferricyanide reaction has no definite end-point, the measurements were discontinued. The histochemical reactions for tyrosine[6] and tryptophan[3] were tried, but gave such poor staining that no conclusion could be drawn.

The striking increase in protein concentration in the cytoplasm of the operated neurones parallels the increase in gallocyanin-stainable material (constituted mostly by ribonucleic acid[7]) previously observed in the same material. This fact is in accordance with the view that the synthesis of the cellular proteins is related to the cytoplasmic ribonucleoproteins[8]. The section of the axon appears to stimulate the cytoplasmic protein-forming mechanism in such a way that the production of both ribonucleins and proteins overrates their consumption. This leads to their accumulation in the cytoplasm. The protein-forming mechanism in the nucleolus seems to be stimulated, too, as indicated by a slight increase in protein concentration; the fact that the gallocyanin-stainable material, half of which is constituted by ribonucleic acid[7], does not vary significantly in the nucleolus, indicates only that the balance between production and consumption is not substantially altered, and does not allow any conclusion about the rate of production itself.

After severance of the axon, there are no significant changes in the concentration of proteins in the nucleoplasm, in contrast with the sharp drop in the concentration of the nucleoplasmic gallocyanin-stainable material, constituted chiefly by deoxyribonucleic and ribonucleic acid. Unpublished experiments suggest that most, if not all, of the decrease in concentration of the nucleoplasmic gallocyanin-stainable material may be accounted for by migration of DNA and RNA from the nucleoplasm towards the inner surface of the nuclear membrane. Various experiments[9] support the thesis that RNA is synthesized in the nucleus in close association with DNA and is continuously released to the cytoplasm. In the neurone stimulated by section of the axon, the migration of DNA and nuclear RNA close to the nuclear membrane would obviously assist the process of transfer of RNA to the cytoplasm. The fact that the proteins histochemically detectable in the nucleoplasm are not substantially altered after section of the axon suggests that they are not an important part in this mechanism.

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RELATIVE CONTRIBUTIONS OF THE THYMUS AND THE BURSA OF FABRICIUS TO THE MATURATION OF THE LYMPHORETICULAR SYSTEM AND IMMUNOLOGICAL POTENTIAL IN THE CHICKEN

By BEN. W. PAPERMASTER* and PROF. ROBERT A. GOOD†

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RECENT reports have indicated that the bursa of Fabricius plays a central part in the ontogenesis of immunological function in the chicken[1-3]. This organ develops as an epithelial cloacal diverticulum on the fifth day of incubation, and becomes a lymphoid organ on the fifteenth day, as described by Ackerman and Knouff[4]. Surgical ablation of the bursa after hatching significantly reduces the antibody-forming capacity of the maturing chicken[1-3] provided that bursectomy is performed before two weeks of age. A more complete reduction in immunological capacity results when eggs are treated with androgenic or progestational steroid analogues, such as 19-*nor*testosterone, early in the incubation period[2,3,5,6]. Chicks hatching from eggs treated with 19-*nor*testosterone on the fifth day of incubation have no bursa of Fabricius and are severely compromised in their ability to form circulating antibody[2] and overcome immunological attack by adult homologous spleen cells[3].

Since 19-*nor*testosterone appeared to retard immunological maturation more than surgical bursectomy, an experiment was designed to test the biological effect of the drug on the lymphoid tissue of the chicken, using the Simonsen assay for immunological competence[7]. A suspension of spleen cells was prepared from 14-day-old normal and 19-*nor*testosterone-treated donors, as described previously[8], and 2 × 10^6 splenic leucocytes were inoculated on to the chorioallantoic membrane of eggs incubated for 10 days. Recipient eggs were opened 4 days after inoculation, and the embryos examined for splenomegaly and white foci on the chorioallantoic membrane, as in the experiments of Boyer[9]. The findings are summarized in Table 1.

That there appeared to be no reduction in the immunological potency of spleen cells from donors treated with 19-*nor*testosterone was surprising in view of the generally poor response of such chickens to diverse immunological stimulation. These experiments led us to consider the role of another lymphoid-epithelial organ, the thymus, in the development of immunological potential in the chick.

Table 1. IMMUNOLOGICAL COMPETENCE OF SPLEEN CELLS FROM 19-*nor*TESTOSTERONE-TREATED CHICKS

Spleen cell donor	No. of recipient embryos with: Chorioallantoic membrane foci	Splenomegaly
Normal 14-day old chicks	9/10	5/10
19-*nor*testosterone-treated 14-day old chicks	7/10	5/10

To compare histogenesis of the thymus with that of the spleen, bursa of Fabricius, liver, gut tract, skin, and bone marrow, whole embryos were fixed in 10 per cent formalin or absolute alcohol, serially sectioned, and the sections stained with hæmatoxylin and eosin or methyl green-pyronin. Such sections were obtained from embryos at 10, 12, 14–18, and 21 days of incubation. Hatched chicks were killed at 1, 5 and 12 days of age, and spleen, gut tract, thymus, skin, liver and bone marrow sections were prepared. By this simple technique, the morphological development of all the lymphoid tissue in the animal could be compared.

In the material examined, lymphocytogenesis began in the thymus by the twelfth day and the thymus was a lymphoid organ by the fourteenth day of incubation, whereas lymphocytogenesis did not begin in the bursa until the fifteenth day (Fig. 1). Similar observations on the thymus have been recorded by Ruth[10] and by Schrier and Hamilton[11]. Moreover, lymphoid cells were almost entirely confined to the thymus and bursa at 18 days of incubation (Fig. 2). The spleen appeared to be primarily involved in granulocytopoiesis and the liver and bone marrow in erythrocytopoiesis at that time. Significant lymphoid follicle development in the spleen did not appear until after hatching, when lymphoid

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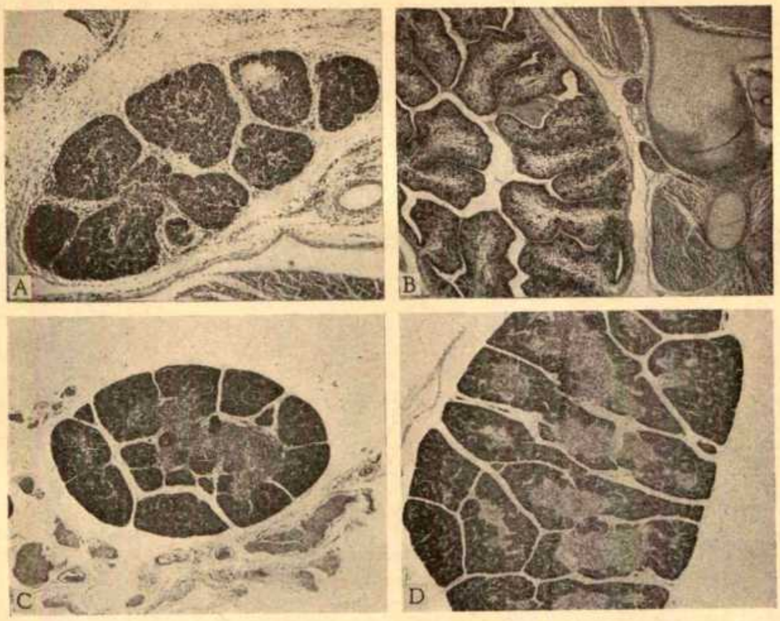

Fig. 1. *A*, thymus from a 14-day chick embryo, showing that this is already a lymphoid organ; *B*, bursa of Fabricius from a 14-day chick embryo. Lymphocytopoiesis is beginning in the epithelial buds; *C*, thymus from a newly hatched chick; *D*, bursa from a newly hatched chick. The thymus and bursa of Fabricius have a similar lymphoid picture at this stage. (× *c*. 18)

cells began accumulating in the venous sinuses and then in the red pulp surrounding the small arterioles. Lymphoid follicle development in the gut was also delayed until after hatching.

These observations, coupled with the earlier finding that the thymus was reduced in size but not absent following 19-*nor*testosterone treatment of embryos[2,3,6], suggested that any immunological potential remaining after treatment of eggs with this drug might be related to the presence of small amounts of thymus tissue. Indeed, the thymus and bursa of Fabricius in the chick may act synergistically as sources of immunologically competent cells in the chick during the neonatal period. Cells arising in the bursa and thymus might populate the spleen, bone marrow and other sites.

Spleen sections from 19-*nor*testosterone-treated chicks killed at 4 and 14 days after hatching showed reduced lymphoid follicle development compared with normal controls. This correlated with the reduction in spleen weight previously reported[2,3,6]. Although limited, this follicular development probably accounted for the presence of immunological potential in spleen cells from treated chicks. Spleen cells from mice thymectomized in the immediate neonatal period do not produce a graft versus host response, and this correlates well with the lack of lymphoid follicle development in the spleens of thymectomized mice[12].

In mammals, the thymus may be the most important organ supplying lymphoid cells to other organs in the reticuloendothelial system, as originally postulated by Ruth[13]. It may also influence the *de novo* development of lymphoid cells in such organs as the spleen and intestine through humoral factors influencing lymphocytopoiesis. However, the observations cited here, experimental work with thymectomized mice[12], and the high proliferation-rate of lymphoid cells in the thymus during the neonatal period in mammals[14], point to the bursa of Fabricius and the thymus as centres of lymphoid cell production in the neonatal period. Cells emigrating from the thymus and bursa could enter the spleen, gut tract, and other areas of lymphoid cell accumulation, and continue to proliferate and/or differentiate into immunologically competent cells. Thus, 19-*nor*testosterone treatment in the chick may reduce the total amount of available reactive lymphoid tissue, render the animal unable to produce measurable amounts of antibody[2] or to reject the impact of injected adult homologous cells[3], but not suppress lymphoid development completely because of the remaining thymic tissue. If both thymectomy and bursectomy could be performed early enough in the chick, such animals might compare with thymectomized mice[12]. The lymphoid development of the thymus and bursa in the chick are similar, and the functions of these two organs in the chick may also prove to be similar.

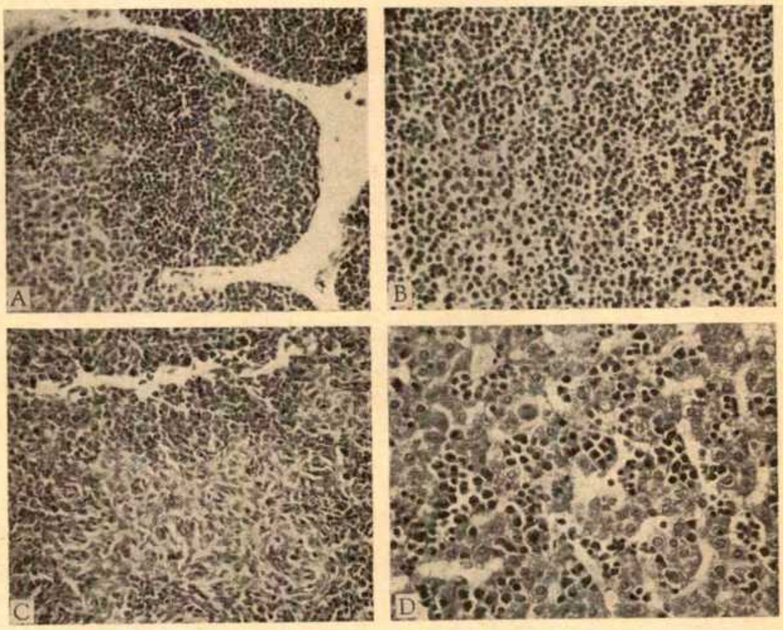

Fig. 2. *A*, lymphoid-epithelial bud from bursa of an 18-day chick embryo. Note accumulation of lymphocytes in the bud; *B*, section through the thymus of an 18-day embryo. Cellular population is nearly all lymphocytic; *C*, section through the spleen of an 18-day embryo. Small vessels and sinusoids are lined by developing granulocytes, fibroblasts, and reticular cells; *D*, section of liver from 18-day embryo. The cellular developmental pattern in the sinusoid is erythroblastic. (× *c*. 188)

Morphological studies of the developing lymphoid tissues have been completed in the mouse, rabbit, guinea pig and dog. These support the foregoing conclusions and will be reported elsewhere.

This work was supported by grants from the National Foundation, the U.S. Public Health Service, the Minnesota Heart Association, and the American Heart Association.

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EXCITATION AND INHIBITION OF BRAIN-STEM NEURONES BY *NOR*ADRENALINE AND ACETYLCHOLINE

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THE technique of iontophoretic application of drugs by micro-pipettes introduced by Nastuk[1] and developed by del Castillo and Katz[2] was successfully adapted for an examination of the responses of spinal neurones by Curtis and Eccles[3]. The precise analysis of the pharmacological properties of single neurones which can be achieved with this technique gives great promise for the study of drug action and chemical transmission in the central nervous system.

It is natural that the known peripheral transmitters, acetylcholine and *nor*adrenaline, should be tested for actions in the central nervous system and neurones which respond to iontophoretic application of acetylcholine have been discovered in the spinal cord[3], inferior colliculus[4] and cerebral cortex[5,6]. More recently Salmoiraghi and Steiner[7] have found that acetylcholine has both excitatory and inhibitory effects on neurones in the brain-stem. *Nor*adrenaline, so far as it has been tried[4,8,9], has been found to be ineffective or to have a non-specific inhibitory action. Previous investigations with other techniques (see review[10]) have suggested that adrenaline and *nor*adrenaline can act on neurones in the brain-stem and Bradley and Mollica[11] found that intra-carotid injections of these substances excited some neurones and inhibited others.

In the experiments recorded here a survey has been made of the responses of neurones in a limited region of the brain-stem of decerebrate cats to the local application of acetylcholine and *nor*adrenaline. Four barrelled electrodes of overall tip diameter 2–12μ were used. One barrel was filled with 4 M sodium chloride for extracellular recording of unit activity; current could be passed through the other three barrels, one of which was routinely filled with 0·9 per cent sodium chloride acidified to *p*H 3 with hydrochloric acid, while the other two contained 10 per cent acetylcholine hydrochloride and 3 per cent l-*nor*adrenaline bitartrate, both at *p*H 4. A small negative backing current of 3 n.amp or less was used to prevent diffusion of the positive ions from the electrode tip. To expel the ions the current was reversed and increased to not more than 100 n.amp. The electrode was inserted under visual observation at positions in the rostral third of the medulla and caudal half of the pons within 3 mm from the mid-line. Each neurone encountered as the electrode was moved inwards was tested for its response to current passed through each barrel. The neurone impulse frequency was sampled every second and the mean frequency over a 10-sec period was plotted against time (Fig. 1). A positive current applied to the control barrel (acidified saline) usually depressed neuronal activity but occasionally had no effect or produced a slight excitation. By carefully adjusting the current, these effects could be distinguished from drug effects by their magnitude and time course.

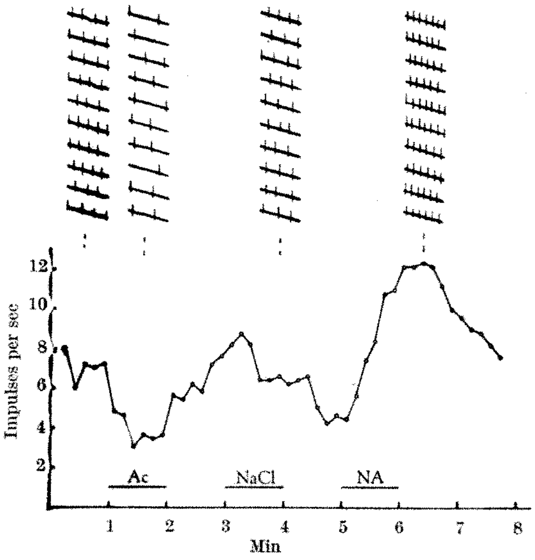

Fig. 1. Above, oscillograph records of neurone activity at times indicated; below, graph of impulse frequency against time. The black bars indicate the period of application of drugs using positive currents of 50 n.amp (electrode tip 10μ)

More neurones were found to be affected by l-*nor*adrenaline than by acetylcholine. Of 95 neurones tested, 44 (46 per cent) showed a response to this substance and of these 30 (31 per cent) were excited and 14 (15 per cent) inhibited (Figs. 1 and 2). The

(*Continued on page 873*)

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MAN IN SPACE

Aerospace Medicine

Edited by Harry G. Armstrong. Pp. xiii + 633. (London: Baillière, Tindall and Cox, Ltd., 1961.) 144*s*.

Environmental Effects of Consciousness

Proceedings of the First International Symposium on Submarine and Space Medicine, United States Submarine Base, New London, Connecticut, September 8–12, 1958. Edited by Karl E. Schaefer. Pp. x + 146. (New York and London: The Macmillan Company, New York, 1962.) 5.50 dollars; 41*s*.

THE recent advances in aviation and space technology have stimulated great interest in the physiological problems associated with manned flight. Both of the books under review reflect this interest, one as a text-book and the other as a collection of papers presented at a symposium. Although the text-book is entitled *Aerospace Medicine* it deals primarily with the medical problems associated with manned flight within the Earth's atmosphere. The symposium, on the other hand, was an attempt to exchange information between various groups of investigators interested in the physiological aspects of sealed environments.

In *Aerospace Medicine* Armstrong has drawn together the contributions of some twenty-one American authors, each of whom is a recognized authority in his own field of interest. Following a brief historical review of the subject, six chapters are devoted to describing the clinical examination of aircrew with respect to their fitness to fly. These chapters contain unnecessarily detailed descriptions of standard techniques used in clinical medicine, and, in certain instances, the useful information contained in them could well be added to later sections in the book devoted to cardio-vascular and ophthalmological problems. The clinical chapters are succeeded by sections in which the environment of flight and the physiological effects induced by exposure to low barometric pressure, radial and linear accelerations, high and low temperatures and acoustic energy are described. Luft has contributed a lucid account of effects of acute hypoxia. The chapter on administration of oxygen in flight does not, however, treat this important subject adequately. There is no discussion of the basic functional requirements which must be met by aircraft breathing equipment and there are erroneous statements with regard to the occurrence of ozone in pressurized aircraft at high altitude and the mechanism of the cerebral disturbance produced by exposure to oxygen at 2–3 atmospheres. There are comprehensive chapters on decompression sickness and the physiological aspects of pressure cabins. The effects of radial and linear accelerations are adequately described, although the chapter on linear accelerations contains several lengthy descriptions of individual sledge track runs which, it is felt, are out of place in a text-book.

Probably one of the best sections of this book is that written by Nuttall, in which he deals in a clear and concise manner with the many physiological problems associated with escape from aircraft and the subsequent survival on land and in the sea. The public health aspects of air travel, both with regard to crew and passengers, are dealt with in some detail. Finally, Strughold's contribution outlines the specific medical problems involved in manned space flight.

The appearance of this comprehensive text-book will be welcomed by both students and practitioners of aviation medicine. It stands as a real addition to the literature of this subject. It is hoped, however, that in the next edition the editor will attempt a further integration of the work of the various contributors.

Environmental Effects on Consciousness is a collection of the papers which were presented at one session of the first international symposium on submarine and space medicine which was held at New London, Connecticut, in September 1958. Several of the papers deal with the effects of changes of the partial pressure of oxygen, carbon dioxide and nitrogen on the functions of tissues at the cellular level, while others describe the effects on man of exposure to various gaseous environments. Pugh contributed a concise and lucid account of the effects of acute and chronic hypoxia on consciousness, and Behnke and Taylor discussed the mechanism of nitrogen narcosis. A third group of papers was devoted to the effects of sensory deprivation on consciousness, the part played by the vestibular apparatus in orientation in space and the effects of the gravity free state. The theory presented in the latter paper has now been supplemented by the results of the recent manned satellite experiments. This book also contains a verbatim report of the panel discussion which followed the formal papers. Although this discussion contains many points of interest, it suffers in the written form from the presence of many superfluous phrases. The impact of the arguments which occurred during the discussion could have been increased considerably in this book by judicious editing. Although the contents of this book will be of interest to physiologists concerned with the effects of the gaseous environment on man, its value is reduced by the period of four years which has elapsed between the presentation of the papers and the publication of the book.

J. ERNSTING

STUDIES IN ASTRONAUTICS

Vistas in Astronautics—1960

Vol. 3: Proceedings of the Third AFOSR Astronautic Symposium, jointly sponsored by the Air Force Office of Scientific Research and the Society of Automotive Engineers, Inc., October 1960. Edited by Dr. E. R. Van Driest, Emanuel Haynes, Lt.-Col. C. W. Craven, C. W. Guy, W. W. Withee and N. D. Sanders. Pp. vi + 266. (New York: Society of Automotive Engineers, Inc.; London: Pergamon Press, 1961.) 55*s*. net.

THIS book records the papers and discussion at the Symposium on Astronautics held in Los Angeles in October 1960. The contents are divided into six sections: on vehicle utilization, propulsion, bioastronautics, the interplanetary environment, communications and guidance. Many of the papers are slight, and serve merely as introductions to their subjects. Some are so slight that they scarcely deserve recording, and the same can be said of the luncheon addresses and of the discussions, which consist of a question and a non-committal answer like "Offhand, I don't have the exact factor. . . .". Of the more substantial papers, several were valuable at the time they were presented, but have now been overtaken by events.

There remain a few, however, which are still of considerable interest, chiefly those on advanced methods of propulsion, such as nuclear rockets, ion rockets and magneto-hydrodynamic propulsion. Two papers deserve particular mention. The first, by J. Green, deals with the application of geology to the problem of man's survival on the Moon: this is a thorough and admirable survey (though it bears a striking resemblance to the same author's article in *Advances in Space Science and Technology*, Volume 3). The second notable paper is a stimulating discussion by S. T. Demetriades and C. F. Young on an "Orbital Refuelling Satelloid"—a large manned vehicle with an air intake, designed to fly at orbital speed at a height of about 70 miles. This satelloid would function as a ramjet, about half the air it swept up being accelerated by magneto-hydrodynamic methods to provide enough thrust to overcome the air drag; the other half of the incoming air would be liquefied so as to be available as propellent for refuelling other spacecraft. Much of the cost of space travel by conventional methods will be attributable to the need to transport fuel from the Earth's surface into orbit, which this futuristic scheme might avoid. Despite such felicitous papers as these, the book as a whole cannot now be recommended. It fulfilled the purpose of the Symposium by providing a satisfactory (though imperfectly edited) introduction to astronautics in 1960; but its senescence has been rapid.

D. G. King-Hele

FURTHER PAPERS OF ISAAC NEWTON

Unpublished Scientific Papers of Isaac Newton

A Selection from the Portsmouth Collection in the University Library, Cambridge. Chosen, edited and translated by A. Rupert Hall and Marie Boas Hall. Pp. xxi + 416 + 5 plates. (Cambridge: At the University Press, 1962.) 60*s*. net.

It is well known how difficult it is to form any clear idea of Newton's philosophy from the seemingly contradictory statements contained in his published writings. The publication of new documents casting light on this fundamental aspect of his complex personality will therefore be hailed with gratitude by professional historians of science, and with interest by everyone curious of science and its wider human implications. Yet these feelings must be tempered by serious misgivings when the volume under review is more closely scrutinized. That it should only bring a selection from the mass of the Newton papers deposited in the Cambridge libraries is inevitable, but the nature of the selection gives rise to some concern. The authors tell us that they have been primarily interested in collecting evidence on Newton's ideas of the structure of matter; but they warn us that the collection they offer of relevant documents (valuable as it is) makes no claim to being complete. On the other hand, they have thought fit to present other documents quite unrelated to the main theme, just to illustrate the wealth and variety of information still dormant in these unexplored deposits; here again, then, all we get is a very fragmentary survey of the existing evidence. Indeed, incompleteness seems to be the mark of this book: even the list of books and articles dealing with the Newton papers, which is given in the introduction, "is probably far from complete"; and we shall see presently that the authors' documentation about the known sources of Newton's ideas is apparently deficient in one important respect. What do they hope to achieve by this kind of 'butterflying'? One may fear that their example will invite others to imitate them (this has happened already), and that such unco-ordinated effort may further jeopardize the long overdue undertaking of a really systematic and exhaustive examination and soundly selective publication of the Newton manuscripts.

This being said, it must readily be admitted that the selection now published is an excellent one; of the samples we are offered of Newton's versatile activity—ranging from extended essays to mere jottings of thoughts and observations—none is trivial and several are of the first importance. About the editing there can only be praise: each document is provided with a commentary containing every desirable information and elucidation; an effort is also made to assign a date to the document, but the result (through no fault of the authors) is often inconclusive or vague. The Latin texts are followed by a translation—a heavy tribute to the barbarians! The documents are distributed on six sections (mathematics, mechanics, theory of matter, manuscripts related to the *Principia*, education, notes) each preceded by a substantial introduction, generally very competent and well founded (there are occasional slips; thus, the comparison of Newton's differential concepts with those of Leibnitz is somewhat muddled up). This arrangement of the material is not very happy, inasmuch as the documents pertaining to the theory of matter, the most important theme, are in fact (as the authors themselves indicate) scattered over all the sections.

The main contribution of the authors to the analysis of the material is their introduction to the section on the theory of matter, in which they make a fresh attempt at solving the enigma created by Newton's continual vacillation between the points of view of action at a distance (through an immaterial agent) and transmission of force by contact through an appropriate medium. They have undeniably made a serious study of the problem; but their argument is vitiated by their rather surprising neglect of a circumstance which, in my opinion, is of decisive importance, namely, the influence exerted on Newton's thinking by Henry More (the famous "Cambridge Platonist" receives only casual mention). This influence, to which Prof. Fierz has directed attention in a brilliant essay (*Gesnerus*, 11, 62; 1954), is, I think, the real clue to an understanding of the deepest motivation of Newton's qualms and hesitations. The conflict which tormented him was one between the conception (suggested by More's ideas) of God's acting on matter "within his boundless uniform Sensorium" and his inability to derive from the phenomena any cogent picture of the mechanism of such action. The authors suggest that he rejected the æther as a possible medium for transmission of force because he saw that every such mechanical transmission implied an infinite regress (the interaction of the particles of æther requiring another medium for their transmission, and so on *ad infinitum*): this is a mere guess, however, and not a likely one. What really worried Newton is that the density of the æther, on the evidence of the planetary motions, must be so small as to make it difficult to understand *quantitatively* how it could transmit gravitation. There can be no question, on the other hand, of Newton, as the authors would have it, abandoning the æther conception in despair and committing himself to the idea of action at a distance directly dependent on God's intervention. In their eagerness to defend this untenable view, they give to some of Newton's utterances an interpretation which I do not think these can bear when considered against their proper background.

My main reason for being so critical of what is, after all, a welcome addition to our knowledge of Newton's thought, has been to caution unwary readers, all too prone (as experience reveals) to imagine that the commentary exempts them from reading the text, or at least from independently making an effort to recapture, in studying these old documents, the struggle of a powerful mind towards truth and harmony. Yet, just in this effort lies the fascination of history. L. ROSENFELD

ADVANCES IN NUCLEAR SCIENCE

Annual Review of Nuclear Science

Vol. 11. Edited by Emilio Segrè, in association with Gerhart Friedlander and Walter E. Meyerhof. Pp. vii + 513. (Palo Alto, Calif.: Annual Reviews, Inc., 1961.) 7 dollars.

THE eleventh annual volume in this series of reviews is still very much the mixture as before. The editors have been sufficiently catholic in their selection of topics so that at least half the articles are of value to any scientist who has anything to do with nuclei. In this volume the depth of different articles differs rather markedly, but since they are meant to be topical rather than definitive this is to be expected. There are three reviews of fundamental particle and high-energy physics problems. "Statistical Methods in High Energy Physics", by Kretzschmar, is essentially a review of a theoretical physics technique. "Strong Interactions and Reactions of Hyperons and Heavy Mesons", by Morpurgo, does not include the recent surprising work on pion–pion resonances, and hence many of the conclusions may now be upset. The review of "Nucleon–Nucleon Elastic Scattering Theories", by Moravcsik and Noyes, is an excellent account of a most confused and difficult field. Though this is one of the most fundamental problems in all nuclear physics, the rate of progress toward a complete solution has been somewhat disappointing.

"Nuclear Orientation" (Roberts and Dabbs), "Neutron Capture Gamma Rays" (Bartholemew), and "Neutron Diffraction" (Wilkinson, Wollan and Koehler) are three excellent reviews of special fields of nuclear physics where the advances have been interesting and significant. Neutron diffraction is nowadays primarily a technique in the investigation of the solid and liquid state.

One of the most interesting features of these annual reviews are the articles on border-line fields which link several specializations. Arnold's review of "Nuclear Effects of Cosmic Rays in Meteorites" is one of these, in which radioactivity analysis of extreme sensitivity can throw light on theories of cosmic rays and on the problem of the origin of meteorites. Another review of this type of great topical interest is "Detection of Nuclear Explosions", by Latter, Herbst and Watson. The major problem of the control of nuclear weapon tests has led to very interesting scientific investigations of the physical effects of such explosions on the environment. An article of much practical use to the nuclear physicist is Lindenbaum's "Shielding of High Energy Accelerators". The heavy-ion accelerators of the world are surveyed by Hubbard. "Isotope Effects in Chemical Reactions" by Weston is essentially physical chemistry of considerable importance to nuclear physicists. Libby's article on "Industrial Use of Isotopes" is rather an arbitrary selection of some ways in which radioactive isotopes are used.

This volume also includes an index of the reviews in this series of the past five years, and should be of considerable appeal to those interested in nuclear science. E. B. PAUL

A DICTIONARY OF NUCLEAR PHYSICS AND TECHNOLOGY

Dictionary of Nuclear Physics and Technology

English—German—French—Russian. By Ralf Sube. Pp. 1,606. (Oxford, London, New York and Paris: Pergamon Press: Berlin: VEB Verlag Technik, 1961.) 400*s*.

HERR SUBE'S dictionary provides English, German, French and Russian equivalents of some 15,000 terms. His net has been cast fairly widely, and one might quarrel with the inclusion of quite a number of such entries as *to act*, *crackling noise*, *optics* and *thick*. The book consists of four sections, one for each language, in which the entries in the first column are in alphabetical order of that language, followed by three columns giving corresponding expressions in the other languages. In addition, each term has a letter and number derived from its English alphabetical position; the compiler explains that the purpose of this code is for use with complementary volumes to be published in other languages, but it could, presumably, just as well have been used to achieve a considerable reduction in the size of the present volume.

Gender and number are given where appropriate. Terms not recommended for standard usage have a cross-reference to a more acceptable term. American and British forms are generally distinguished. As usually happens in polyglot dictionaries, every term has its counterpart in all the other languages, and the user is left wondering how many of these are documented and how many provided by the compiler, especially when the 'equivalent' is a lengthy descriptive paraphrase. Apart from this, the dictionary is remarkably complete, accurate and free from errors. It is inevitable that a few terms should have escaped attention, for example, *flute instability*, *megaton*, *Mössbauer effect* and *whole-body counter*; but they are a small minority. The compiler's English has occasionally led him into writing, for example, 'go critically' and including 'growth of grain' but not 'grain growth'; and in general the most complete explanations are given in the German column, so that the reader acquainted with German will be at an advantage even when translating between other languages.

The dictionary ends with a two-way index to the Cyrillic spelling of geographical names relevant to nuclear physics and technology. Herr Sube is to be congratulated on his enterprise and industry; one regrets only that the British publisher has seen fit to raise the price so high.

J. B. SYKES

CHINESE PHYSICS

Science and Civilisation in China

By Dr. Joseph Needham, with the collaboration of Dr. Wang Ling, and the special co-operation of Kenneth Girdwood Robinson. Vol. 4: Physics and Physical Technology. Part 1: Physics. Pp. xxxiv+434+plates 96–123. (Cambridge: At the University Press, 1962.) 84*s*. net.

THIS is the first part of the fourth volume of Needham's *History of Science*; it is concerned with physics. The main chapters treat of light, of sound and of magnetism. There are briefer sections on mass and mensuration, on dynamics, surface phenomena, heat, luminescence and electrostatics. It is a very comprehensive and well-illustrated survey.

Throughout the book the author is pre-occupied with the question why the Chinese did not develop scientific theories in spite of the many practical devices they invented. This failure is indeed as important for our understanding of science as the success in classical Greece. Dr. Needham suggests the lack of an alphabetic language as one reason. He remarks, too, that atomism is a crucial stage in the growth of physics which the Chinese never reached, while the Greeks very quickly arrived at it.

I think this is very true and, in fact, points to an explanation. After all, the growth of science is a natural phenomenon; it belongs to the mental, social and biological evolution of mankind. The development of a scientific idea is in some ways analogous to the process by which the individual human being, from infancy on, learns to cope with, and to know, reality. All our ideas arise from primitive phantasies that turn into myths and fables, and slowly become more refined, rational and even abstract. The history of mathematics, from Pythagorean number phantasies to formal axiomatics, is an obvious example. The progress of physics has certainly been in the direction of ever-increasing abstraction that was made possible by the use of the steadily improving, mathematical symbolism. The symbolism was capable of improvement because the Pythagoreans represented number as a geometrical pattern. This enabled them to split their symbols into content and form, to 'formalize' them, and to repress the primitive phantasies attached to them originally. Thus a set of, more or less. empty symbols is obtained which can be filled with new content, resulting from observation and experiment. Physics expanded through 'mathematization'; and science as a whole was enlarged, and made secure, through formal, logical reasoning which is the offspring of mathematics.

The Chinese ideograms are then clearly seen to be insufficient for the purposes of science; the symbols —though later turned into radicals—are too closely bound to their original, primitive meanings. They are schematized, but not sufficiently formalizable so as to allow the representation of experiences that are more complex and unusual—like the laboratory experiment—than those of everyday life. In physics, the Chinese started, as the Greeks did, with the concept of continuum. The *Chhi* and the Greek concept resemble each other, naturally; but the Greeks made their ideas more and more abstract. Thus they managed to overcome the horror they felt concerning the idea of change by seeing it as the motion of indestructible though invisible atoms in the void. This involved the idea of cause, *aitia*, which was abstracted from the primitive belief in the inevitability of the relation of crime and punishment; and so an orderly, lawful cosmos was created that could be the object of scientific inquiry. The path to the atom and to the later *pneuma* was not straightforward as can be seen from the Pre-Socratic and Stoic writings. The Chinese never took it because they remained too close to their original phantasies; they never separated as sharply as the Western thinkers phantasy from fact, internal from external reality. This comes out clearly in the present book. Their greatest success—the magnetic compass—always remained bound up with pseudo-science, with divination and geomancy even after it had been in use for the purposes of navigation.

Why did the Greeks succeed in subduing their phantasies and in becoming more rational while the Chinese failed ? One reason may be this. The Greeks, at the crucial time in their development, managed to throw off the divinity of kingship and similar beliefs—and to institute (more or less) democratic régimes. The Chinese did so only in 1912.

The book, like its predecessors, is carefully edited and beautifully produced. It will give much pleasure and stimulation to the historian and philosopher of science.

ERNEST H. HUTTEN

THEORY OF THE SOLID STATE

Wave Mechanics of Crystalline Solids

By Prof. R. A. Smith. Pp. xv+473. (London: Chapman and Hall, Ltd., 1961.) 84*s*. net.

THE increasing scope and importance of solid-state physics has been accompanied by a rapid increase in the number of books on the subject. Prof. Smith's book has been written with a particular aim which makes it a particularly valuable addition to the list. To quote the blurb: "The underlying theory both of the forces which bind solids into their crystalline form and of the motion of charge carriers through the solids is by no means simple, and is frequently beyond the experience and training of experimental research workers in this field. The number of such workers is now very great and this book is an attempt to make some of the basic theory of crystalline solids more readily available to experimental physicists and engineers. Its aim is to treat the fundamental theory of wave motion in solids, both that of the lattice and of the electrons, in as elementary a way as is possible".

In my opinion the book is exceptionally successful as a feat of lucid exposition of the elements of the subject—the standard theory of lattice vibrations and the motion of electrons in crystal lattices—which are covered in the early chapters of the book. The order and method of presentation have been carefully thought out, simple models are studied in patient detail which is not allowed to become wearisome, points of difficulty are never hastily passed over or quietly ignored, and (a particularly happy feature) the text contains a good number of exercises chosen so as to encourage the reader to make his own contribution towards the development of the subject.

The book's success is achieved at a price. Its scope is severely limited, and large but difficult branches of the theory, such as ferromagnetism, cohesion and many-body theory, are scarcely mentioned at all. Also—and this gives the book a slightly old-fashioned air—the discussion of transport and optical phenomena which occupies the later chapters of the book leans heavily on over-simplified models based on the concepts of relaxation times and effective masses; here the author's predominant interest in the properties of semiconductors has been allowed to determine the approach. One would have liked to have seen some account of the recent exciting successes in establishing the form of the Fermi surface in metals, and some appreciation of the consequent re-orientation of emphasis in the theory of metals towards general geometrical and topological features of the energy surfaces.

But one must not ask the impossible: and to survey the whole field of solid-state theory adequately in a single book is no longer possible. As a workmanlike introduction to the subject, which will provide beginners—budding theoretical physicists no less than the experimentalists whom Prof. Smith has primarily in mind—with a sound basis from which to advance to the more abstruse ramifications, the book is much needed and is highly welcome.

E. H. Sondheimer

DEVELOPMENTS IN PHYSICAL ELECTRONICS

Advances in Electronics and Electron Physics

Vol. 15. Edited by L. Marton, assisted by Claire Marton. Pp. x+412. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 93*s*.

PHYSICAL electronics has long been dominated by the generation or emission of charged particles, their motion through a vacuum, gases or semiconductors and their impact on a collector. Recently, however, much attention has been directed to quantum electronics, which exploits energy-state transitions in atoms or molecules, as a means of generating and amplifying high-frequency signals, and it is appropriate that three articles should now be devoted to the new subject in this series of reviews—one of the oldest devoted to the physical sciences.

The first article in the latest collection shows how a transition between hyperfine-structure levels in atomic cæsium provides a standard of time of much increased accuracy compared with that based on the orbital motion of the Earth around the Sun. The second describes maser developments, of which there have been many since Gordon, Zeiger and Townes demonstrated the ammonia beam oscillator eight years ago. Tuning, not possible in that oscillator, came with the use of paramagnetic crystals used at sufficiently low temperatures to permit useful inversions of populations between two energy-levels. The extension of the principle to visible and infra-red frequencies opens a field which seems certain to react on many branches of science. The article is recommended, particularly for its summary of the history of maser work. The third article, on relaxation in paramagnetic salts, though more for specialists, has direct ties with the second.

The steady evolution of techniques for handling millimeter waves is the basis of the next article; generation is only briefly described, more space being given to transmission, total reflexion and measurements. The distribution of electrons in the ionosphere—a fascinating but complex subject—is the subject of the fifth article, attention being given also to motion and recombination or charge transfer. Rockets and satellites offer a new means of investigation and are likely to add much information quickly to that obtained less directly in the past. The last article tells how the autodyne, an oscillating detector, assisted the discovery of paramagnetic resonance. This detector, like others using feedback and non-linearity, is simple to construct and is sensitive, but it is difficult to analyse and sometimes difficult to use with precision; the article attempts analysis but takes much for granted on the part of the reader.

The future of this valuable series may soon be in jeopardy unless some substantial contraction of coverage is made, for six articles annually cannot cover the continuously expanding subject.

J. R. Tillman

ANALYSIS OF SYMMETRY

Group Theory and Its Application to Physical Problems

By Morton Hamermesh. (Addison-Wesley Series in Physics.) Pp. xv+509. (Reading, Mass.: Addison-Wesley Publishing Company, Inc.; London: Pergamon Press, 1962.) 105*s*. net.

THE tradition that mathematics, especially the useful sort, is essentially 'the calculus' dies hard. This fact and the wealth of highly sophisticated algebraic methods used by theoretical physicists to-day make the gap between the undergraduate course and the research paper applying algebra alarmingly large. Certainly it can no longer be bridged by a single volume of reasonable size and any author in this field must decide whether to write a general introduction or to single out a class of special applications for detailed treatment. Dr. Hamermesh has chosen the former alternative and he writes as a physicist speaking to physicists. That is, he has in mind a reader who knows quantum mechanics but no group theory. He is particularly concerned with preceding the mathematical chapters by motivation and following them with physical illustrations. As for the mathematical core, this consists of an introduction to groups (discrete and continuous) and their representations, with a detailed treatment of the symmetric group (including many useful techniques of constructing characters and representations) and of the linear groups in n dimensions. Interspersed with these mathematical topics are accounts of the symmetry groups in space, the classification of spectral terms (selection rules), the splitting of atomic levels in crystalline fields, angular momentum analysis and other related applications.

The very pragmatic approach to the subject will obviously appeal to the student meeting it for the first time and he will be able to read further into this book than into a more abstract treatment. The theoretical part has been kept down to a minimum so as to allow as much space as possible for the discussion of particular examples. Valuable though these may be, their very profusion may bewilder the reader and make him wish for a more complete discussion of the general theory. This is true particularly of the treatment of Lie groups, where, after a brief general introduction, many points of technique are merely mentioned in the form of an example, sometimes with rather scanty explanation (this ellipsis leads to an incomplete classification of three-dimensional Lie algebras on p. 306 which may worry some readers). Of course, this is merely the author's way out of the dilemma of compressing the incompressible, and it is only fair to remark that he has succeeded in including a large number of techniques in easily digestible form. As such, the book should be of particular value to anyone wishing to acquire a working knowledge of group theory for atomic and nuclear structure. P. M. COHN

SEMICONDUCTORS

Semiconductor Statistics

By J. S. Blakemore. (International Series of Monographs on Semiconductors, Vol. 3.) Pp. xii + 381. (London and New York: Pergamon Press, 1962.) 84*s*. net.

Thermal Conduction in Semiconductors

By Dr. J. R. Drabble and Dr. H. J. Goldsmid. (International Series of Monographs on Semiconductors, Vol. 4.) Pp. ix + 235. (London and New York: Pergamon Press, 1961.) 60*s*. net.

Photo and Thermoelectric Effects in Semiconductors

By Jan Tauc. Translated by Margaret Laner. (International Series of Monographs on Semiconductors, Vol. 2.) Pp. ix + 248. (London and New York: Pergamon Press, 1962.) 60*s*. net.

THE book by J. S. Blakemore deals with the statistics of mobile charge carriers in semiconductors. It is divided into two parts, dealing with quasi-equilibrium and non-equilibrium statistics respectively.

Part 1 starts with a historical review of the theories explaining the behaviour of electrons in metals beginning with the Drude and Lorentz theories and leading to a description of the electronic band theory of solids. Chapter 2 discusses the effects of varying carrier concentrations on the Fermi-level including the effects of temperature and magnetic field. Chapter 3 deals with the case most often found in practice of the semiconductor the behaviour of which is controlled by impurity-levels. The case of semiconductors containing several localized levels is also dealt with. This part of the book can be expected to be of great value to the practical device designer, since the behaviour of devices is often governed by the presence of several localized impurity levels of the type discussed. There is a short section on irradiation damage in semiconductors which is a topic of increasing interest at the present time, particularly in relation to solar cells.

The second part of the book discusses semiconductors containing excess carriers. Although, as the author states, it is necessary to discuss quasi-equilibrium conditions first, the second part of the book is devoted to the study of effects which are of the greatest interest to most workers in the field now. In many cases of practical interest in the device field non-equilibrium conditions occur in which injected 'minority' carriers greatly outnumber normal majority carriers. The mechanism of carrier movement through the semiconductor, and the more simple processes by which the excess carriers recombine, are treated in detail in Chapters 5 and 7. Chapters 5 and 6, dealing with radiative and radiationless recombination and band-to-band recombination, are particularly useful. More complex recombination mechanisms involving decay through a set of impurity-levels which may be monovalent or multivalent are treated in Chapters 8 and 9. Chapter 10 is devoted to a discussion of the factors governing spatial distribution of excess carriers, including a brief discussion of the effects at junctions and contacts. In general, however, the book makes no attempt to discuss in detail the properties of junctions in relation to carrier distributions in the semiconductor. Although much of the material contained in the book has been treated elsewhere in as great detail, the integrated manner in which the information is presented here will undoubtedly be of great use to workers in both fundamental and applied semiconductor physics. The style of presentation is good, and although much of the text is necessarily mathematical in nature, the book remains easily readable.

The two authors of the second book under review (Dr. J. R. Drabble and Dr. H. J. Goldsmid) have spent many years in the field of semiconductor physics. They are especially well qualified to write on the subject of thermal conductivity in semiconductors since their own work has been largely in this field. Although at first sight the subject may appear to be a very highly specialized one, its implications are more far-reaching than the limited field of semiconductor physics. The authors state in their preface that the treatment is restricted to semiconductors, but nevertheless there is much basic material in the book which is of use in the more general study of thermal conduction in solid media.

The subject of thermal conduction in semiconductors, as well as being of interest in elucidating conduction mechanisms in solids in general, is of great topical interest in connexion with refrigeration and power generation from semiconductor thermocouples. The book, therefore, should find ready acceptance by a wide range of workers in solid-state physics.

It begins with a short introductory chapter on the development of theories of thermal conduction in dielectrics, metals, and insulators. This is followed by a very complete description of methods of measuring thermal conductivity in semiconductors, including dynamic as well as static experiments.

Chapter 3 is largely devoted to the general problems of energy transfer in solids. After dealing with the statistics of electrons in crystals and the physics of the crystalline lattice, the Boltzmann equation for electrons and for phonons is considered in detail in relation to energy transfer processes through solids.

The final chapters discuss the electronic part of the thermal conductivity in semiconductors and the lattice component of thermal conductivity.

The layout of the material in this book is excellent, and the treatment throughout is comprehensive, considering both theoretical and practical aspects of thermal conduction in solids. The subject-matter is clearly and concisely laid out and the text is easily read. A full list of references is included at the end of the book.

This work is to be recommended to all who have an interest in thermal conduction processes in solids.

The book by J. Tauc was originally published in Czechoslovakia in 1958. It has been revised and brought up to date before being translated into English by Mrs. M. Laner.

Its aim is to examine and explain the basic phenomena in semiconductors which lead to the generation of electromotive forces, and it is intended for physicists who are about to undertake research or development work on such phenomena.

In order to make it self-contained, the first two of the five chapters deal with basic semiconductor concepts and carrier transport effects respectively. These are presented in a manner which could make them difficult for the new graduate to understand. However, this need not be a serious disadvantage since the newcomer to the field now has a wide choice of sources of information on these topics from which to draw before proceeding to the subsequent chapters.

Chapter 3 deals with photovoltaic effects in semiconductors. The theoretical aspects of this subject are fairly completely covered; but the practical difficulties in the design of photovoltaic devices are not discussed in any detail. In this respect the chapter appears incomplete. References are given in the text which allow the reader to find the principles of device design from other sources. In this chapter are collected together descriptions of the less-well-known photovoltaic effects such as those observed at metal–semiconductor contacts, the lateral photoeffect, and photoeffect at surfaces. This provides some valuable material which is not easily found in collected form from any other source.

Chapter 4 is concerned with thermoelectric effects in semiconductors, and here a good deal of useful theoretical information is to be found. The same criticism can be made here, however, that the influence of the basic factors on device design has not been emphasized. The final chapter covers photoelectromagnetic and thermogalvanomagnetic effects in semiconductors.

In addition to the numerous references in the text, there is a comprehensive bibliography at the end. This work cannot be described as an easy book to read, and the style in places leaves something to be desired. This, of course, may be due to the undoubted difficulty in translating a work of such technical complexity. It will be found of use as a reference book for physicists working on photoelectric and thermoelectric phenomena; but in my own opinion it is unlikely to be regarded as a standard work in this field.

A. A. SHEPHERD

A HISTORY OF ELECTRICAL ENGINEERING

A History of Electrical Engineering
By Dr. Percy Dunsheath. (Technology Today and Tomorrow.) Pp. 368+48 plates. (London: Faber and Faber, Ltd., 1962.) 50*s*. net.

THE *History of Electrical Engineering* is one of a series entitled *Technology Today and Tomorrow*, the intention of which one may suppose is to reveal the relations of technology to the fundamental sciences on one hand and to social needs and trends on the other. Doubtless the expansion of technological education up to the highest level has provided the main stimulus to its inception. Since, as Dr. Dunsheath reminds us in his preface, "history is essentially an account of the work of men", the historical approach is one worth using to the full, though not, as the title of one of the series recognizes, as an exclusive means to the end envisaged. The writing of such a history presents formidable difficulties: it must set an example of meticulous scholarship in regard to the marshalling of the evidence and the demonstration of mutual influences between technological trends, social drives and the preoccupations of scientists; and it must resist the temptation to dramatize the narrative in the absence of supporting evidence. But it must be 'contemporary history' in the sense that, while not obsessed with finding 'anticipations' of all the clever things we do to-day, it will be seen as 'relevant' by a young man or woman whose primary aim is rightly to 'enlarge the bounds of human empire'. For the achievement of such a task it is doubtful whether anyone could have been found better fitted in respect of knowledge and experience than Dr. Dunsheath. For myself the reading of this book has been a liberal education in a field of which I can for the most part claim only a peripheral knowledge. Nevertheless, since, as the publishers claim, this book is the "first of its kind", it may be proper to point out a few aspects in which it is misleading.

Though the fundamental work in electrodynamics is related with rather more than usual insight, the part played by the pioneers of electricity as a science *uberhaupt* is not set where it rightly belongs—in the Royal Society, where from the remarkably ingenious experiments on discharge at low pressures by the elder Hauksbee (who is not mentioned) the Society acted as a clearing house for almost every significant advance. Moreover, this includes the beautiful demonstration of the conservation of charge by Franklin, who, rather than Watson, introduced 'plus' and 'minus' as viable terms together with several others. It may be doubted also whether the pressure brought by George III against Franklin's point-conductors was wholly political; the scientists (notably Benjamin Wilson) disagreed, not altogether without reason, for disaster could and did follow the installation of pointed conductors inadequately earthed. Passing to the nineteenth century we are surprised that Joseph Henry is referred to only in connexion with the improvement of electro-magnets; for he quite probably recognized the appearance of induced currents before Faraday, and quite certainly was the first to understand the nature of self-induction, as is recognized in the name of the international unit of inductance. Incidentally, Dr. Dunsheath might have made a little more of the origin of such terms—Ohm, himself, does not occur in the index; and a little help to the general reader in connexion with inductance, reluctance, oersteds, gauss and the like would have been welcome. Though I myself when an undergraduate would have been scandalized at the suggestion that the Daniell cell "instead of releasing the objectionable free hydrogen gas *caused it to combine at the separator with the copper sulphate*" (p. 45, italics not in text), I welcome the prominence given to the high importance of the demands of electrochemistry in the search for more powerful means of current generation: it is significant that it was to 'sell' his brother's patent to Elkingtons, the pioneers of electroplating in Birmingham, that the nineteen-year-old Wilhelm Siemens came to England to start his astonishing career. Here, as elsewhere, Dr. Dunsheath has overcome with great skill the difficulty of showing the men (and what giants many of them were) as part of the movement, without obscuring the lines of development of the latter.

Dr. Dunsheath has wisely concentrated on the heroic age of the second half of the nineteenth century when lighting, traction, telephones and oceanic cables became established, and the attendant problems of generation and insulation were in the main solved. Of 'electron engineering' he tells us less (and nothing of J. L. Baird) owing to the great acceleration and specialization of recent years. But enough is said to point out the moral that the development of electrical theory in respect of thermionic emission and semiconductors was almost wholly the 'result' of technological novelty and not the reverse. The book concludes with chapters on "Professional Organization" and "Social and Historical Background"; a valuable "Chronological Table" and a less-valuable index, from which many useful references to the text are omitted. Documentation and illustration are well chosen.

There are a few typographical slips, of which the repetition of a line of type on p. 93 is the only serious one. "Baronetcy" on p. 215 should be 'Barony'; and readers must be warned that Dr. Dunsheath's reference to Maxwell's famous tribute to Ampère (*Treatise*, 2, 164) is *not* a quotation. W. P. D. WIGHTMAN

PHYSICS FOR STUDENTS OF ELECTRICAL ENGINEERING

Physics for Electrical Engineers

By Prof. W. P. Jolly. (Physical Science Texts.) Pp. xii + 308. (London: English Universities Press, Ltd., 1961.) 21*s*. net.

THIS is a very readable little book, published at a modest price, and claiming to cover all the physics required by electrical engineers for the examinations of the Institution of Electrical Engineers and the London B.Sc., and to be of value to students preparing for the graduate examination of the Institute of Physics and for the Higher National Certificate in physics and applied physics.

Students are always grateful for any text-book which gives them exactly the material they need for their examination and nothing more. This book can, therefore, be sure of an appreciative welcome from the harassed student although it may be questioned whether a book which attempts to satisfy the needs of students for so many different examinations can exactly meet the need of any one of them.

The author divides his work into four sections: on the structure of matter, its electrical properties, radiation of energy and thermodynamics. This is a wide canvas and each succeeding section appears to be more condensed. Thermodynamics is so often taught as a separate engineering subject that it might well have been omitted from this book in order to allow a corresponding expansion of the other three sections. This criticism also applies to some extent to the treatment of electromagnetic waves and of Maxwell's laws, which is very heavily compressed and might better have been omitted, since the subject is usually adequately taught as electrical engineering rather than physics.

Otherwise, the author succeeds in maintaining the interest very well, although forced at times to a somewhat dogmatic treatment in order to avoid introducing mathematics which would be of a too advanced character for his readers.

At the end of each chapter the author gives a number of problems with solutions on the same page—surely an undesirable practice. The problems are mainly taken from examination papers and are generally disappointing. The numerical questions are usually trifling and do not ensure an adequate understanding of the preceding chapter. The theoretical questions add nothing to the text. The book could be greatly improved by carefully thought out problems designed to clarify and expand the author's treatment of the subject. Examination questions have a different purpose.

The publishers have laid out the text in an attractive fashion, but the number of misprints is rather greater than is usual in a scientific work. Fortunately, few of them are of such a nature as to be likely to mislead the student.

These minor criticisms should not obscure the real merit of the book. It is likely to be useful to graduates as well as to those who still have their examinations ahead of them. A. H. M. ARNOLD

POWER SYSTEM ENGINEERING

Power System Engineering

An Introduction. By F. de la C. Chard. Pp. xii + 288. (London: Cleaver-Hume Press, Ltd., 1962.) 50*s*.

THE rate of increase of the volume of literature dealing with the generation, transmission and distribution of electrical energy remains high. The steady growth of demand for electricity has required increased capacity of generating stations, transmission over longer distances and the interconnexion of larger networks, and has produced new problems and accentuated old ones. To meet these demands new techniques have been devised, and more powerful methods of design and analysis have been developed. These advances are represented in the literature by substantial groups of specialized texts.

Prof. Chard's book lies outside these specialist categories, being a general text of an introductory character. The book aims at giving a general picture of power system performance built up from consideration of the characteristics of the basic components of the system but dealing only, and in as simple a way as possible, with phenomena which are of fundamental importance. The major topics are treated in five chapters.

Chapter 3, entitled "Synchronous Machines, Performance and Control", deals with the basic characteristics of synchronous machines and their behaviour under steady-state and under transient conditions. The next chapter—the transmission line—after a brief treatment of the parameters of three-phase lines and of the equivalent single-phase system, develops the line equations for steady-state transmission and considers the phenomena of reflexion. Thereafter the technique of the power-voltage diagram is developed. Transformers, switch gear and protective gear, constitute the subject of Chapter 5, while Chapters 6 and 7 deal with the important topics of short circuits and faults together with steady-state and transient system stability. The first two chapters of the book give the underlying circuit theory and set the pattern of treatment. Matrix methods are introduced almost immediately and are utilized throughout the book. The final chapter examines the more important features of extra-high-tension transmission and includes a short section on high-voltage direct-current transmission.

In a book having so wide a coverage there is a serious risk that the treatment of some topics may be so sketchy or superficial as to be almost valueless. The risk can be minimized by very careful selection of material, meticulous attention to its presentation and care to direct the reader's attention, wherever necessary, to its limitations. Prof. Chard has satisfied these criteria to a remarkable degree. The presentation is clear and precise at those levels of difficulty at which this is practicable; but in those instances in which the logic of the step cannot be demonstrated this is stated. References at the end of each chapter direct the reader to more detailed and advanced treatments of the various branches of the subject. The standard of mathematics utilized comes well within the compass of an undergraduate course in electrical engineering.

This book should be of real service to undergraduate electrical engineers, not on account of its presenting topics in a particularly novel way, nor because it offers material not otherwise readily available, but because it selects and treats in one volume the range of phenomena which are of the greatest significance in the behaviour of a power system.

In a foreword written by Mr. J. L. Egginton, chief transmission engineer of the Central Electricity Generating Board, it is pointed out that a comprehensive introduction to power system problems, such as this, has a real value also to practising engineers who are not specialists but who require, for purpose of reference, a sound, general treatment.

The book is well printed and produced, the clarity of the diagrams and the mathematical portions of the text being particularly good. JAMES GREIG

GYROSCOPIC PHENOMENA

Gyrodynamics and its Engineering Applications

By Prof. Ronald N. Arnold and Prof. Leonard Maunder. Pp. x + 484. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 100s. net.

FOR many years gyroscopes have been used in stabilizers and compasses for ships and aircraft and, although some interesting alternatives are being investigated, there appears to be no prospect of gyroscopes being displaced by other devices during the next decade. In recent years there have been spectacular improvements in constructional techniques which have led to the practical attainment of systems of great precision; inertial navigation, which is dependent on gyroscopes having drift-rates of a few minutes of arc per hour is now in use in ships, aircraft, missiles and land-vehicles.

Elementary theory which can be used to explain simple gyroscopic phenomena is insufficient for an adequate understanding of the problems that arise with these more precise systems, and this book will be of great value to those who wish to study the subject fully. The treatment is mathematical, and the authors expound the view that the majority of readers will profit more from this approach than from physical reasoning alone. There is a great deal of truth in this; however, the book does not neglect practical applications or the physical interpretation of the analytical results. The reader is required to be able to handle linear differential equations.

The book is based on lectures given in the Post Graduate School of Applied Dynamics in the University of Edinburgh, and in the early chapters there is an air of a class 'brushing up its dynamics'. It is somewhat surprising to find in a book which will, in general, be read by graduate engineers an exposition of Newton's laws of motion or an introduction to vectors, but it is by no means merely a collection of lecture notes. The first six chapters deal with classical dynamics and much of this matter will be familiar to the reader. Nevertheless they are easy to read, and may be found useful for reference.

The remaining ten chapters, which form the bulk of the book, are concerned with gyroscopic phenomena and systems utilizing gyroscopes. Much of this is new or has not been collectively published and, in addition to being of considerable value to the engineer concerned with design and development of these systems, it will be of general interest to a wider circle of readers. The Coriolis acceleration and its effects are treated clearly and fully and there is a comprehensive analysis of the behaviour of several gyroscopic configurations. The ship's gyrocompass is discussed in some detail (nearly 40 pages are devoted to it). and as a natural follow-on from gyroscopic vibration absorbers the early Schlick and Sperry ship's gyrostabilizers are briefly analysed and compared. These devices are, of course, of no practical interest to-day, and it must be assumed that the authors included them solely because of their analytical interest. There is a brief (rather too brief) chapter on gyroscopic effects in aircraft, but no reference to autostabilizers or autopilots (in which the gyroscope plays an important part).

Inertial navigation is treated at considerable length. There is intensive activity within many countries in this subject for military application and the outcome of much of the work is not freely available, but the basic principles and problems are clearly set down in this book, and the reader who has mastered it will be well equipped for further work in this field.

The book's value to the student is enhanced by the inclusion of a set of problems relating to each chapter. W. O. BROUGHTON

DERIVATION OF ASYMPTOTIC EXPANSIONS

Asymptotic Approximations

By Prof. Harold Jeffreys. Pp. 144. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 30s. net.

THIS is an introductory tract on asymptotic approximations in which special emphasis is placed on uniformity of approximation over regions in the complex plane. A brief chapter covering Poincaré's definition, multiplication, division, integration and differentiation of asymptotic series is followed by the central core of the work, a closely related group of four chapters dealing successively with the derivation of asymptotic expansions for a function expressed as a definite integral (mainly by steepest descents), the derivation of asymptotic expansions of Stokes and Green types for a function satisfying a given linear differential equation of the second order, application to Bessel functions, and applications to confluent hypergeometric and parabolic cylinder functions. The remainder of the text consists of short chapters on Mathieu functions, estimation of the remainder in an asymptotic expansion, and wave problems in three dimensions.

The treatment of the material forming the central core of this tract is masterly. The writing is clear and concise, and rendered particularly stimulating by the introduction of recent work—only sketched, but in context for the first time in a systematic survey. Coverage is also good, especially in the approach from differential equations. The most material omission is that of the part played by Lagrange's theorem in developing full asymptotic expansions of functions expressed as integrals.

The chapters on Mathieu functions and on wave problems in three dimensions are illuminating, though possibly too highly condensed for all the individual topics to be clearly grasped.

It must be made clear that this tract deals almost exclusively with techniques for deriving asymptotic expansions, particularly the first few terms, and scarcely at all with such problems as what partially illicit processes cause series of this divergent type to appear, the structure of the later terms, whether such series are inherently approximate or exact but formalistic, and instability in form when all terms are of the same sign and phase. Consequently, the short sections on topics partly linked with such aspects—for example, the discussions on the definition of an asymptotic approximation, on the Stokes phenomenon, and on the estimation of the remainder—do not quite match up to the high standard of elucidation otherwise maintained throughout the text.

R. B. DINGLE

TRANSIENTS

Introduction to Transients

By D. K. McCleery. Pp. xi+232. (London: Chapman and Hall, Ltd., 1961.) 42*s*. net.

THE author of this book believes that Heaviside's operational methods provide a better introduction to transients for students of limited mathematical attainments than the more rigorous approach provided by the Laplace transform. It is quite clear that this subject is very near his heart, and his twenty-years experience as a teacher makes him well qualified to produce a book which is sure to have a great appeal to students starting on this difficult subject. It is not necessary to agree entirely with his method to appreciate the merits of this book. He expounds his subject clearly, and his numerical problems are well chosen to develop the student's understanding of what he has read. The Laplace transform is banished to the last chapter. His introductory chapter is a masterpiece of the art of making a difficult subject look easy, in order to engage the interest of the reader, before introducing him to the great advances made by Heaviside.

Rather unexpectedly in an elementary work, the author expounds to his readers the deficiencies of Fourier's method of analysis when applied to periodic waves with a discontinuity, such as a square wave. It is all too seldom in modern engineering books that Gibbs's spike is discussed. A perfunctory note may be given about the mathematical limitations of Fourier's analysis; but the student who takes the trouble to plot a few terms of Fourier's series for a square wave is likely to be surprised at the very poor resulting approximation. If his curiosity leads him to evaluate a few more terms he is likely to be still more surprised at the apparent lack of improvement.

Now in mathematical works it is proved that, notwithstanding these limitations, a Fourier approximation for a given number of terms provides a better approximation to a square wave than any other series of sine waves—yet it may very easily be verified that an infinite number of different sine waves series may be developed which will approximate as closely as is desired to a square wave and show not a vestige of Gibbs's spike. It is only necessary to develop a Fourier series for any periodic wave with no discontinuities which approximates closely to a square wave.

This appears to suggest that the mathematical criterion for a good approximation is unsatisfactory since it is unable to detect any error due to Gibbs's spike, although this spike is quite unacceptable in practice. It would be very interesting if the author could develop a new criterion for judging the closeness of approximation of a sine wave series to a square wave which would make it possible to decide on the best series which was free of Gibbs's spike. This might be a powerful weapon for him in his war against mathematicians.

One possible criterion is that the distance of that point on the approximate curve which is farthest from the nearest point on the square wave should be reduced to a minimum.

A. H. M. ARNOLD

VIEWS OF A GEOLOGIST ON THE ORIGIN OF LIFE

The Geological Aspects of the Origin of Life on Earth

By Prof. M. G. Rutten. (Elsevier Monographs: Geo-Sciences Section. Subseries: Geology.) Pp. vii+146. (Amsterdam and New York: Elsevier Publishing Company, 1962.) 25*s*.

THIS is a small but very important book. Theories of the origin of life have been much debated in recent years, but almost exclusively by physicists and biochemists with a sprinkling of astronomers. Here is a work by an experienced geologist who has martialled the evidence which can actually be found on the Earth and in its rocks. He has taken the evidence and used it to examine the theories on the origin of life which have been put forward on other grounds.

The major thesis centres around the evidence for an early atmosphere not containing oxygen. Hitherto, this has been postulated in order to account for the first formation of simple organic compounds, by means of ultra-violet light from the Sun unstopped by the present-day ozone layer, itself derived from molecular oxygen. What evidence there was for this reducing atmosphere was derived from the spectroscopically proved presence of the hydrogenated compounds such as ammonia and methane on the larger planets, and from the difficulty of finding any concentration of oxygen on the minor planets. It is also *a priori* probable on account of the high reactivity of molecular oxygen; but this is a weak argument.

The other type of argument is the chemical experimental argument which shows that, from a mixture of these gases with water vapour, it is possible to synthesize, by the use of various radiations, elementary biogenic compounds of the type of amino-acids and purines. Is there more evidence of a more definite character for this early period? Prof. Rutten thinks there is and expresses it on two lines of evidence, both of them geochemical: one, the presence of easily oxidizable pyrites (FeS_2), in sands of a sufficiently early date, of the order of two thousand million years, and the other the presence of red beds, which he considers indicative of an oxygen atmosphere, dating from a very early date, but not as early as the first. It would put the origin of the present atmosphere somewhere between one and two thousand million years.

Prof. Rutten finds these earliest forms of life where they would be expected to occur, on geological grounds, in the old shields of Fennoscandia, Africa and Canada. The earliest occur in the so-called Bulawayo–Dolomite formations which contain interestingly banded structures attributed to fossil algae but without showing any actual organic features beyond fine and wavy bands. It is, as he says: "The fine laminar structure of these deposits which is so typically biochemic. This not only strongly resembles the structure of the lime deposits formed by later algae and other organisms, it is moreover quite distinct from any structure which may develop in limestone through inorganic processes".

The age of these limestones is given by radioactive dating as 2·7 billion years, preceding, for reasons to be mentioned later, the appearance of life in atmospheric oxygen. What Prof. Rutten refers to as the oldest real fossils were those described by Tyler and Barghoorn in the gunflint formation of the Canadian old shield in southern Ontario. Here the evidence of structure is very much stronger and is well illustrated in photographs some of which have not previously been published. They would appear to be of the nature of algae or fungi, a long series of cells bearing occasional spores preserved as silica.

It is notable that some of the photographs show objects very much resembling those recently found by Claus[1] in meteorites. This resemblance would either serve to justify the meteorite-life hypothesis or it would validate the organic character of the gunflint deposits according to taste. Their date, moreover, is now becoming better known and is now estimated to be one thousand six hundred million years.

Rutten thinks these organisms must have grown under present-day oxygenated conditions. The same is true of another biogenic deposit of the Pre-cambrian age of the Hoggar in North Africa for the so-called reefs of *conophyton*. Here we have another form of lime-depositing alga; but it is continuous with forms as late as those of the Ordovician, and clearly belongs to modern forms of life. They are of the Pharusian period but the date is uncertain, between one and two thousand million years. Rutter concludes: "So, although the remains of early life found up to now are scanty, they are very revealing. . . . [they] have taught us a great deal about early life on earth. We have advanced very much in our knowledge during the last two decades".

The other type of geological evidence cited by Prof. Rutten is of quite a different character, being purely mineralogical. It is indication of the change of the type of weathering which occurs in the very earliest formations of the Pre-cambrian. We are all very familiar with present forms of weathering which, starting with an igneous rock containing, say, quartz, felspars and iron-containing minerals like mica, breaks them down into soluble materials which later form clays and leaves insoluble quartz behind in the form of sand. Sand from all modern and even later Pre-cambrian deposits always contained an enormous preponderance of silica and very little, for example, of felspars. It happens in various parts of the old shields, Finland, Canada, South Africa, that sands of an entirely different kind are found, often in reef rock containing gold or uranium. In these sands, the principal minerals are rarely silica, and more often pyrites or pitchblende, uranium oxide. These occur, however, as Prof. Rutten points out, in deposits of a typical water-borne character with rounded grains and a careful sorting of the grains as in a placer deposit. Now, sulphide ores would certainly be oxidized to magnetite or hæmatite under present conditions and the existence of pyrites is evidence that they were not exposed to the oxygen. Nevertheless, they were deposited by streams and therefore not very far from the contemporary atmosphere. The rounding is evident even in those grains that have shown a subsequent crystal growth, the fresh, outer crystal being marked by a clear boundary from the inner rounded one. Similarly, the uranium compounds occur in the less oxidized forms than those which are characteristic of pitchblende. Collaborative evidence is found in the study of the Finnish granite weathering, which shows an actual reduction in the ferric–oxide content, quite the contrary from what is found in modern granite weathering. Even more interesting is the account given by Ramdohr of the attack on these pyrites grains of what appears to be some form of micro-organism, a pyrites bacterium. Their age is given as a minimum of one thousand eight hundred million years.

Further evidence is given by the existence of iron ores whether in a reduced form, as in some of the earlier Pre-cambrian beds in Lake Superior region, or in the much more common later red-beds, which occur in almost all parts of the older shields, including the Torridonian sandstones of Scotland and the Jotnian sandstones of Feno-Scandinavia. The tentative age for the youngest of these red-beds is one thousand million years. It is on this evidence that Prof. Rutten places the transition to the present atmosphere as between one and two thousand million years.

Rutten agrees that the external conditions of the globe, apart from the chemical composition of the atmosphere, cannot have varied much over the whole length of geological time. They were always less than the differences that occurred at any time between the poles and the equator.

It will be seen that, on the whole, Prof. Rutten's evidence supports the now classical picture of biogenesis on Earth, that of Oparin and Haldane with its stage of oxygen-free and oxygen-containing atmospheres. However, he maintains a fairly sceptical attitude towards the biochemical evidence itself. He says: "The facts gleaned from geology make it probable that the environment postulated by that theory had indeed been present. This is not, of course, proof that biogenesis occurred. It only proves the feasibility of the theory".

Though sceptical of the extent of our present knowledge, the author is optimistic about the possibility of investigations revealing far more than we know at present about the origins of life. He hopes: "that further research will bring to us more of these valuable discoveries of early fossils, be they from the primeval pre-actualistic period or from the dawn of our present life. . . . Moreover, we may legitimately hope that more intensive mapping of the Old Shields will yield better correlations between the series of rocks by which they are built up. With the availability of more absolute datings it will then be possible to assign more reliable absolute ages to these fossil finds, both old and new".

His last words are that: "The findings of geology are in complete agreement with modern biological views on an origin of life on earth through natural causes. Although, of course, such an agreement offers no proof that the newer biological theories are correct, at least it offers us a most pleasant ending to our studies into one of the most intriguing problems of present-day science".

This pithy book gives us a welcome fresh light on one of the most exciting problems of the day, and account will certainly have to be taken of its findings in any future discussions of the origin of life.

J. D. BERNAL

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[1] Nagy, B., Meinschein, W. G., and Hennessy, D. J., *Ann. N.Y. Acad. Sci.*, 93, 25 (1961).

WERNER'S MINERALOGY

On the External Characters of Minerals

By A. G. Werner. Translated by Albert V. Carozzi. Pp. xxix + 118. (Urbana: University of Illinois Press, 1962.) 4.50 dollars.

ABRAHAM GOTTLOB WERNER was an outstanding figure in the geological world during the last quarter of the eighteenth century and the early years of the nineteenth century, the period that saw the rise of modern geology and mineralogy. From 1775 until his death in 1817 he taught mineralogy and mining geology at the School of Mines at Freiberg, in Saxony. Here his inspiring lectures and attractive personality built up a school of mining and geology that drew students from all over Europe. His fame rests not so much on his contributions to mineralogy and geology, which, for the most part, have not stood the test of time, as on the interest and enthusiasm that he aroused in his students, at a time when these subjects were beginning to emerge as separate and definite sciences. Many who attended his lectures afterwards made contributions to geological and mineralogical science of more permanent value than the doctrines taught at Freiberg.

Shortly before Werner commenced lecturing at the School of Mines he had published his *Von der äusserlichen kennzeichen der fossilien* (Leipzig, 1774). *On the External Characters of Minerals* is a new translation of this book which is, essentially, a determinative mineralogy. In it the author advocates the recognition of mineral species by careful and systematic examination of all their external characteristics. Werner realized that any mineral system must ultimately be based on chemical composition; but he was writing for practical mining engineers and miners to whom chemical analyses were not readily available, and at a time when the instrumental aids now taken for granted had not yet come into use. In its time, therefore, the book was one of great importance, as a serious attempt to bring some sort of order into a subject in which, previously, much confusion had existed.

Apart from the fact that earlier editions of the book are not easily obtainable, this new translation is of particular interest because it has been made from Werner's personal, annotated copy of the original edition. This was recently acquired by the library of the University of Illinois, where the translator, A. V. Carozzi, is professor of geology. Prof. Carozzi has established that the extensive annotations, alterations and additions are in Werner's handwriting; and he suggests that they were made with the intention of publishing a second edition which, in fact, never appeared. Internal evidence indicates the alterations were made over a considerable period of years. The actual translation presented here incorporates these alterations and is, therefore, virtually a second, revised edition of the original work. From a historical point of view this seems regrettable, because all the several earlier translations into English or French incorporated modifications based on notes made by Werner's students, and we are still without a translation of the original text. Prof. Carozzi states that his intention was to publish such a translation, but that editorial and printing difficulties that arose from the attempt to include Werner's alterations in footnotes made it impracticable. He has, however, annotated the text, and added an interesting biographical and bibliographical introduction. The book is a valuable addition to the series of historically important scientific texts and translations published by the University of Illinois in recent years.

V. A. EYLES

WORLD MORPHOLOGY

The Morphology of the Earth

A Study and Synthesis of World Scenery. By Prof. Lester C. King. Pp. xii + 699 + 14 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1962.) 84*s*. net.

THIS important and attractive book is characterized by the author as an inquiry to find order, system and, if possible, explanation amid the major topographical features of the Earth. It is a synthesis comparable, though not identical in scope, with Suess's *The Face of the Earth* or Umbgrove's *The Pulse of the Earth*. It is not chiefly or even largely concerned with land sculpture, in the ordinary sense. There are many side references throughout to geophysical and petrological topics, though one regrets the absence of reference to Jeffrey's great work, *The Earth*. The book begins with a brief summary of the structure of the crust—the evidence of seismology, isostasy, radiothermal energy, etc., and it comes quickly to an affirmative position in respect to continental drift and sub-crustal convection. In an interesting and significant section on "Some Dead Wood", the author dismisses the classic theory of a cooling and shrinking Earth and the supposed "crustal shortening". He also dismisses permanence of continental and ocean basins and regards the present concept of sialic roots to mountain ranges as over-simplified.

He then in succession examines the unity of Gondwanaland and its fragmentation, continental structure (shields, mobile welts, etc.). In the second part of the book there is a clear summary of Prof. King's own distinctive and valuable views on pediplanation, slope formation and the "ages of land surfaces". There follows a long chapter on the geomorphology of Africa, with shorter accounts of the rest of Gondwanaland, North America, Europe and Asia.

The Palæozoic, Neozoic and modern mountain ranges and the ocean basins are then reviewed.

In a last summary chapter, "Towards a Model of the Earth", emphasis is laid on the role of occluded volatiles in the mechanism of earth-movement. The book is clearly written, tautly organized and well illustrated. It is not necessary to agree with all the author's speculations and conclusions to find it stimulating and indeed exciting. There can be no doubt that it attains the status of an indispensable major work.

S. W. WOOLDRIDGE

A NEW HISTORY OF CHEMISTRY

A History of Chemistry

Vol. 3. By Prof. J. R. Partington. Pp. xxiii+854 (71 illustrations). (London: Macmillan and Co., Ltd., 1962.) 126*s*.

THE scope and general character of Prof. Partington's massive four-volume work have been indicated in a review of Volume 2 (*Nature*, 194, 222; 1962). That volume, the first to appear, dealt mainly with the period 1500–1700; it is now followed with surprising promptitude by Volume 3, which is chiefly concerned with the development of chemistry during the crucial eighteenth century. The first three chapters show how the flowering of chemistry in France during that century had its bourgeoning in the preceding century through the labours of a series of laborants, teachers and writers, many of whom had a leading interest in the application of chemistry to medicine: among them may be named Beguin, Davisson, C. Bourdelin, Blaise de Vigenère, Le Févre, Glaser, Thibaut and N. Lemery. A mere glance at the later galaxy of chemists who furthered the progress of chemistry in France in the course of the eighteenth century enables one to achieve some understanding of the distant roots of that provocative opening of Wurtz's *Histoire des Doctrines Chimiques* (1866): "La chimie est une science française. Elle fut constituée par Lavoisier, d'immortelle mémoire". Lavoisier's crowning achievements are reached of course much later in this volume, after discussions of the antecedent investigations of Black, Scheele, Priestley, Cavendish and others. In the valuable chapter on Lavoisier, account has been taken of recent researches; but here, as elsewhere, Prof. Partington bases his treatment on original sources; moreover, his critical attitude of mind is evident in the estimate he gives of Lavoisier's work viewed in its relationship to contemporary ideas.

The important contributions of Scandinavian chemists are considered in two chapters with the headings "Borrichius to Bergman" and "Scheele". Of Scheele the author truly remarks: "Every chemist who has attempted research will look over the record of Scheele's discoveries outlined above with astonishment and admiration. Astonishment at the great volume of discoveries which he made in his short life in such disadvantageous circumstances; admiration of the way in which he carried out his work and the fundamental importance of it all". An account of eighteenth-century chemistry in Germany contains also a short section dealing with some Dutch chemists of this period; the erudite Boerhaave, however, so prominent a figure among the chemists of the early eighteenth century, finds only numerous scattered references in Volume 3, supplementing the specific notice in Volume 2.

As already indicated, Lavoisier's epoch-making conclusions were based largely on the research work of Black (Scotland), Scheele (Sweden) and Priestley and Cavendish (England). Not the least valuable feature of Volume 3 is the discerning analysis, very fully documented, of the experimentation and writings of these pioneers and of their chief contemporaries: this is a wide and complex field of which Prof. Partington has an unsurpassed knowledge, and his conclusions will be helpful to all students of immediate pre-Daltonian chemistry.

Before taking up the theme of Dalton and the atomic theory, Prof. Partington smooths the path into the nineteenth century by discussing the later aspects of the chameleonic theory of phlogiston, the foundations of stoichiometry, and the position of chemistry at this time in Great Britain and Ireland. Here, a welcome feature lies in an authoritative contribution to the perennial Higgins–Dalton controversy. This, we may perhaps hope, has reached a final judgment in a recent detailed review of the evidence which has been undertaken by Prof. Partington in collaboration with Prof. T. S. Wheeler of Dublin. To quote from that judgment: William Higgins's claims to have anticipated Dalton, "put forward seven years after the publication of Dalton's theory, really originated in statements by Davy in 1811 . . . Dalton was led to his atomic theory quite independently of Higgins . . . Higgins did not attach any importance to the atomic *weights*, which are a cardinal feature of Dalton's theory, nor did he propose any methods by which they could be determined".

From what has been said, it will be clear that in this volume Prof. Partington has produced a masterly exposition of the development of chemistry during the eighteenth century. His distinctive approach, blending the topographical and biographical aspects, emphasizes two leading features of historical science which are often overlooked: these are the international character and the humanistic appeal of this great discipline.

JOHN READ

LAVOISIER ON AIR

Lavoisier—The Crucial Year

The Background and Origin of His First Experiments on Combustion in 1772. By Henry Guerlac. Pp. xix+240+11 plates. (Ithaca, New York: Cornell University Press; London: Oxford University Press, 1961.) 36*s*. net.

IN three important memoranda Lavoisier recorded the experiences which convinced him only in 1772 that reagent air was involved in combustion and calcination: *le pli cacheté* of November 2, a partial preliminary draft of October 10 and a later form which the great French chemist modified for publication. From a reconsideration of these with all related events and writings, Prof. Guerlac advances two chief conclusions. First, that Lavoisier, certainly influenced by Hales and perhaps also by Priestley's *Directions* (1772) on the preparation of soda-water, was quite unaffected by Dr. Black or any other pneumatic chemist across the Channel; secondly, that the youthful Parisian genius was led to postulate a chemical role for air by contemplation of calcination rather than combustion.

The author of such confessedly 'intricate argument' inevitably conflicts with other experts such as Meldrum and Metzger (who share his dedication), Fourcroy, Speter and McKie; indeed, at one point (p. 102), he must even "set aside . . . testimony from Lavoisier's own pen". On innocence of Dr. Black he may perhaps protest too much; thus at one place (p. 23) it is doubtful if Lavoisier "had ever heard of him" in 1772, while at another (p. 93) the Frenchman in August is hustling out a priority note against the great Scot's views on latent heat.

On these and other issues the evidence on both sides is fully and fairly set forth. There are some most interesting re-appraisals of de Morveau, Turgot, Sage, Mitouard and other contemporary philosophers. Print, illustration and index are first class; the appendix reprints leading texts from the actual manuscripts.

The value of such an exegesis remains historical rather than scientific. *Les plis cachetés* and other furtive, variable memoranda should never determine precedence in scientific discovery against another's public avowal of his views and his willingness openly to abide the question. Those who plot priority gain little. "We own they're prudent, but who owns they're good?" It is sad to be so often reminded by this erudite and educative discussion that Lavoisier's reputation ("*j'étois avide de gloire*") might have been still more splendid had it given him less concern. Here, too, he had something to learn from Dr. Black. ANDREW KENT

HISTORICAL ASPECTS OF CHEMISTRY

Chymia

Annual Studies in the History of Chemistry, Vol. 7. Edited by Henry M. Leicester. (Edgar F. Smith Memorial Collection: University of Pennsylvania. University of Pennsylvania and Division of History of Chemistry, American Chemical Society.) Pp. 152. (Philadelphia: University of Pennsylvania Press; London: Oxford University Press, 1961.) 40*s*. net.

THE regular appearance of this annual publication solely devoted to the history of chemistry is very commendable and must undoubtedly be of considerable assistance to students in that field. In reviews of earlier volumes attention was directed to the high standard of the articles and their interest to chemists generally, and these features are well maintained in the present issue. It contains an essay on biochemical concepts among the ancient Greeks by H. M. Leicester; three articles by M. Levey on Indian alchemy, the Muslim developments of the atomic theory, and an aspect of Arabic chemical technology; a continuation by R. Rappaport of her article on Ruelle in the last volume; "a curious Lavoisier episode" related by H. Guerlac; some Russian material on Berzelius assembled by Y. I. Solov'ev; an account of American editions of Davy's book on agricultural chemistry by W. H. Miles; and a survey of early studies of the influence of time on chemical and physical changes by E. Farber. Levey thinks previous publications on Muslim alchemy tend to underestimate the technical achievements, and he translates part of a manuscript by Ibn Bādis (A.D. 1007–61) giving recipes for various kinds of inks, including one which writes black on white and white on black.

Solov'ev's article on Berzelius has some interesting material on G. I. Hess, one of the founders of thermochemistry who spent a short time in Stockholm with Berzelius, on Fritsche, who worked with platinum metals and rediscovered and named aniline, and Klaus, who discovered ruthenium. Berzelius corresponded extensively with contemporary chemists and the exchange of letters between him and the Russian chemists presented here is of interest. Farber's essay on the influence of time in chemical and physical changes assembles some little-known material, for example, the publications of Proust (1810–14) on the influence of composition on the rate of burning of gunpowder, and Liebig's (1849) on the acceleration of esterification by mineral acids.

This volume should be of interest to chemists, and some of the articles have a wider appeal. The printing and binding are very good. J. R. PARTINGTON

METHODS IN CHEMICAL ANALYSIS

Chemical Analysis

The Working Tools. Edited by C. R. N. Strouts, H. N. Wilson and R. T. Parry-Jones, with the assistance of J. H. Gilfillan. Vol. 1: Pp. xvi + 467 + 4 plates. Vol. 2: Pp. x + 479 + 13 plates. Vol. 3: Pp. xii + 273 + 1 plate. Revised edition of "Analytical Chemistry" first published in two volumes in 1955. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 147*s*. net the set of three volumes.

Treatise on Analytical Chemistry

Edited by I. M. Kolthoff and Philip J. Elving. With the assistance of Ernest B. Sandell. Part 2: Analytical Chemistry of the Elements. Vol. 2. Pp. xx + 471. Vol. 9: Pp. xvi + 491. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1962.) 135*s*. each volume.

MOST œnologists would agree with the trite saying: "It is not the label on the bottle that matters, it is the quality of the wine which counts".

(1) This remark could equally well apply to this recent, revised, three-volume edition of that well-known vade-mecum on chemical analysis which was first published seven years ago by the Analytical Chemists' Committee of Imperial Chemical Industries, Ltd. The title of the new edition has, indeed, been slightly changed, but the excellence of the contents will undoubtedly retain the approbation of those perceptive analysts who sampled the earlier vintage.

It is one of the tribulations of continued scientific progress that many of our analytical "working tools" can become obsolescent or greatly modified with the passage of a very few years. Periodic revision of authoritative scientific publications is thus an accepted necessity, and no discipline has grown or expanded so rapidly as that complex field which includes both chemical and instrumental analysis.

Volume 1 of the new edition deals lucidly with the application of statistical methods, accuracy, sampling procedures, measurement of physical constants, apparatus and its calibration, etc., the textual notes being embellished by a wealth of informative practical laboratory detail. Paper, columnar, gas-solid and gas-liquid chromatographic techniques are given a detailed description in this volume, which also includes a new chapter on vacuum fusion and extraction methods for the determination of gases in metals. Isotope dilution methods, titrimetry in non-aqueous solvents, and microscopy receive brief mention.

Approximately one-third of Volume 2 is devoted to the quantitative applications of conductimetric and electrometric methods, including the simpler Heyrovsky and cathode-ray polarographic techniques. Coulometric methods are also described and, because of the exactness of such determinations, they are obviously worthy of much attention by the up-to-date analyst.

The volume concludes with lucid theoretical and practical sections on visual colorimetry, fluorimetry and absorptiometry. The chapters on ultra-violet absorption, infra-red spectroscopy, flame photometry, X-ray diffraction and on the popular X-ray fluorescence analysis method are ably written and provide a wealth of instruction for the tiro and a refreshing recapitulation of principles for the skilled analyst. The tabulated spectroscopic data are both informative and labour saving.

Volume 3 is concerned with ultimate organic analysis of macro- and micro-type, for example, the Schutze–Unterzaucher direct oxygen determination, the Grote method for halogens, sulphur, etc. Diagrammatic illustrations of considerable clarity simplify the understanding of the experimental procedures. A useful section deals with practical studies of unsaturation, the determination of active hydrogen, etc.

The final chapters describe in much detail the more refined methods of molecular weight determination utilizing limited amounts of material. The Rast procedure is included as a rapid method of limited accuracy.

The application of complexans in analysis receives only brief mention in this edition, but this may be a deliberate omission in view of the numerous publications already devoted to ethylenediamine tetraacetic acid type reagents. Some prominence should, however, have been given to the Schoniger 'oxygen flask' type techniques which are now of such ubiquitous academic and industrial interest.

There is, however, little doubt that this new edition of *The Working Tools* will become a well-thumbed aid on the bookshelves of most practising analysts. There is no authority which is so prepollent as that of experience and these volumes are based on the practical activities of the members of the Analytical Department of one of our major industrial chemical organizations. This speaks for itself.

(2) Part 2, Vol. 2, of this book covers the general chemistry of the gallium–indium–thallium trio, and contains adequately referenced chapters on the analysis of compounds of silicon, germanium, iron, cobalt and nickel. The most sensitive qualitative tests are enumerated, for example, phenyl fluorone as a reagent for germanium.

Normal quantitative procedures are described which have a basis which is titrimetric, gravimetric, spectrophotometric, polarographic, radiochemical, etc. There are some obvious shortcomings, for example, in describing the Weisz ring oven test for iron, some reference could have been made to the ubiquitous presence of iron as impurity in the papers used. Perhaps this was regarded by the authors as too obvious.

Although written essentially in review form, sufficient detail is generally given to enable the competent analyst to proceed with a separation scheme, and to be aware of probable pitfalls, for example, the determination of silica in the presence of fluoride ion, borates, etc.

Part 2, Vol 9, is devoted completely to the general and analytical chemistry of uranium and the transuranium actinide elements. Booman and Rein of the Phillips Petroleum Co., Idaho, have written a most comprehensive and detailed review of uranium; analysis of radioisotopes, alpha-energy analysis by magnetic spectrometry, fluorimetric analysis, etc. Nearly a thousand references are quoted in the up-to-date bibliography which accompanies this excellent review.

Metz and Waterbury of Los Alamos provide a similarly extensive and informative treatment of actinide chemistry, together with much advice on handling problems, glove boxes, toxicology, etc. Plutonium receives considerable mention and although the criticality hazard problem is clearly described, the significance of some of the numerical parameters which are given are not too clearly expounded in the text. This catastrophic topic is certainly worthy of lucid practical illustrations and laboratory examples.

Density and phase data for metallic neptunium, plutonium and americium are tabulated. Precipitation, solvent extraction, cation exchange and related methods are described for the separation of these predominant actinides. Radiochemical, spectrophotometric, coulometric and other methods of analysis are given detailed treatment. Methods for the isolation of associated fission products in pile irradiated Pu are succinctly enumerated. The properties of the lesser-known elements from curium to lawrencium receive small but reasonable coverage in the final chapter.

These two books, like their progenitors, are printed on excellent paper, and constitute useful additions to the specialist review volumes of this series which have already been published. Like all modern, well-produced editions, they are expensive.

D. T. LEWIS

NEW VIGOUR AND RIGOUR IN INORGANIC CHEMISTRY

Advanced Inorganic Chemistry

A Comprehensive Text. By Prof. F. Albert Cotton and Prof. G. Wilkinson. Pp. xv + 959. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1962.) 105*s*.

IT is now well established that inorganic chemistry has in the past twenty or so years undergone an impressive renaissance: the subject has acquired renewed life and is growing vigorously. Apart from the initiation of this process in the development of atomic energy this may be ascribed largely to two factors: first, the increasing application of physical techniques in the study of inorganic systems and, secondly, the use of the quantum concepts of theoretical chemistry to interpret, rationalize and to provide a framework for the understanding of experimental fact. The scope of the subject nowadays is very wide and increasing, ranging in extremes from the study of semiconduction to that of the reactivity of biologically important co-ordination compounds, for example, the prosthetic groups of enzymes.

What may be termed the 'new inorganic chemistry' has few really up-to-date text-books to serve it and few that give, for example, a balanced treatment of non-transitional and transition elements. Though there are now many good works of a review or specialized nature it is difficult to find a balanced modern treatment of the subject in a one-volume text-book. Transition metals particularly tend to be inadequately treated.

It is clearly a formidable task to give an adequate treatment of the subject in one volume. This task has been attempted by Profs. Cotton and Wilkinson, and the result is the work under review. It is intended to meet the needs of the honours-level student at British universities and his counterpart in the United States.

The book is divided into three parts with page allocations as follows: Part 1, dealing with general theory (11 per cent); Part 2, the chemistry of the non-transitional elements (42 per cent); Part 3, the chemistry of the transition elements (47 per cent).

One can immediately see where the authors' interests lie.

The first part deals briefly with the electronic structure of atoms; ionic compounds and electrostatic bonding. The valence bond and molecular orbital treatments of bonding are then described and this part concludes with a discussion of topics such as bond energies, electronegativities, bond lengths, bond and molecular polarity, force constants and van der Waal's radii.

The second part of the book deals with hydrogen and the first-row elements (incidentally, an excellent short account of the hydrogen bond is given) and then a chapter on molecular stereochemistry is interpolated. At first sight one might have anticipated finding this in the section on general theory: the justification for its inclusion here is that only after the first-row elements need one consider more than four electron pairs in the valence shell: the stereochemistry of the heavier elements is more varied and complex. The non-transition metals of groups I–VII and zinc, cadmium, and mercury are then treated briefly but on the whole adequately, with high-lighting of features of particular interest. The use of reaction diagrams with radiating arrows are useful as summaries of the reactions of various elements and important compounds.

The last, and largest, part of the book deals with the transition elements and it is in this field that the book is particularly valuable for here, as one might expect from this authorship, is one of the best modern general accounts of transition metal chemistry. This part starts with an introductory chapter on the electronic structure and configurations of the transition metal atoms and ions and their relation to magnetic properties. The following chapter deals with complexes of the transition metals; types of co-ordination; types of ligand; structure, isomerism and nomenclature; the stability of complex ions and the kinetics of their reactions (ligand replacement and electron transfer processes). An account of the theory of the metal–ligand bond is next given and the crystal field, ligand field and molecular orbital theories are considered in turn. The older ideas of the valence bond approach are summarized.

The complexes of π-bonding ligands CO, CNR, PR_3 and NO and organo-metallic compounds (of π- and σ-type) form the subjects of the next two chapters, and the work closes with chapters on the elements of the first, second and third transition series; the lanthanides and the actinides. There are appendixes covering, *inter alia*, atomic structure (Bohr's treatment, and the solution of the wave equation); problems on the first two chapters; hybridization and molecular orbitals; the experimental determination of stability constants.

A feature of the book is the provision of well-chosen and up-to-date reading references at the conclusion of each chapter. In a single-volume work selection is necessary, and there are omissions: the inorganic chemist interested in radiochemistry and related topics will find scant reference to his subject, and the solid-state chemist also will find this treated too briefly. There is no reason, however, why a text-book should not to some extent follow the interests of the authors, and such comments are minor criticisms of a work that is stimulating and modern.

The authors are to be congratulated on completing a difficult task so successfully. This must surely be the best single-volume inorganic text-book available at present.

R. H. PRINCE

MALTING TECHNOLOGY

Barley and Malt

Biology, Biochemistry, Technology. Edited by A. H. Cook. Pp. xiv + 740. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1962.) 150*s*.

THE growing of barley and the making of malt were successful human skills at a time when botany was in the hands of herbalists and chemistry the prerogative of alchemists. Major advances in biology and biochemistry are now impinging on the malting industry, and in this book Dr. Cook and his collaborators survey both the ancient arts and the new vistas opening to technology through the application of scientific method. To appreciate this, one should first read the description of malting practice and learn what maltsters do—and, broadly, always have done—and then examine the account of evaluation of malting barley. Although no answer is given to the question of what is 'good' malting barley, except that it makes 'good' malt and, possibly, good beer, this chapter contains a most stimulating account of methods being explored, with considerable success, in order to estimate the potentialities of a living and therefore variable raw material.

Fundamentally malting depends on biochemistry. Herein, however, lies a practical difficulty, for although knowledge of the biochemistry of germination is incomplete the maltster must control not only well-understood patterns of metabolic change but also many ancillary and possibly unsuspected reactions which occur in growing grain and which may affect the finished malt. What is known about this basic biochemistry is surveyed in two dynamically written chapters, on structural chemistry and on enzymes; these chapters show remarkable breadth of vision and they will be of value both to inquiring maltsters and to biologists investigating problems related to the physiology of germination.

Inevitably, perhaps, there is overlap between the account of enzymes and the dissertation on the nature of the malting process: it is interesting to read the accounts of different authorities of the same process, but careful hybridization and selection from these two chapters might have freed space for other topics. Thus, though one hesitates to suggest additions to a 750-page volume, a separate chapter on crop physiology would have provided a useful connexion between biochemistry and the more descriptive botanical aspects of barley. As it is, general agronomy is treated perfunctorily: only six references are quoted compared with thirty-six for diseases of the crop and more than seven hundred for its chemistry. However, there is a useful account of the morphological development of the plant in the field, under the misleading and rather grandiose title of "Botany of the Barley Plant" and a good discussion of criteria used to distinguish different cultivars. Excellent illustrations accompany the chapter on identification; these contrast favourably with the rather oddly labelled diagrams of the anatomy of barley which are presented on pp. 3 and 9. Rounding off the botanical section is an incisive survey of barley genetics in which the difficulties of breeding new varieties for an industry which cannot yet state its requirements precisely are critically surveyed. This is a model essay on applied genetics, neatly linking biology with technology.

All who are concerned with barley, whether as a crop, as an industrial raw material or as a tool for physiological research will find much to interest them in this book. It is particularly recommended to biochemists: not only will it convince them that malt is more than a convenient source of amylases, but also it will surprise many to learn how substantial a contribution to fundamental knowledge has been made by students of malting technology.

ANNA M. MACLEOD

BIOCHEMICAL LABORATORY TECHNIQUES

Laboratorní technika biochemie
Edited by Bořivoj Keil and Zora Šormová. Pp. 872. (Prague: Publishing House of the Czechoslovak Academy of Science, 1959.) Kčs. 71.00.

THE appearance of the *Laboratory Technique of Biochemistry* (exact translation of the Czech title) will be welcome to all chemists and biologists, students as well as teachers, research workers and the like, at least those who can master the Czech language. It is a unique book in its scope and presentation, and it is to one's regret that it will not be accessible to most biochemists.

The book was edited by two able Czech biochemists, Drs. B. Keil and Z. Šormová, from the Chemical Institute of the Czechoslovak Academy of Science. Despite the fact that almost every section of each of the seventeen chapters was written by a different author, the editors have made a good job of putting together a smooth and a concise volume which can serve both as a text-book and a reference. Besides the editors, the following authors collaborated on the book: Otakar Mikeš, Blahoš Sedláček, Arnošt Kleinzeller, Josef Říman, Ivan Rychlík, Karel Šebesta, Dozider Grünberger, Luboš Matoušek, Vladimír Tomášek, Otto Knessl and Jiřina Černá.

Since this is the first book of its kind to be published in Czechoslovakia, the editors were careful to select those common routine laboratory procedures which are not usually described in scientific journals, as well as certain highly specialized procedures, on the basis of their relative importance, degree of difficulty and application. Almost every section of the book is accompanied by numerous references to monographs and original articles (the field is covered until 1957). The field of proteins and nucleic acids is especially well documented. It is interesting to note that most citations besides those of the authors' own work are to American journals.

Laboratorní technika biochemie comprises two main parts. The first part is of a general nature and includes chapters on the equipment of the biochemical laboratories (26 pages), basic laboratory procedures (116 pages), physico-chemical methods (59 pages), manometric and diffusion methods (38 pages), radioactive isotope tracer techniques (29 pages) and statistical evaluation of data (6 pages).

The chapter concerning the basic laboratory techniques includes sections on grinding and homogenization, filtration and centrifugation, extraction, dialysis and ultrafiltration, evaporation and freeze-drying, adsorption, chromatography, ion exchangers, electromigration, and mechanization and automatization of laboratory work. In the chapter on the physico-chemical methods there are sections on the principles of work with laboratory instruments, measurement of temperature, measurement of *p*H, measurement of conductivity, polarography, spectrophotometry and colorimetry, polarimetry, refractometry, measurement of surface tension, determination of diffusion constants, electrophoresis, viscosimetry, osmometry, sedimentation analysis, light scattering, electron microscopy and other methods used in the investigations of macromolecular substances. Among the manometric and the diffusion methods, the Warburg and the Van Slyke apparatuses and their use, the Thunberg technique and the Conway microdiffusion methods are described in detail. It is regretted that of the various radioactive isotope techniques only autoradiography and the use of isotopes carbon-14, sulphur-35 and phosphorus-32 are described.

The second portion of the book is more specialized and includes chapters on the properties of proteins and nucleic acids (34 pages), preparation of biochemical substances (100 pages), analytical methods (112 pages), methods of determining the structure of proteins and their alteration (57 pages), enzymatic activity (41 pages), techniques of cellular biology (34 pages) and other methods of biochemical interest (34 pages). Included in this part are also very useful tables of buffers (9 pages), tables of characterized protein preparations (29 pages), tables of basic physico-chemical constants of proteins (26 pages) and finally the tables of composition of protein hydrolysates (29 pages).

The chapter dealing with the preparation of biochemical substances includes sections on amino-acids and peptides, proteins, saccharides, phosphorylated sugars, nucleotides, nucleoproteins and nucleic acids, coenzymes, lipids and other useful materials. The analytical methods are given for basic elements and ions, amino-acids, proteins, sugars and sugar derivatives, phosphorylated sugars, nucleic acid components, nucleic acids, coenzymes, lipids and simple metabolites. The chapter on enzymes covers both theoretical and practical aspects of enzymology. Tissue slices and homogenates, cell suspensions, cell fractionation and enzyme isolation from cellular structures are the main topics of discussion in the chapter dealing with cellular physiology. Among other methods of biochemical interest are the discussions of the localization of enzymes, microbiological methods, immunochemical techniques and finally the preservation of biochemical preparations.

Of greatest interest to the biochemist are the detailed and thorough discussions dealing with proteins and nucleic acids, the field of the authors' interest and the field in which they excel. The emphasis on these two subjects forms also the basis of work at the Chemical Institute in Prague.

This book is furnished with a table of contents and in addition every chapter is preceded by a detailed list of titles of numerous sections and subsections. The book has a subject index but lacks an author index. However, since the lists of references which follow most chapters and sections are arranged in alphabetical order a lack of the author index is not a big handicap. The book is richly illustrated with graphs, photographs and diagrams. There is also an abundance of formulæ and chemical equations throughout the text.

Although the scope of the book did not allow a detailed description of topics as some monographs do and the coverage of some topics was extremely scanty, this volume will be of great use to every biochemist, serving as a ready one-volume reference and a guide. As there is no counterpart to this volume in English, it is hoped that the book will stimulate British and American chemists and biologists to collaborate on a comparable work and, furthermore, that some linguist–biochemist will venture to translate the present volume.

M. RECHCIGL, JUN.

LIFE OF VERTEBRATES

The Life of Vertebrates

By Prof. J. Z. Young. Second edition. Pp. xv + 820. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 70s. net.

THE second edition of a path-finding book is always received with a heightened sense of occasion, because it is expected to show whether the path outlined by the author was the right one. Success or failure of the author's 'idiosyncrasies and ideas' in the presentation of his subject become evident by the degree of change in 'plan and basic balance' which a confident or disappointed author has felt obliged to introduce after the book's prolonged maiden voyage through the archipelago of criticism.

Prof. Young is confidently launching his *Life of the Vertebrates* on its second voyage in the full and—may it be said straight away—justified consciousness of success. No fundamental alteration in approach and treatment has had to be made. Some critics objected against the free combination of embryological, anatomical, physiological, biochemical, palæontological, and ecological aspects of vertebrate life within the covers of a single volume. It was stated that each of these facets was bound to suffer the insufficiency of cursory treatment. What was overlooked was the phenomenon of hybrid vigour, and what was underestimated was the thirst of the present-day student of biology, or for that matter of any other scientific subject, for synthesis: and thus this book was welcomed by teachers and students alike and has established itself as indispensable during the first dozen years of its existence.

This does not mean that its friends have not grumbled at times about this and that error of fact or about one or the other omission or too scanty a treatment of something considered essential: and those who have mistakenly tried to use the work as a reference book were undoubtedly among the most disappointed of its readers.

Meanwhile, the author has had his ear to the ground, and a considerable number of improvements have been introduced into the second edition. Notable among them are the thoroughly revised and amplified chapters dealing with sense organs and sensory function and with the function of the endocrine machinery. The study of echo-location and hearing in bats and the functional morphology of the ear in cetaceans have found their proper place in the relevant chapters. The chapters on electrical and luminous organs in fishes might perhaps have been given a little more attention in view of the many new findings in these fields. However, there are undoubtedly many more chapters about which similar wishes could be voiced. But a fulfilment of all these would be manifestly impossible in the framework of a single volume.

A number of technically less satisfactory illustrations have been replaced by redrawing or by new ones, and additional text figures have made their appearance.

The friends of the book will welcome the treatment for teething troubles which it has received from its author, and some of its adverse critics may be converted by the steadfastness of purpose with which its author has guided it forward to the second phase of its existence.

O. LOWENSTEIN

SCOTTISH AND OTHER PLANKTON

Nature Adrift

The Story of Marine Plankton. By Dr. James Fraser. Pp. v + 178 (40 plates). (London: G. T. Foulis and Co., Ltd., 1962.) 45s. net.

WHEN Sir Alister Hardy's *The World of Plankton* appeared a few years ago its comprehensive excellence might have led one to suppose that there would be no occasion for another British book on plankton for another decade or more. Dr. Fraser has shown that one would have been wrong; in *Nature Adrift* he has produced a worthy companion volume to the earlier book. Since the two books are on the same subject they inevitably overlap a good deal, but there is sufficient difference in emphasis and content to make them complementary rather than conflicting, and anyone who owns one of them will find it pleasant, as well as profitable, to acquire the other.

Nature Adrift is an account of the plankton around the British Isles; only passing references are made to the tropics, the ocean depths or higher latitudes. The book begins with a really elementary introduction, which is followed by a good description of plankton apparatus and methods. Then come five chapters which survey the plants and animals of the plankton; these are very good indeed and provide a great deal of purely botanical and zoological information, including such an advanced topic as the complicated life-histories of the Doliolida and other Thaliacea. Anyone who really needs the elementary introduction may well find this part rather heavy going, but if he is thereby inspired to amplify his reading in a zoological text-book, so much the better, perhaps.

The last chapters include descriptions of plankton behaviour and distribution, and a masterly account of the subject that Dr. Fraser has made so much his own, the use of indicator species in interpreting water movements on a wide geographical scale, and the effects of these movements on the fisheries off Scotland and in the North Sea. The last chapter is a salutary discussion of the possible uses of plankton as a source of food for man.

The book is very well and copiously illustrated from a variety of pens and cameras, notably those of J. D. Milne, P. David and D. P. Wilson; it was presumably the question of expense that made it necessary to keep the coloured photographs so small. There is one curious defect in the illustrations which might well be remedied in the next edition; whereas all the animals and plants in text-figures are meticulously supplied with scales of magnification, those in the plates are not. An unsophisticated reader might find it difficult to realize that the *Ianthina* in Plate XV, for example, is not in fact as big as the beer bottle in the neighbouring photograph.

The book is very well produced and a pleasure to read.

J. S. COLMAN

LOW-TEMPERATURE BIOLOGY

Biological Effects of Freezing and Supercooling

By Dr. Audrey U. Smith. (Monographs of the Physiological Society, No. 9.) Pp. xi+462. (London: Edward Arnold (Publishers), Ltd., 1961.) 55*s*. net.

IT is an exciting thing for those of us who were working on low-temperature biology in the 1920's to follow the striking advances that have been made in this field during the past decade.

In those days reports of the recovery of function of animal tissues after freezing were regarded with healthy scepticism, and it was not until the critical experimental work initiated by W. B. Hardy that the circumstances in which tissues such as muscle could be frozen and thawed with recovery of function were precisely defined. Hardy's efforts were directed to the identification of the reasons for the changes which, when irreversible, resulted in the death of the cells. This 'early' work is briefly reported in Dr. Audrey Smith's book, as also is that of R. B. Haines on bacteria. The essential outcome of this work—the damage to the proteins of the cell generically described by the term denaturation is, however, overlooked, and it is fairly obvious from the contents of the book as a whole that workers in her own, the medical, field have moved off in entirely different directions. It is not easy to understand why this early lead has not been followed up more energetically in the areas of applied research outside those concerned with the preservation of food by cold. Modern cryobiology arises in fact from the chance observation[1] that glycerol protects human spermatozoa against the effects of low temperature. This book provides ample evidence of the energy, skill and imagination with which this observation has been developed in medical and cognate fields. It covers the viability of frozen spermatozoa, ovarian and other mammalian tissues and organs; the effects of freezing and thawing on micro-organisms and red blood cells; and the effects of glycerol on all these systems. It then describes the revival of animals from temperatures below zero.

A frustrating feature of the protecting action of glycerol (and such near relations to glycerol as cane sugar) is the unpredictability of its efficacy; or conversely, the fortuitous differences in the response to freezing of tissues and organisms which differ in no obvious respects. This becomes easier to understand, however, if we go back to the earlier work, in which freezing damage was clearly related to the sensitivity of proteins to denaturation as a result of changes in the ionic environment, especially by increase in ionic strength. If we accept the view of Dr. M. L. Anson that "there are many different kinds of proteins which can be denatured by many different procedures, that both native and denatured protein can exist in many different forms, and that denaturation involves many structural changes in a large complex molecule" (Solvay Council, Brussels, 1953) we shall better understand why the spermatozoa of closely related species, closely similar bacteria, and closely similar tissues can react so differently to identical freezing and thawing treatments. Furthermore, we shall be able to understand the mechanism of the protective action of polyhydric alcohols in providing a nimbus of hydrophilic functions, maintaining the configuration of the native protein, when water molecules are removed from its environment by freezing or drying. This protective action was known to us in food investigation, but its use in foods is limited because of the sweetness imparted by the high concentrations of the agent necessary for effective action. One example of its use, however, was in 'sugar-dried egg', a product introduced during the War and still being manufactured, which retains in full the aerating power of undenatured fresh egg proteins.

Apart from the action of polyols in protecting proteins from denaturation, they can also protect cells against freezing both by lowering the freezing point of the aqueous medium and by promoting the separation of ice external to the cells. The formation of ice within the cells is much more damaging than when it forms outside, water then being merely withdrawn from the cell to the extracellular sites of crystallization. An immense amount of work following R. Plank in Germany and Chambers and Hale in Great Britain has been done on extra- and intracellular ice formation in relation to rates of freezing and other physical circumstances. In this connexion the name of Luyet is outstanding. For many years, until in fact quite recently, his theory of vitrification as the explanation of the beneficial effects of rapid freezing has been much favoured; but there is now evidence that the water is in fact frozen as ice but in exceedingly minute crystals giving merely a vitreous appearance. In many systems it is the attainment of a critical rate of freezing which is important in preventing cellular damage, as the early workers were so quick to appreciate.

In spite of the breadth of its title the book does not deal at all with the effects of freezing on plants. It is essentially a book for the animal physiologist, and even more particularly for the medical physiologist. As such it is an exceedingly competent and timely review of the work of the past decade. It marks not only a phase of achievement but also opens up the prospect of remarkable discovery in the immediate future. What is so striking in this account is the number of misconceptions about the effects of cold which have been current. The resuscitation of frozen mammals, now so amply demonstrated, would have been dismissed a few years ago as a fabrication. Many limitations are, of course, still there, but they are understood and may not be insurmountable. The author scrupulously avoids any unwarrantable claims in this respect. Her facts are allowed to speak for themselves and it is left for her readers, who should be many, to make their own extrapolations from them.

E. C. BATE-SMITH

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[1] Polge, C., Smith, A. U., and Parkes, A. S., *Nature*, 164, 666 (1949).

DISTRIBUTION OF BRITISH FLOWERING PLANTS

Atlas of the British Flora

Edited by F. H. Perring and S. M. Walters. Pp. xxiv+432+12 transparent overlays. (London and Edinburgh: Thomas Nelson and Sons, Ltd., 1962. Published for the Botanical Society of the British Isles.) 105*s*. net.

THE *Atlas of the British Flora* represents a tremendous factual contribution to the knowledge of the distribution of flowering plants within the British Isles at the present time. As such it is scarcely possible to over-estimate its value in the future, not only to botanists, but also to scientists of many kinds whose work touches directly or indirectly on the flora of the British Islands. It also reflects very great credit on the widespread interest taken in field botany in Britain that it has been possible to organize such a comprehensive survey in so short a time, and in particular to find such a large body of collaborators with the necessary knowledge and enthusiasm to carry it out. The lack of such support would have produced unevenness in the recording of the common species, and considerably lowered the high standard of reliability of the whole work.

About 1,700 species are mapped in the present volume, and this provides an interesting basic estimate of the content of the British flora, shorn of the innumerable segregates of the major critical genera such as *Rubus, Rosa, Alchemilla, Hieracium, Euphrasia*, etc., but at the same time including quite a number of introduced species. Hulten, in his *Atlas of the Distribution of Vascular Plants in N.W. Europe* (1950), which covers a much greater land area, gives maps for more than 1,800 species, but this does contain a considerable number of critical segregates, so that it is scarcely possible to assess these figures together, in all fairness. It is wonderful, however, to be able to compare maps of the British and Scandinavian distribution of so much of the British flora for the first time.

One matter for regret perhaps is that in the present work it has not been considered advisable to give even some general indication of the wider distribution of the species contained in the *Atlas*, on the grounds that a considerable part of the interest and value of any work of this kind lies not only in the analysis of distribution in a particular area but also in its relationship to the total area of the species concerned. It is clear, however, that the main intention is to deal only with the accurate representation of present knowledge of species within the British Isles and to leave interpretation of the facts to others.

The editors have chosen 1930 as the main basis for their existing survey, while at the same time utilizing earlier records when it is clear that a species has declined in frequency or that the present survey has been unable to confirm the continued existence of a plant in previously known stations. It is with the recording of species before 1930 that one feels on less secure ground, and that in many cases the inclusion of earlier records might have been more extensive in view of the considerable volume of literature available. A select bibliography is given and it is stated elsewhere that local Floras have been used (but not which), also the *Reports of the Botanical Exchange Club*, apparently only from 1930 onwards. It is not clear if the earlier *Reports* of this Society have also been abstracted although one presumes that this is so. The volume of literature on the British flora is so considerable that a more detailed bibliography would have been welcome for purely practical reasons, although it is obvious that time could not permit much more work in this direction.

Lists are given of species which are regarded as aggregates, or which have only been regarded as provisionally mapped, for various reasons, and these are appropriately marked in their context. The indication of these aggregate species appears to be rather irregular, and it is hoped that in the future volume on critical segregates many of these will be expanded, including others which have not been so characterized. Many botanists will be uneasily conscious that certain species, such as *Thalictrum minus* and *Viola tricolor*, *Sedum telephium* and even the naturalized *Lysimachia punctata* might surely be regarded as aggregates, yet there is no indication that this is so. One wonders why it should be difficult to separate records for *Ledum palustre* and *L. latifolium*, and why the maps for *Calystegia sepium* and *C. silvatica* should be marked as 'Provisional' but not that of *C. pulchra* (which is obviously under-recorded for the north of England and western Scotland).

The provision of twelve transparent overlays to assist in the analysis of different factors which have contributed towards distribution is an inspiration and of immense value and the introductory remarks on the interpretation of these maps extremely interesting. In considering the distribution of the introduced species *Fuchsia magellanica* in relation to frost, one might suggest that this map is a little misleading, possibly due to a reluctance to record a species usually accepted as an escape from cultivation.

That there should be omissions in recording is obvious and inevitable, in the circumstances, but in no way disastrous. Now that the work has seen the light of day, practically every field-botanist with specialized knowledge of a particular area will lose little time in pointing them out to an already much-tried editor. This will of course be beneficial, and in the nature of filling in gaps, but the major distribution is already drawn out and we can now look forward to the production of the forthcoming companion volume on critical segregates with great confidence and very considerable interest.

C. W. MUIRHEAD

GROWTH OF TREES

Tree Growth

Edited by Prof. Theodore T. Kozlowski. Pp. ix + 442. (New York: The Ronald Press Company, 1962.) 12 dollars.

THIS book is a collection of papers contributed to the International Conference on Forest Tree Growth, held in 1960 at the University of Arizona. 'Tree growth' has been interpreted generously, and there will be few botanists and foresters to whom the book will not have either an intrinsic appeal or a use as a reference book. It is difficult to think that the subject could have been much more widely covered than by the twenty-seven articles which make up the book.

Physiological and ecological aspects of the subject, as might be expected, occupy a good proportion of the whole, and several contributors deal with the relation of various climatic and edaphic factors to tree growth; in a paper on the relation between ageing and environment an interesting case is mentioned in which young beeches, growing in very heavy shade, lose the juvenile character of leaf retention. On the genetical side consideration is given to evidence of hybrid vigour, the selection of superior forest trees and to geographical variability in growth. The paper on ecological variability considered in relation to taxonomy of forest trees should warn the over-enthusiastic systematist of the dangers of too much splitting and the creation of unnecessary sub-species. Several of the contributions are concerned with methods of measuring or predicting the growth of trees as individuals and as stands, while other authors deal with development of tree rings and with dendrochronology in relation to archæology. The vascular cambium is, of course, the subject of several articles, which include the physiology of cambial activity, and auxin gradients and the regulation of cambial activity. It is a pleasure to find Prof. Melin's name among the contributors, with a paper on mycorrhiza, a subject which has received his attention over the past forty years.

An up-to-date book on trees, comprehensive enough to cover the modern morphological, physiological, ecological and genetical aspects, in fact a book which will consider in all aspects the tree as a life-form, is much to be desired. *Tree Growth* goes part of the way to satisfying that need, although a collection of papers by a number of authors must clearly lack the homogeneity of a unified work. Its editing, however, has been in experienced hands, for Prof. Kozlowski is the joint author of the modern book *Physiology of Trees*. If the desired book on trees comes to be written, *Tree Growth*, with its wealth of information and valuable bibliographies, will be an essential source-book for the author. F. W. Jane

RECENT ADVANCES IN PLANT PHYSIOLOGY

Annual Review of Plant Physiology

Vol. 12. Edited by Leonard Machlis, in association with Winslow R. Briggs. Pp. vii + 502. (Palo Alto, Calif.: Annual Reviews, Inc., 1961.) 7 dollars.

The twelfth *Annual Review of Plant Physiology* follows the lines of previous volumes, and the twenty contributions it contains cover a wide range of topics. Under the general title of cell structure and function, mitochondria are dealt with by F. L. Crane and cell walls by G. Setterfield and S. T. Bayly. Two aspects of mineral nutrition are considered: minor element nutrients and soil–plant relationships in ion uptake. The former is discussed by D. J. D. Nicholas, who pays particular attention to the part played by micronutrient elements in nitrate metabolism and phosphate metabolism. A consideration of iron metabolism is also included. In their consideration of ion uptake from the soil, M. Fried and R. E. Shapiro indicate the processes involved in the movement of ions from their place in the soil to the place in the plant where they are utilized.

Nitrogen metabolism is represented by an article on amino-acid synthesis by a leading worker in this field, A. I. Virtanen, and one on protein synthesis by G. Webster in which the complexity of the mechanism of protein synthesis is emphasized. Here it might have been as well if the writer had defined the abbreviation *s*RNA, as this contraction is of comparatively recent introduction and may not be familiar to readers who have not followed the rapid developments in the study of protein synthesis which have occurred during the past few years. An article under the general title photosynthesis by G. Hoch and B. Kok deals mostly with recent work on aspects of the photosynthetic mechanism, while J. F. Talling reviews the subject of photosynthesis under natural conditions. The biosynthesis and function of carotenoids, a subject on which much new information has been obtained during the past three or four years, is considered by T. W. Goodwin. Other articles dealing with metabolic aspects of plant physiology are on phosphorylation in higher plants by E. Marré, the biochemistry of senescence by J. E. Varner and plant water deficits in relation to physiological processes by Y. Vaadia, F. C. Raney and R. M. Hagen.

Six contributions are included under the general heading of growth and development. Three of these are concerned with growth regulators, auxins by C. H. Fawcett, gibberellins by N. W. Stuart and H. M. Cathey, and kinetin and allied substances by C. O. Miller. The remaining articles in this group are on photoperiodism by F. B. Salisbury, the physiology of mitosis and meiosis by J. H. Taylor and geotropism by H. Rufelt.

The review generally contains one or two articles on less familiar topics. In the present volume there are two which may be regarded as coming into this category. They are on photochemical air pollution damage to plants by J. T. Middleton and the physiology of wilt disease by T. S. Sadasivan.

The volume concludes with very useful cumulative indexes of authors and titles covering the last ten volumes of the *Annual Review*. W. Stiles

A COMPENDIUM OF BANANA RESEARCH

The Evolution of the Bananas

By N. W. Simmonds. (Tropical Science Series.) Pp. xii + 170. (London: Longmans, Green and Co., Ltd., 1962.) 42s.

In 1922 the disastrous wilt known as Panama disease threatened with extinction the banana industry of the West Indies. The foundation of the Imperial College of Tropical Agriculture in that year was also the beginning of a research project which had as one of its aims the breeding of bananas resistant to the disease. At the same time it was realized that lack of knowledge of the genetics, cytology and taxonomy of the bananas was a serious handicap to scientific breeding. The succession of botanists who, with vision and versatility, have investigated these topics during the past forty years have now compiled a wealth of information on which the banana breeder may draw for guidance.

The same information may also be used to attempt a reconstruction of the evolutionary history of both the numerous wild species of *Musa* and the cultivated varieties derived from them. N. W. Simmonds, whose own contribution in the research project has been very considerable, has in this book summarized with admirable clarity the discoveries which assist this reconstruction. He assesses the relationships between the wild bananas in terms of morphology, geographical distribution, cytology and the results of interspecific hybridization, and derives a scheme of evolution which fits the evidence, though he is careful to avoid unwarranted inferences about the ancestors of modern bananas. Turning then to the evolution of cultivated bananas he discusses at some length the origin of the two features which are essential in a successful edible variety, namely, parthenocarpy and seedlessness. There is also an evaluation of the part played by interspecific hybridization and triploidy, and finally, a discussion of the spread of bananas from their centre of diversity in South-East Asia to Africa, America and elsewhere. On all these subjects there is substantial evidence to support the speculation inevitable in phylogenetic arguments, and the picture which Simmonds draws is remarkably complete.

The book is certain to be of great value to those working with bananas, but it is strongly recommended also to all students of experimental taxonomy and of the origin of cultivated plants.

J. N. Hartshorne

NEW METHODS IN CELL PHYSIOLOGY

Weiterentwicklung der Zellphysiologischen Methoden

Angewandt auf Krebs, Photosynthese und Wirkungsweise der Rontgenstrahlen, Arbeiten aus den Jahren 1945–1961 (New Methods of Cell Physiology: Applied to Cancer, Photosynthesis and Mechanism of X-ray Action. Developed 1945–1961). Von Otto Heinrich Warburg. Pp. xv + 644. (Stuttgart: Georg Thieme Verlag; London: John Wiley and Sons, Ltd.; New York: Interscience Publishers, 1962.) 138 D.M.; 259*s*.; 34.50 dollars.

The title of this book is a misnomer, for it is a collection of 95 papers, 20 of them hitherto unpublished, from the Max Planck Institut in Berlin, the work of Prof. Otto Warburg and his assistants from 1945 until 1961. Several of the papers repeat each other with minor variations. Apart from a chemical actinometer, which Prof. Warburg himself gave up using in 1954, the new methods are numerous improvements to the constant-volume manometric apparatus, culminating in the 'universal flask' of 1960, which has a single side-arm, a side-arm with twin inter-communicating bulbs, and a third side-arm leading to a trough above the centre well.

I myself am not one of those who think that the Warburg flask finally died with the introduction of the photo-multiplier. There is a continuing place for a tool so quick, easy and flexible for measuring rates of gas exchange. The limitation of the manometric method is that it will give useful measurements over only a relatively small range of rates; it cannot be scaled down, and thus often cannot satisfactorily be married to other analytical techniques. This does not matter so much, perhaps, when one is using homogeneous populations of micro-organisms or cell fragments; with more complicated tissues it can raise almost unanswerable problems of cell damage in slices, or of proportions of cell types, as in much tumour work.

A difficulty which confronts experts in a versatile technique is that they become almost prisoners of the method. It is saddening to observe that in the present collection of papers, isotopic tracers and paper chromatography raise their heads only once each. Other methods of measuring gas exchange—carbon dioxide or oxygen electrodes, nuclear magnetic resonance—are not discussed, a pity, since an objective appreciation by such a master of technique would be very valuable. This reticence extends even to such relatively trivial matters as the use of diethanolamine or carbonate/bicarbonate mixtures for maintaining a constant concentration of carbon dioxide in the flask.

The establishment of the quantum efficiency of photosynthesis in *Chlorella* at around 4, using the technique of over-compensation, is a triumph which sweeps many of these criticisms aside; it has opened the way to much valuable research in the past few years. The controversy over respiration and tumour genesis, on the other hand, seems to have reached a dead end, since Prof. Warburg himself has changed his ground by admitting that measurements of aerobic glycolysis are too untrustworthy to be meaningful. The rate of anaerobic glycolysis in cells is not necessarily related to respiration-rates at all. As for aerobic glycolysis, pyruvate and lactic dehydrogenase is such a splendid system for measuring redox potentials that Holzer and others have used it to estimate the $NAD/NADH_2$ ratio *in vivo*. Manometer users in general, mesmerized by the bicarbonate ion, might have paid more attention to the concentrations of intermediary metabolites in tumour and wondered whether the defect in carbohydrate metabolism lay in the pyruvic oxidase step; might have wondered also whether pyruvate was acting as a general hydrogen acceptor for $NADH_2$.

There may well be a relative lack of cytochrome *c* in tumour cells; but one is reminded of phage-infected bacteria, in which synthesis of cellular protein ceases, except for that necessary to provide energy for phage reduplication. This is not to suggest that cancer is caused by a virus, rather that in these growing cells, self-starved as it were, every reserve protein molecule is catabolized and the limiting factor becomes the supply of prosthetic groups.

Umbreit, Burris and Stauffer's work, in spite of its limitations, will retain its pride of place, since this is not a laboratory manual. Its chief charm lies in the evocation of the years 1920–40, when Warburg, Weiland and Willstätter, Meyerhof, Embden and Parnas marched and wheeled on the plains of Germany, searching for the whites of each others' eyes before firing off their highly individualistic cannon.

J. H. Ottaway

CELL BIOLOGY

International Review of Cytology

Vol. 12. Edited by G. H. Bourne and J. F. Danielli. (Prepared under the auspices of the International Society for Cell Biology.) Pp. viii + 424. 1961. 107*s*. 6*d*. Vol. 13. Pp. ix + 393. 1962. 107*s*. 6*d*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd.)

The editors' interpretation of the scope of cytology is, legitimately, a wide one, embracing biochemistry, biophysics and molecular biology simultaneously with gross histology, and this eclecticism would seem to imply that the series is intended for the non-specialist and must be so judged. I feel, therefore, that authors may reasonably be expected to offer comprehensive critical surveys of recent literature, summaries of older work, assessments of the importance of their subjects to cytology, and, ideally, novel points of view or interpretations; the subjects chosen should be those in which there have been recent advances of general significance to cytology. Such reviews could be most valuable correctives to the present fragmentation of cytology into specialist disciplines.

Some authors come close to this ideal: Hamerton with a masterly survey of sex chromatin, Hsu with a lively, if excessively verbose, treatment of aneuploidy. Yčas (the coding hypothesis), Taylor (chromosome reproduction), Flickinger (gene action and protein synthesis) and Evans (chromosome aberrations) provide a sensible and useful group of topical reviews. Some, less ambitiously, provide workman-like essays in limited, but important, fields: Austin (fertilization *in vitro*) and Finean (myelin structure), although 'workman-like' is an inappropriate adjective for Finean's electron micrographs; others deal with perhaps too recondite topics: Steffensen (chromosomes and metal ions), Yamamoto (fertilization in fishes). Some other authors fail to consider the needs of the non-specialist although their subjects are of general importance; Schweiger (erythrocyte metabolism) and Ball (mitochondrial composition), for example, seem to me, as a non-biochemist, to be writing primarily for specialists. Here, editorial control appears to be inadequate, as also in Felts's excessively detailed and turgid treatment of bone implants *in vivo*, in Bertalanffy's superficial and uncritical review of cell renewal, and in Bessis's premature attempt to describe the electron microscopy of blood cells. Similarly, Wilbrandt should have been persuaded to expand into something more thorough his brief lecture on cation transport, originally delivered in 1960 and now reprinted, little changed, after two years. Nath on the cytochemistry of the Protozoa, however, gives sheer joy; he is off to a flying start with the statement that the work of the Oxford cytologists (and Nath) has "more or less solved the Golgi problem in Metazoa", and the pace thereafter never slackens.

The criticisms I have expressed reflect, inevitably, my personal prejudices and interests, but I think few readers of these volumes will escape similar feelings, although perhaps with different foci of irritation. No cell biologist will find these volumes devoid of interest; few will want them on their own bookshelves. I feel regretfully that given rigorous editorial control the *International Review of Cytology* could be much more valuable. T. Vickers

EXPLORING THE CELL

General Cytochemical Methods

Vol. 2. Edited by Prof. J. F. Danielli. Pp. xi + 297. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 80s. net.

THIS is the second volume of a series edited by Prof. Danielli, and a general pattern is beginning to emerge. The individual articles are highly specialized, but they are all concerned with physical or chemical cytology and despite their apparent diversity the series as a whole possesses a degree of coherence often lacking in others of this nature. Certainly anyone working with cells would find it necessary to have at least some acquaintance with most of the topics discussed, if only to assess the value of work by other scientists.

The first article by Ross deals with immersion refractometry of living cells. It gives a useful account of how both phase contrast and interference microscopy can be used for measuring the solid concentration and dry mass of living cells.

The next three chapters give authoritative accounts of the Cartesian diver and related techniques. Zeuthen deals with the Cartesian diver balance, Holter with the standard diver and Zajicek and Zeuthen with the use of the ampulla diver for determining cholinesterase activity in single cells. The emphasis in these chapters is mainly practical, but examples of applications ranging from the growth of single amœbæ to the quantitative estimation of cholinesterase in single bone marrow cells or parts of single neurones are discussed.

Daoust describes a method that may have widespread applications in the study of many intra-cellular enzymes, though the present article is restricted to deoxyribonuclease. A tissue section is covered by a thin film of gelatin containing an appropriate substrate. After a suitable exposure this film is stained to reveal areas attacked by the enzyme. The technique has many features in common with autoradiography.

McManus in his article on periodate oxidation techniques gives a good account of some aspects of carbohydrate histochemistry. Dimedone blocking, which is very useful for distinguishing between glycogen and other periodic acid–Schiff-positive substances, is not mentioned, and I believe that the use of saliva rather than amylase for removing glycogen may give misleading results in some cases.

The last two articles are concerned with aspects of protein histochemistry. Barnard discusses acylation and diazonium coupling and Maddy describes the use of fluoro-2 : 4-dinitrobenzene as a cytochemical reagent. These articles show how well-known chemical reactions can be adapted for histochemistry and indicate some of the special difficulties that may be encountered. It is clear that the day of the amateur in this field is passing, and future progress will depend on a thorough understanding of chemical theory and methods.

Altogether this is becoming a solid and satisfying series. Prof. Danielli is wise to avoid the pitfalls of annual publication. If he continues to bring out further volumes as necessary he will render a useful service to cytology. R. Barer

FRESH FRAMEWORKS FOR DEVELOPMENTAL ANALYSIS

New Patterns in Genetics and Developments

By Prof. C. H. Waddington. Columbia Biological Series, No. 21.) Pp. xiv + 271 + 24 plates. (New York and London: Columbia University Press, 1962.) 80s., 10.00 dollars.

PROF. C. H. WADDINGTON is well known for his excursions into art, architecture and philosophy and his readiness to adopt notions and nomenclature from these fields into his home-ground of genetics and embryology. Embryologists cannot thank him for introducing more vocabulary into a subject already confused with foreign 'coinage', but they will have to admit that some of the ideas included in this new book cannot be expressed except in the special jargon in which they originated. The book, which is a transcription from lectures delivered in Columbia University in 1961, deals with developmental processes from the molecular level up to the multicellular and multi-organ levels of complexity. The 'new patterns' indicated in the title are patterns of thought, derived from biochemistry, microbial genetics, thermodynamics, statistics, architecture and even social science. Patterns of structure, revealed by means of the electron microscope, are also discussed: but it is the causes underlying them and the causal systems they represent, rather than any description of them in static terms, that occupy the author's attention.

Because of the inevitable time-lag before publication, Waddington's first chapter, dealing with molecular synthesis, now lacks freshness of impact. We have already read elsewhere much clearer, crisper accounts of enzyme induction and repression, of the 'operon' system for gene action proposed by Jacob and Monod, and of possible genetic coding mechanisms. However, this chapter is still useful in reminding us of the relevance of these ideas to development, as well as for its examples of known mechanisms of gene action. The next chapter begins with some mathematical expressions for the relationship between interacting autosynthetic processes—a theme that Waddington broached in his earlier book *The Strategy of the Genes*. Now he takes it further, using Goodwin's equations to express these developmental (or 'epigenetic') processes in thermodynamic terms. We are now asked to accept the old (also 'Waddingtonian') term, 'competence', re-defined as a condition in which 'epigenetic temperature', θ, is not much greater than 'epigenetic kinetic energy' (T). As 'determination' proceeds, the difference between θ and T is supposed to increase. These expressions may hold some fascination for people with sufficient mathematical knowledge to understand them, but the rest of us are left musing somewhat bitterly that such theoretical elaborations can, of course, be plausible because of our great ignorance of the actual events. In sudden contrast, the rest of this chapter switches to a consideration of chromosomal structure and of possible interchanges between nucleus and cytoplasm during the differentiation of specialized cells such as those of the insect's eye and the newt's notochord. A vesicular 'ergastoplasm', apparently continuous with the nuclear membrane, appears during these processes. These descriptions, as well as others in later chapters, are illustrated by clear text drawings, much better than the group of plates placed inconveniently in the centre of the book.

Chapter 3 is undoubtedly the most excursory and provocative part of the book; here Waddington proposes describing patterns and forms in living organisms in terms of the mechanisms causing them to arise. He distinguishes unit-generated forms, instruction-generated forms, template-generated forms and condition-generated forms. The last type depend on continuously interacting conditions in the developing cells, and require somewhat complex mathematical consideration. Reference is made to Turing's mathematical demonstration of how alternate peaks and troughs of concentration of a morphogenetic substance could arise in an originally homogeneous ring of cells. Waddington suggested this ten years ago as a possible explanation of metameric segmentation, but has met with criticism recently from Maynard-Smith. It is most unfortunate that Turing has not survived to take the argument further himself.

In the second half of the book an immensely wide range of morphogenetic processes is described and discussed, at the level of single cells, groups of cells and the orderly groupings of organs that comprise an appendage or a head. A final chapter discusses examples of gene-controlled patterns, chiefly in insects: most of these are already familiar from Waddington's previous writings, but he adds new examples of the stabilization of variants by selection. This is an entirely 'genetical' chapter—control mechanisms at the somatic level are not known in these cases. In describing the control of organ development our continued ignorance is obvious, too, for Waddington has to revert to the old, enigmatic idea of 'field' action. As he admits in his "Epilogue", embryologists have for a long time failed to achieve any major break-through in the explanation of morphogenetic events at the multicellular level. His own persistence in introducing new patterns of ideas that are seldom fully acceptable to his colleagues may perhaps goad them into the clearer thinking from which some such major break-through can come.

E. M. Deuchar

ARCHÆOLOGY OF EASTER ISLAND

Archaeology of Easter Island

By Thor Heyerdahl, Edwin N. Ferdon, Jr., William Mulloy, Arne Skjölsvold and Carlyle S. Smith. Edited by Thor Heyerdahl and Edwin N. Ferdon, Jr. (Reports of the Norwegian Archæological Expedition to Easter Island and the East Pacific, Vol. 1. Monographs of the School of American Research and the Museum of New Mexico, No. 24, Part 1, 1961.) Pp. xi + 560 + 96 plates. (London: George Allen and Unwin, Ltd., 1962.) 231*s*.

For many who have been tantalized by Thor Heyerdahl's two popular volumes *Kon-Tiki* and *Aku-Aku* this report has been awaited with interest. Now the first weighty volume is before us, and the evidence—or at least part of it—is available. The authors have certainly not spared any detail. The accounts of the excavations are such that, though they contain much of interest, they will deter even the most avid reader.

The first two problems tackled by the expedition were of absolute dating and the culture sequence. In the light of these careful excavations and of carbon-14 dates we can now speak with assurance of the early occupation of the Island already by the fourth century A.D. Thereafter the archæological history divides into three periods, early, middle and late. The early period extends to about A.D. 1100, and is as yet not well known; the middle period, dating from *c*. 1100 to 1680, is one of development, with certain culture changes and an underlying continuity from the early; the late period begins with the great fire at the Poike ditch and marks a decadent phase extending into the last century. The third problem concerned the age of the various masonry styles and the origin of the stone-building tradition. The minute survey of excavated masonry and of reconstruction work supplies a clear answer. The specialized masonry style appears in the earliest stages, and later declines. On origins, my mind is still open, although the authors consider that the evidence favours Peru. The next problem concerned the origin of the Easter Island figures. Here, too, I feel that the problem awaits solution, as does the whole question of cultural origins and migrations.

My impressions of this book are curiously mixed. In spite of its lavish production and great length, and the fact that it is only the first of several volumes, my expectations are unsatisfied. I am perplexed to find repeated quotations from earlier writers emphasizing so often their errors rather than contributions; the language of the text is often turgid; detail is frequently repeated, apparently pointlessly (for example, significant as the several types of fish-hooks may be, why illustrate quite so many fragments at full size ?). The results here published are undoubtedly a real contribution in the same way as the much less ambitious research and publication of Suggs on Nuku Hiva[1]. But while that writer found in the Marquesas complete vindication for the west-to-east movement of Polynesian culture effectively ruling out New World origins, the present research at Easter Island supports the view that here, at least, certain traits derive from South America—perhaps far more. I feel, in short, that the complete picture cannot rest in Easter Island alone and hope that in one of the coming volumes the authors will provide a more integrated and digested analysis of these problems, perhaps coupled with a more sympathetic use of the rapidly growing evidence from other parts of the Pacific. F. R. ALLCHIN

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[1] Suggs, R. C., *The Archaeology of Nuku Hiva, Marquesas Islands, French Polynesia* (New York, 1961).

EASTERN PAPUAN POLITICS

Politics of the Kula Ring

An Analysis of the Findings of Bronislaw Malinowski. By J. P. Singh Uberoi. Pp. xv+162. (Manchester: The University Press; New York: Humanities Press, Inc., 1962.) 25*s*. net.

THIS book is a digest of some of the literature on the islanders of the Eastern Division of Papua for the period 1900–30 considered from the point of view of political economy. The 'Kula' is one name of the area for exchanges of shell currencies or ornaments and other goods between trade friends of different local residences. Uberoi's point that trade has a political side to it is an axiom which he takes to mean that trade relationships between individuals covered wider areas than their homes. He gives no digest of figures of trade between Papua and Australia, Great Britain, Japan and Germany to exemplify his point.

A common view of Malinowski's works on the Trobriand Islanders of 1914–18 is that they discuss the 'Kula', among other things, but not world trade or Neolithic society. In mentioning Neolithic society in the Trobriands, Malinowski fabricated evidence about the number of the wives, the extent of the sphere of influence and the amount of the wealth of the eighteenth- and nineteenth-century predecessors of one chief among several he met during 1914–18. The domains of chiefs and of their home groups of about thirty men and their wives and children are not wide in area. They are areas of about a thousand acres, nine hundred of which are lying fallow in secondary growth in every season and which are cultivated by slash and burn agriculture. Malinowski wrote without much knowledge about agriculture and agricultural economics. One of the economic questions is whether the importing of chemical fertilizers can be made to pay or not, and he does not discuss this question or offer evidence pertinent to it.

There is much that is unknown about the history of these islanders, but they plant the yam and taro, which have not grown in the wild-type in north-east Africa or in south-west Asia since the late Miocene. They speak languages which are proto-Semitic in family and they very possibly have sailed from south-west to south-east Asia to Papua since the dates of the origin of agriculture in south-west and in south-east Asia. In the territory of New Guinea, stone pestles and mortars have been found resembling those excavated at Jericho by Miss Kathleen Kenyon from a seventh millennium B.C. site. A technique of making portrait skulls in plaster on bone from early Jericho is paralleled by a technique for making portrait skulls in baked clay on bone in nineteenth-century New Guinea. Clay spindle whorls from early Jericho have no analogues in New Guinea.

Uberoi's essay does not treat Goodenough, Ferguson and Normanby Islands adequately, or treat agricultural economics. It is written in disagreement with Malinowski on disputable and sometimes fabricated evidence or opinion about the chiefs of north Boyowa Island and in discussion of some of the trade of the area. R. F. FORTUNE

ANNUAL ENCYCLOPÆDIA OF SCIENCE

McGraw-Hill Yearbook of Science and Technology

1961 Review/1962 Preview. Compiled by the Staff of the *McGraw-Hill Encyclopedia of Science and Technology*. Pp. 558. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1962.) 175*s*.

IT has been said—many times—that we live in an age when the man in the street knows less and less about more and more, while the specialist knows more and more about less and less. This is a book intended to help the scientific worker to know more about more and to inform him of recent work in all fields of scientific and technical activity. It is the first of a series of supplementary volumes produced to keep the *McGraw-Hill Encyclopedia of Science and Technology* abreast of the times.

The subjects treated are arranged alphabetically and bibliographies are supplied with most of them. There are many cross-references. Most articles deal with very specialized matters and so valuable criticism of them could only come from experts in these fields, but there are quite a number which will have a much wider appeal and which describe in outline how problems of general interest are being tackled. Among these are articles on such varied topics as nuclear explosion detection, land subsidence, warning systems for tidal waves, tenderizing of meat, oil and gas pipelines, vertical take-off aircraft and space travel. Few people will not have given thought to some of these.

The alphabetical section is preceded by a "Preview of 1962" which consists of five feature-length articles on subjects of widespread interest written by "the spokesmen of their fields". These are, in the main, good. "Man in Space" and "Man and the Sea" combine interesting records of past achievement with speculations as to future progress. The records impress with the success achieved in face of the very complicated problems which have been faced. The speculations are the sober judgments of responsible experts in their respective fields and are by no means to be judged as science fiction. "The Mind of Man" includes interesting accounts of experiments about animal learning and the information they provide for the psychologist. "Basic Research" reviews the planning and financing of scientific work in the United States. These are all well illustrated. However, the article about computers is less successful. We have heard many times of the wonderful things that machines can now be made to do: discussion of these occupies most of the space. It would have been more interesting to have had an explanation of the working of a computer of a very simple type and an indication of how it 'learns' and 'remembers'. The photographs of large rooms filled with meters and consoles are not instructive and could well have been omitted. They are, in fact, illustrations of computers but might equally well be accepted as refrigerators or filing cabinets.

The general impression is that this is a useful and reliable supplementary volume and that it amply achieves the purpose for which it is intended.

K. W. LYON

A SCIENTIST LOOKS AT ART

Darwin and the Naked Lady

Discursive Essays on Biology and Art. By Alex Comfort. Pp. vi+170. (London: Routledge and Kegan Paul, Ltd., 1961.) 18*s*. net.

THIS is an entertaining, provocative and illuminating set of essays. It is, at the same time, serious minded. It invites us to remember and allow to enter into the consideration of our playing and working, art and the enjoying of art, our knowledge of what we are. We know, but have not yet implemented our knowledge, that we are the products of evolution, tormented by our attempts to keep our true motives and emotions hidden from ourselves. We pay lip service to Darwin and Freud, but go on theorizing as though they had not given us their key concepts of natural selection and the unconscious. Once more, philosophers of art are exhorted to consider the facts and to look at art as one among many human activities, fulfilling or failing to fulfil human needs. More important perhaps, intelligent lookers-on at the human scene are exhorted to look more closely at what people, as a matter of fact, enjoy in their reading, looking and listening, and to think less about the standards of the art and literature enjoyed by the cultured few.

The implication is that if *this* is what satisfies, *this* should supply the standards of excellence in art. At least, this is what one is led to expect, but Dr. Comfort shies away from his own conclusion. It is as if one of Plato's philosophers, re-entering the cave, were to fall under the old spell all over again, and join in the praise of those who were adept at noticing resemblances among the shadows. But we cannot have it both ways; either we think that art, like any other human activity, is to be understood in terms of its causes and its functions, or we think that both its causes and its functions are irrelevant to its true nature, that we are, in effect, to look for reasons and not causes in justifying our evaluations. We must also be prepared to accept the implications of the former view, that if art has a function, it is expendable. Dr. Comfort accepts this implication, and even seems prepared to suggest a substitute for art.

Dr. Comfort presents us with a distinction among thinkers, the soft- and the hard-centred, comparable with William James's tough and tender minded. The crucial difference is said to lie in the willingness of the soft and the tender to accept emotionally satisfying conclusions without waiting on the facts. Dr. Comfort, however, seems to fall into the common scientists' mistake of thinking that what makes a belief scientific is the kind of fact held to be important rather than the attitude towards facts. One can hold unscientific attitudes towards germs and electrons and be scientific about beauty and immortality. A corollary of this mistake is to have a vague general belief that some kinds of statements do not need the support of facts. There is a valuable treatment of Indian erotic art, which, with other chapters, should lead to the conclusion that since they have this adequate substitute release, people of the Indian culture would have less need than do Europeans of actual erotic experience, and that they would be less anxiety ridden. Is this so? There is no hint that some facts are needed here—fact and hypothesis are left in the air.

Dr. Comfort makes the further scientists' mistake of extending the sphere of scientific authority from fact and theory to opinion and belief. The final essay, in its occasional insights, and its enormous self-satisfaction, is a model of scientific over-simplification and arrogance. Has Dr. Comfort, after all, forgotten his Freud? Do we not all still have to enter the world by the same route? Do we not all have to find our place in a family where the jealousies and resentments of the established members are only to be matched by the arrogance of the newcomer, who behaves as if the whole family were his slaves? Scientists, like everybody else, show the contrast between wise talk about the family, and intolerant and irrational behaviour in its midst. Scientists are able to keep the illusion that they would be wise rulers only because they so rarely descend into the political arena.

R. L. SAW

STATISTICAL TABLES

Handbook of Statistical Tables

By D. B. Owen (Addison-Wesley Series in Statistics.) Pp. xii+580. (London and Paris: Pergamon Press: Reading, Mass; Palo Alto: Addison-Wesley Publishing Company Inc., 1962) 70*s*.

THIS handbook contains one of the most extensive sets of statistical tables so far published. Its twenty sections include tables relating to all the common distributions, including the multivariate normal and the hypergeometric. Almost one-third of the contents is devoted to non-parametric statistics, with 36 pages given to Wilcoxon tests and 20 to Kolmogorov–Smirnov statistics. Most of the tables have been specially computed; the methods of computation are briefly described, some indications of the use of each table are given and references are made to a bibliography with 251 entries. Some information is conveyed in the form of graphs or charts, a practice which could usefully have been extended.

Almost all the tables have been reproduced photographically from the output sheets of digital computers, with the aim of eliminating transcription errors. Legibility is good and adequate headings have been inserted by hand. The most serious drawback is the small format; the page size is only 9 in. × 6 in. and the type-area is usually considerably smaller, so that most tables extend over several pages.

The author states that, in the choice of tables, he has stressed inaccessible material at the expense of that readily available and himself points out that very little space is given to (for example) the Poisson distribution. The normal distribution provides another example—the usual quantities P and Z, together with Q/Z, P/Z and the first three derivations of Z are tabulated for $X = 0$ (0·01) 3·99, but the inverse tables giving X and Z as functions of P are so sketchy as to be of little but illustrative value. There are no tables of working values and weights for maximum likelihood probit calculations, or for the other common transformations of proportions, even though the table of $\sin^{-1}\sqrt{p}$ is to $5S$ and occupies ten pages. In all, this will be a useful volume on the library shelf, but not one likely to supplant existing collections of tables for day-to-day use.

M. J. R. HEALY

SHORT REVIEWS

A Journey Through Space and the Atom

A Course of Selected Lectures in Astronomy, Space Rocketry and Physics. Edited by Prof. S. T. Butler and Prof. H. Messel. Pp. 495. (Sydney. Shakespeare Head Press, Pty., Ltd., 1962.) n.p.

THIS volume records the texts of four sets of lectures given at a Summer Science School at the University of Sydney in January 1962, and intended for "fourth-year High School students". The first contribution, by Prof. H. Bondi, "The Structure of the Universe" (77 pages), is a satisfying introduction to relativity and cosmology; the second is a lively speculative essay on "Life in the Galaxy" by Prof. R. N. Bracewell (12 pages). In the third contribution, of 181 pages, Prof. W. von Braun provides a lavishly illustrated guide to "Space Rocketry", which has particularly valuable sections on electric propulsion and on the U.S. *Saturn* rocket programme. Finally, the editors, Profs. S. T. Butler and H. Messel, contribute 209 pages on "Elementary Atomic Physics and Applications of Atomic Energy", which, according to their preface, constitute a slightly condensed version of a text already published in two previous books of theirs, to which they frequently refer.

Although the individual contributions can confidently be recommended, the book as a whole has many flaws. The sections differ in tone and treatment and are unco-ordinated, so that repetition occurs and obvious cross-references are not made Some lectures are apparently printed verbatim: "located right there in Huntsville, Alabama" comes well from a lecturer with map and pointer, but reads oddly. Some of the visual aids, too, are unsuitable for a book, for example, the half-page illustration consisting of: (1) the words 'Rocket Propulsion', (2) a diagram of a large key, and (3) the words 'Key to Space'. Other irritations abound: the photographs occupying pages 182, 214 and 279 are identical; there is no index or price; misprints, etc., are unduly numerous. These editorial deficiencies inevitably detract a little from the worth of the book.

D. G. King-Hele

Advances in Cryogenic Engineering

Vol. 7: Proceedings of the 1961 Cryogenic Engineering Conference, University of Michigan, Ann Arbor, Michigan, August 15–17, 1961. Edited by K. D. Timmerhaus. Pp. x + 582. (New York: Plenum Press, Inc., 1962.) 17.50 dollars.

IN the latest volume of *Advances in Cryogenic Engineering* a new store of authoritative information on topics of low-temperature physics and engineering has been released. Almost all the 73 papers given at the conference are printed, together with brief summaries of the discussions. Comparing successive volumes there is evidence of gradually increasing quality. Most of the contributions are from American industrial sources although about 20 come from U.S. Government laboratories. Very few emanate from universities.

Among the four invited papers it is pleasing to note that one describes the work of Sixsmith on miniature turbines which he originated at the University of Reading and later took to the Boulder Laboratory. The other three papers deal with the contribution of cryogenic calorimetry to solid-state chemistry, the U.S. Helium Conservation Programme and, finally, the behaviour of cryogenic liquids in the absence of gravity.

The contribution of cryogenics to the 'space race' is evident in several groups of papers under the headings "Space Simulation", "Equipment" and "Heat Transfer Phenomena". Detailed results and design methods are given relating to most aspects of the storage, pumping, and transfer of liquid oxygen and hydrogen.

There is a significant and welcome increase in the number of papers dealing with low-temperature phase equilibria—including the improbable mixture oxygen/hydrogen. There are also further valuable additions to the published data on the physical properties of metals and non-metals at low temperatures, including adhesives and elastomers. Those who hope to find in the latter paper details of a new wonder material maintaining elasticity at low temperatures will be disillusioned. They will find, however, an exact design method for 'O' ring seals which will probably remain gas-tight on cooling. The volume concludes with a useful cumulative subject index embracing Volumes 6 and 7.

G. G. Haselden

Fundamentals of Electrical Science

By C. T. Baldwin. Pp. 336. (London: George G. Harrap and Co., Ltd., 1961.) 25*s*. net.

THIS book is intended as an introduction to the subject of electrical measurements for Higher National Certificate students and should meet their need extremely well. The author succeeds in maintaining a uniform standard throughout, and the selection of material is well chosen. There is an adequate number of problems, either worked out fully in the text or given at the end of each chapter, for the student to attempt. Numerical answers are given at the end of the book.

It is inevitable that if the book is to meet the need for which it was written it should lack surprises. The merit is in the presentation rather than in the originality of the material. After a chapter on units and standards, the various types of deflecting instruments are described in seven chapters with a further chapter on integrating meters.

Then follows d.c. and a.c. bridge measurements, electronic instruments, high-frequency, high-voltage and magnetic measurements. Instrument transformers have a chapter and there is a final chapter on calibration of measuring equipment.

Electrical measurement of non-electrical quantities receives no more than three pages as an appendix and the bibliography refers only to books, fifteen in all, and none more recent than 1958. Transistors do not appear to be mentioned.

This, then, is scarcely a book to stimulate the imagination and to encourage further reading. It is a workman-like book designed to help students through their examinations. It appears admirably suited to this purpose, and should be sure of considerable popularity.

A. H. M. Arnold

Zahlenwerte und Funktionen aus Physik, Chemie, Astronomie, Geophysik und Technik

Von Landolt-Börnstein. Sechste auflage. Herausgegeben von verschiedenen Fachgelehrten. II Band: Eigenschaften der Materie in ihren Aggregatzustanden. 4 Teil: Kalorische Zustandsgrössen. Bearbeitet von verschiedenen Fachgelehrten. Herausgegeben von K. Schäfer und E. Lax. Pp. xii + 863. (Berlin: Springer-Verlag, 1961.) Moleskin geb. 438 D.M.

THIS is the fourth of the ten parts planned for Volume 2 of the whole work, now in its sixth edition, and covers a subject area which occupied less than a hundred pages of the fifth edition and its supplements. The tables include chronological summaries of the values that have been obtained for the fundamental constants of thermochemistry, such as the mechanical equivalent of heat, the universal gas constant and Planck's constant. The majority of the book is devoted to values for molar heat, entropy and enthalpy of selected inorganic and organic substances, including their dependence on temperature. A useful summary is given of methods of calculation of undetermined thermodynamic functions of organic compounds from consideration of molecular vibrations. Included also are the Joule-Thomson and isothermal Drossel effects, the magnetocaloric effect in relation to paramagnetic salts at very low temperatures and thermodynamic functions of mixtures and solutions. The absolute joule is used throughout, in accordance with the recommendations of the ninth General Conference on Weights and Measures (1948). The copious literature references are recorded up to 1959, and are therefore more complete than in any comparable work at present in publication.

As the price of 438 D M. for this part alone suggests, purchase of the whole work will be an expensive proposition, feasible only for the larger libraries; but the part under review is self-contained, and will be of great value as a source of primary information to all users of thermodynamic data. S. P. COOPER

Volume and Integral

By Prof. Werner W. Rogosinski. Second edition. (University Mathematical Texts.) Pp. xi + 160. (Edinburgh and London: Oliver and Boyd, Ltd.; New York: Interscience Publishers, Inc., 1962.) 10*s*. 6*d*.

THE author's preface to this second edition states: "Only a few minor inaccuracies in the text have come to my notice. These, together with some misprints, have now been amended. Otherwise, apart from occasional added remarks, the new edition is unchanged".

There is consequently nothing which I can usefully add to my review of the first edition of this book (*Nature*, 173, 608; 1954). I still have the same high opinion of it as an excellent introduction to content, measure, and the Riemann and Lebesgue integrals.

R. G. COOKE

Curvature and Homology

By Samuel I. Goldberg. (Pure and Applied Mathematics: A Series of Monographs and Textbooks, Vol. 11.) Pp. xvii + 315. (New York and London: Academic Press, 1962.) 68*s*.

THE study of the relations between the local and global properties of a surface, beginning with the Gauss-Bonnet theorem, widened considerably with the application of tensor analysis to generalized spaces, and received a further stimulus about 1940 by the now classical work of Hodge on harmonic forms and Betti numbers; Bochner, Yano and Lichnerowicz have been responsible for much research in this field, where the calculus of exterior forms and the theory of Lie groups are important tools. The theme of Goldberg's book is the relationship between the curvature properties of a Riemannian manifold and its homology structure; in the earlier chapters the local structure and the topology of a differentiable manifold lead to a discussion of families of Riemannian manifolds. Then complex manifolds are set up on lines suggested by Weil, and the introduction of a special type of metric defines a Kähler manifold. An appendix gives a proof of the fundamental existence theorems of de Rham, again using some of Weil's ideas and Leray's sheaf theory.

The book is one for the young specialist rather than the general mathematical reader. The author hopes that those who have had standard courses in linear algebra, real and complex variables, differential equations, and point-set topology will be able to follow his arguments. But this seems a somewhat slender basis for a fairly stiff task, and the reader would be well advised to have a reasonable knowledge of modern work on Riemann surfaces (that gleaned from G. Springer's book would suffice), and at least a nodding acquaintance with the calculus of exterior forms and some algebraic topology; granted this, he will find the book a valuable survey of recent work and of probable lines of future progress.

T. A. A. BROADBENT

Boundary and Eigenvalue Problems in Mathematical Physics

By Prof. Hans Sagan. Pp. xviii + 381. (New York and London: John Wiley and Sons, Inc., 1961.) 76*s*.

THIS book has a refreshing approach to its subject-matter. Mathematical methods, as used in various branches of applied mathematics and theoretical physics, have come in recent decades to consist largely of a collection of analytical tricks and techniques, and there appears to be little unity underlying the myriad of special recipes available in certain fields. But in this book only a few basic concepts are used, such as Hamilton's principle and the calculus of variations or Bernoulli's separation method for solving certain partial differential equations. The student is expected to master these topics and then a unified background exists for the methods of solving problems in fields such as vibration, heat conduction and wave motion.

After a chapter on the calculus of variations there follows a careful account of the theory of the vibrating string and membrane and a treatment of the equations of potential and heat conduction. There is a good chapter on Fourier series, including some of the theory of the series, as well as the details of various applications, for example, the plucked string.

The next chapter is on self-adjoint boundary value problems and this is followed by one on Legendre polynomials and Bessel functions. In an important chapter on the characterization of eigen-values by a variational principle the minimum properties of eigen-values are discussed thoroughly and the Rayleigh-Ritz method is introduced. The last two chapters are on spherical harmonics and the non-homogeneous boundary value problem.

Lecturers in applied mathematics usually find it difficult to streamline their courses so that in the limited time available there is a reasonable coverage of material. The author of this book has solved this problem nicely (the book is based on courses of lectures given over a number of years) in that he has combined a fairly wide coverage with an essential unity of approach. L. S. GODDARD

Hyperconjugation

By Prof. Michael Dewar. Pp. vi + 184. (New York: The Ronald Press Company, 1962.) 6.00 dollars.

HYPERCONJUGATION is the name given to a series of chemical effects. These begin with the observations by Baker and Nathan that an alkyl group can influence the rate of a chemical reaction, and then go on to the further effects of such a group (for example, methyl) on bond-lengths, ultra-violet spectra, heat of formation and other measurable properties of a molecule. It is as if the alkyl group could conjugate with a double or triple bond to which it was attached. But it has recently become very difficult to sort out how much of all this is truly a 'resonance' situation, how much an 'inductive' one, and how much is simply the result of changes of hybridization on one or more of the carbon atoms. Neither theory nor experiment is sufficiently clear-cut, and much confusion surrounds the whole topic.

Prof. Dewar has done a most useful job in setting out the evidence, from as many different points of view as possible. Each chapter of this book is concerned with one aspect of hyperconjugation. First, he examines the arguments in favour of hyperconjugation, and then he shows how inconclusive most of them are. One is left with the feeling that except perhaps for free radicals and certain carbonium ions, the concept has been rather over-emphasized during the thirty years of its existence.

The writing and general style of this book bear the unmistakable imprint of the author's wide knowledge and tremendous enthusiasm. Despite occasional evidence of over-hasty preparation, this is easily the best available account of a very puzzling chemical phenomenon. C. A. COULSON

Microdiffusion Analysis and Volumetric Error

By Prof. Edward J. Conway. Fifth revised edition. Pp. xviii + 467. (London: Crosby Lockwood and Son, Ltd., 1962.) 42s. net.

MICRO-DIFFUSION analysis and Prof. E. J. Conway are almost synonymous, and it is fortunate that he and the publishers are prepared to bring this book up to date at fairly regular intervals. The method is extremely elegant, eminently suited to the determination of micro-amounts, provided that the substance sought can participate in a reaction in which a gas or vapour is quantitatively liberated. For some tastes, the time required for a single determination is rather long, but with serial determinations this is reduced to a few minutes. For this and other reasons, the most important applications are in clinical and biochemical analysis. Most of the new material incorporated in this fifth edition consists of methods and applications in this field.

The second part of the book, that dealing with the errors of volumetric analysis, remains unaltered. Recently, there has been a renewal of activity in this subject, witness the work of Bishop and of Dean, and the alteration in 1961 of the British Standards method for emptying pipettes. Much of this work no doubt stems from Prof. Conway's exposition, which is a classic and should be read by all analysts. The publishers have maintained the high standard of production of previous editions of this unique book. J. F. HERRINGSHAW

Basic Principles of the Tracer Method

Introduction to Mathematical Tracer Kinetics. By Prof. C. W. Sheppard. Pp. xviii + 282. (New York and London: John Wiley and Sons, Inc., 1962.) 60s.

IT should be clearly understood that this book does not attempt to provide information about the techniques available for the use of radioactive and non-radioactive substances as tracers. It is assumed that the reader is already familiar with the basic principles involved, and the book is devoted to an account of the ways in which experimental results can be treated in given systems. The book is thus largely mathematical in its description and handling of various experimental situations.

A treatment is given for one, two, three and multi-component systems of both closed and open types and also for continuous flow systems and membrane processes. These mathematical analyses are clearly and logically developed and are illustrated by figures and diagrams. One chapter is devoted to a description of analogues (electrical and non-electrical) for the various systems and to a discussion of the use of analogue computer techniques.

The mathematical models arise, of course, from real systems which are referred to in the text, and the examples chosen come from the field of physiology and deal largely with the transport of body fluids.

This volume can be recommended to those interested in, and concerned with, the use of tracers in biological systems and should form a useful reference book. It is clearly written, well produced and good value. J. C. ROBB

The Third Law of Thermodynamics

By J. Wilks. (Oxford Library of the Physical Sciences.) Pp. viii + 142. (London: Oxford University Press, 1961.) 15s. net.

THIS monograph contains chapters dealing with the statistical aspects of entropy and others in which various chemical and low-temperature phenomena are discussed in terms of statistical entropy. I had managed, with no great resolve, to do without the Third Law, and therefore welcomed the opportunity of reading Dr. Wilks's book, if only to find out why feelings about the Third Law seem to run so deeply. I now know that the Law provides a test of whether a system will do anything interesting if cooled to lower temperatures. For example, a magnetic material which obeys Curie's law to the lowest temperatures explored must begin to deviate from this at lower temperatures because the Third Law requires that magnetic susceptibility should be independent of temperature at absolute zero. When Dr. Wilks goes beyond this kind of conclusion, as he generally does by the introduction of statistical mechanical ideas, the relevance of the Third Law becomes confused. He points out that the statistical basis of the Third Law is simply the contention that the ground-state of a system is non-degenerate, or at least only somewhat degenerate.

In a situation where one can turn to statistical descriptions this seems to me a more explicit assumption than the Third Law itself. The Third Law (like the other two) should be useful mainly when microscopic knowledge is unavailable or finally irrelevant, and in overlaying most of the topics in the book with statistical mechanical discussions, Dr. Wilks is paying tribute to the Third Law on the cover but deserting it inside. If the applications of the Third Law as such are not very fruitful or exciting this might be the wisest course, and in this light the book is an interesting compendium which includes phenomena (like the behaviour of glasses) which only rarely find their places in text-books.

D. H. MARTIN

South African Animal Life

Vol. 8. Edited by Bertil Hanström, Per Brinck and Gustaf Rudebeck. (Results of the Lund University Expedition in 1950–1951.) Pp. 557. (Stockholm: Almqvist and Wiksell, 1961.) 75 Swedish kr.

THE eighth volume of results of the Swedish Expedition to South Africa during 1950–51 again contains contributions by workers from a number of countries. South Africa itself is represented by H. Andreae (Coleoptera : Cossyphodidae), A. C. van Bruggen (Diptera (Brachycera) : Diopsidae), J. C. van Hille (Coleoptera : Anthicidae), and B. R. Stuckenberg (Diptera (Nematocera) : Thaumaleidae). Swedish contributors are H. Kauri (Opiliones) and B. Tjeder (Neuroptera Planipennia IV : Hemerobiidae). G. Heslop-Harrison (England) deals with the Hemiptera (Homoptera) : Psyllidae and R. Linnavuori (Finland) with the Hemiptera (Homoptera) : Cicadellidae. The Hemiptera (Heteroptera) : Anthocoridae are described by J. Carayon (France), the Coleoptera (Staphylinidae) : Paederinae, by G. Fagel (Belgium), the Hymenoptera (Aculeata) : Vespidae by A. G. Soika (Italy), and the Diptera (Brachycera) : Nemestinidae by F. M. Hull (United States).

The volume is—like its forerunners—excellently produced, and the illustrations are of a commendably high standard. The numerous zoo-geographical maps deserve special mention.

O. LOWENSTEIN

Soil Animals

By Prof. D. Keith McE. Kevan. (Aspects of Zoology Series.) Pp. xv + 237 + 5 plates. (London: H. F. and G. Witherby, Ltd., 1962.) 30*s*. net.

THIS is an introduction to the zoological side of the complex and rapidly expanding subject of life in the soil. It brings up to date the excellent book, *Soil Zoology*, edited by Prof. Kevan in 1955, which has done much to stimulate interest in soil animals. Though not so exhaustive as Prof. Kuhnelt's book, *Soil Biology*, it will serve well those who require a general view of the subject. It is unfortunate that the extremely full and competent account of how to collect animals from soil samples is not accompanied by an equally full account of the statistical problems encountered in population estimation. Many students of soil zoology to-day are more than a little perturbed by the unwillingness or inability of some soil fauna ecologists to use even simple statistical methods.

In the penultimate chapter a praiseworthy attempt has been made to describe how agriculture and land use affects soil animals. Perhaps it is because soil biology is still in its infancy that this and the final chapter, where the effects of animals on the soil are discussed, do not have the clarity of the rest of the book. Selection of the references could have been more critical and the addition of recent work on soil insecticides would have helped. In spite of all the work done, it is still impossible to explain the precise part played by soil animals in the cycles and processes of soil fertility, and to express the significance of their presence in terms of crop yield.

This book will show many people that soil animals are a group the further study of which must be rewarding.

G. W. HEATH

British Miniature Electronic Components and Assemblies Data Annual, 1962–63

Edited by G. W. A. Dummer and J. Mackenzie Robertson. Pp. xvii + 1140. (London and New York: Pergamon Press, 1962.) 140*s*.; 25 dollars.

THE ever-increasing use of the transistor in electronic circuits has in recent years stimulated the development of a wide variety of miniature components and devices of comparable size, and much of the circuit designer's problem is to know what is available. The present annual has been produced to help overcome this problem, and it is envisaged that, together with its two companion annuals covering transistors, diodes and semiconductors, and miniature and sub-miniature valves, it will provide a complete designer's library. The word 'miniature' has been interpreted to mean less than 1 cubic inch in volume, although some larger items have been included which are the smallest of their type.

Since its first appearance in 1961 the annual has more than doubled in size, listing a variety of components not included in the first volume. These include, among many other devices, accelerometers, batteries, crystal devices, fans, hydrophone assemblies, motors, neons, pot cores, semiconductor networks, solid circuits, strain gauges and warning devices, as well as larger sections on those components included originally.

In the new edition the acknowledgments to manufacturers is replaced by a more useful "Index to Manufacturer's Products", but no prices are included.

E. M. DEELEY

Annual Review of Entomology

Vol. 7. Edited by Edward A. Steinhaus and Ray F. Smith. Pp. viii + 536. (Palo Alto, Calif.: Annual Reviews, Inc., 1962. Published in Co-operation with the Entomological Society of America.) 7 dollars.

THE seventh volume of this now well-established series contains the following reviews: Photoperiodism in insects and mites (J. de Wilde); some physical aspects of insect respiration (J. Buck); metabolic aspects of insect diapause (W. R. Harvey); entomological aspects of radiation as related to genetics and physiology (D. S. Grosch); chemical defenses of arthropods (L. M. Roth and T. Eisner); the comparative anatomy of the insect nervous system (J. B. Schmitt); genetics of sex determination (W. E. Kerr); control systems of orientation in insects (H. Mittelstaedt); microclimates and the distribution of terrestrial arthropods (J. L. Cloudsley-Thompson); dispersal and migration (F. Schneider); use of mathematics in population ecology (K. E. F. Watt); ecology of aquatic insects (T. T. Macan); biology and ecology of predaceous Coccinellidae (K. S. Hagen); ecology of Scolytidae (J. A. Rudinsky); significance of parthenogenesis in the evolution of insects (E. Supmalainen); the integration of chemical and biological control of arthropod pests (R. van den Bosch and V. M. Stern); trends in applied biological control on insects (B. P. Beirne); the bionomics and control of *Culicoides* and *Leptoconops* (D. S. Kettle); mosquito behaviour in relation to disease eradication programmes (P. F. Mattingly); uses of bioassay in entomology (W. M. Hoskins and R. Craig); detoxication mechanisms

(J. N. Smith); some recent advances in apicultural research (G. F. Townsend and R. W. Shuel).

The editors in their preface speak of "critical and penetrating reviews in contrast to routine summary accounts", and in the present volume reviews of this type predominate. Indeed, for the general entomologist, this is perhaps the most useful collection that has appeared in this series. Most of the articles are on general topics, and the few that deal with restricted groups are full of ideas that might be applied to other insects of similar habit.

In this volume the clarity of the writing is much better than in some earlier *Reviews*. For example, the account of control systems of orientation in insects, which could easily have become baffling, is presented most lucidly; while the statement in the discussion of mathematics in population ecology that "Because Volterra's model is based on an inadequate set of assumptions it is of no use in showing how best to control insect pests, and in fact is downright misleading in this regard" could scarcely be more direct.

H. Oldroyd

Acrolein

Edited by Dr. Curtis W. Smith. Pp. ix + 273. (New York and London: John Wiley and Sons, Inc., 1962.) 94*s*.

THIS little book is a complete treatise on the chemical aspects of acrolein. The fifteen authors have spared no efforts in making sure that the information is complete, accurate and up to date. The whole is based on a review of published works and patents up to January 1960, and is supplemented by unpublished data made available by several major laboratories.

The book is divided into small, well-written chapters, liberally sprinkled with tables, figures and formulæ with what must be a fairly complete list of references, some 870 in all. With so many contributors, the editor has done well in maintaining continuity of thought and purpose, possibly due to the fact that he and his authors are all members of the same commercial organization.

The history and methods of preparation are briefly dealt with, followed by an extensive treatment of the physical and chemical properties, concluding with a chapter on analytical methods. There is a very useful section on the handling of the substance and its toxicology.

The book is a little too expensive for its size and this will tend to discourage all but those actively engaged in the field from buying it. This, however, does not detract from the fact that this is a mine of information for anyone even vaguely interested in the subject.

W. Forbes

Progress in Experimental Tumor Research

Vol. 2. Edited by F. Homburger. (Fortschritte der Experimentellen Tumorforschung, Band 2.) Pp. xiii + 386. (Basel and New York: S. Karger, 1961.) 72 Swiss francs.

THE subjects covered by this book were selected after scrutiny of the replies to a questionnaire sent to members of certain learned societies in countries all over the world. At first sight, this seems to be a splendid way of planning a book of this kind; however, it has inherent drawbacks. Already the 'band wagon effect' looms dangerously in the determination of programmes of work. For publishers to aid and abet this process by providing the scientific public with the kind of scientific news they want can only make the situation worse.

The ten chapters cover a variety of subjects with varying degrees of success. Those on the carcinogenicity of tannins (Korpassy) and beryllium (Schepers) are useful but diffuse, and that entitled "Critique of Carcinogenic Mechanism" (Nakahara) cuts more corners than it explores. A progress report on cancer chemotherapy (Sugiura) is too incomplete to be of much use to those working in the field. There is a competent and admirably brief survey of human hormone-dependent tumours (Boyland), and a rather longer chapter (Kirby) in which the hypothesis that a "DNA-DN-polymerase functional unit" provides the basis for the progressive series of changes leading to malignancy is put forward. Amano's virus classification based on host-virus relationships acts as a pleasant stimulant after Luse's rather dull conclusion that "electron microscopy has failed to reveal any characteristic peculiar to neoplastic cells and absent from all normal cells". The chapters by Ham and Siminovitch on carcinogenesis by the polyoma and other viruses, and by Bern and Nandi on the influence of hormones on the induction of mammary tumours in mice, are excellent.

All in all this is an interesting volume and one full of useful references.

F. J. C. Roe

Die Cactaceae

Handbuch der Kakteenkunde. Von Curt Backeberg. Vol. 6: Nachträge und Index. Pp. 3545–4041. (Jena: Gustav Fischer Verlag, 1962.) 82.70 D.M.

"EVEN the weariest river winds somewhere safe to sea", and this is true, if only for the moment, even of the indefatigable Herr Backeberg. The sixth, and last, volume of his *Handbuch* to the Cactaceae consists mainly of several hundreds of pages of addenda, corrigenda and supplementary matter. They are preceded by a paper, by G. D. Rowley, on the genealogy of the large-flowered cactus hybrids. Finally, in a postscript, the author discusses several problems such as that of the typification of species, a difficult question because of the nature of succulents, and he urges the advantages of "photographic type material". The index of names, which excludes those of hybrids, carries us from page 3,919 to page 4,041, the end of this truly colossal work.

N. Y. Sandwith

Museum Publications

A Classified List and Index of Books, Pamphlets and other Monographs, and of Serial Reprints. Part 2: Publications in Biological and Earth Sciences. By Jane Clapp. Pp. 610. (New York: The Scarecrow Press, Inc., 1962.) n.p.

THIS is a massive production which certainly indicates the wealth of the publications issued by the museums of the United States and Canada. No other country in the world could even approach such an output. The book comprises a classified bibliography of the publications available from 276 museums. The present volume is Part 2 of the complete work and refers to 9,231 publications on the biological and earth sciences. Included are books, pamphlets, monographs and serial reprints, but excluded are the publications of the United States National Museum and the National Museum of Canada, administrative reports and similar works.

The book includes a complete list of the museums now distributing publications, and this is followed by an alphabetical list of the same museums arranged according to a symbol, for example, 'AkHi' is the Alaska Historical Library and Museum. Books of a general character head the next list and this is followed by details of the literature on the biological and earth sciences arranged according to subject-matter. References to both authors and subjects are included in the index.

Such a book will be of service to many and especially to those engaged in research for it will facilitate the use of authoritative information sources. It will also give much welcome publicity to the museums concerned and bring to the notice of teachers, students and librarians the wealth and variety of museum publications. F. S. WALLIS

Rembrandt and Spinoza

By Dr. Leo Balet. Pp. x+222+4 plates. (New York: Philosophical Library, 1962.) 4.50 dollars.

THE author of this book is an art historian, born in Rotterdam, who has had wide experience in Europe, culminating in the tenure of an official position in Bremen. His subject is seventeenth-century Holland, with its vehement tensions, political, ecclesiastical and artistic. Much of what he has to say is of interest, and well documented. Unluckily, however, his enthusiasm leads him to write a form of English which, in parts, is almost unintelligible, and made still worse by numerous, and most inappropriate, colloquialisms. More serious is an obsession for polemics, enough to weary the patient reader.

The central theme is the battle for freedom, both intellectual and artistic; hence the two great figures, Rembrandt and Spinoza. Neither was prepared to come to terms with society as he found it. Rembrandt exposed human character with a ruthlessness almost unrivalled, whereas Spinoza carried the quest for truth to a degree of refinement seldom, if ever, equalled. There is always a certain heroism about Jewish philosophy, and Spinoza's thought—atheistic as it was—is in line from Avicenna via Maimonides. Where the two great men of the Netherlands resemble one another is in the totality of their message. To dissect the genius of either of them is to destroy it. The drawings of Rembrandt illustrate this better than the well-known pictures which Dr. Balet describes.

If one could penetrate beneath the tiresome style, there is substantial erudition to be discerned, especially the attempt at the end to see the essentials in terms of the algebra of relations. Here, a capital Y seems to be missing, which makes the demonstration not too clear. F. I. G. RAWLINS

Scientific Russian

By Dr. A. Holt. Pp. xv+195. (London: Chapman and Hall, Ltd., 1962.) 36*s*. net.

TO begin with outward appearance, this book is clearly printed on good quality glossy paper, an important fact for the novice meeting the strange Cyrillic alphabet for the first time. The discreet use of dark and light types is also an aid to comprehension and to memorizing.

Part 1 (pp. 1–78) contains 22 lessons, dealing systematically with the main features of Russian grammar, each lesson being illustrated by a passage from an appropriate scientific text. The grammatical survey begins with nouns and adjectives and their declensions and ends with verbs with their aspects, tenses, participles and gerunds. The illustrative Russian passages for reading and translation are all taken from works on chemistry and they have been chosen with care and understanding.

Part 2 (pp. 79–195) contains short notes on transliteration and Russian chemical nomenclature, a concise summary of grammar, 37 translation exercises drawn from the fields of chemistry, physics and biology and finally a glossary and index.

This book undoubtedly has good qualities but it invites criticism by the very fact of its aiming to teach 'scientific' Russian. Much of this 'scientific' Russian is in fact an international language which every scientist, with the ability to transliterate from the Cyrillic alphabet, could, in fact, guess. The most useful approach for a scientist wishing to read or translate Russian scientific literature will always be an elementary knowledge of the Russian language and access to a good dictionary of scientific terms—there are dictionaries for each branch of science.

Accepting the fact that there is a public which demands text-books of scientific Russian, this one is a good one and to be recommended especially for chemists, for whom it is primarily compiled.

S. I. TOMKEIEFF

Essays on Probability and Statistics

By Prof. M. S. Bartlett. Pp. viii+127. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1962.) 21*s*. net.

THIS welcome little volume contains eight survey articles and occasional lectures written by Prof. Maurice Bartlett during the period 1949–56. Some of these have previously been published in the records of symposia and the like, but to most readers not on the author's mailing list the material collected here will be new. The author considers that they represent collectively a consistent and comprehensive view of statistical theory, whatever the field in which it may be applied. As such they are of special value because of the author's unique preoccupation with both the dogmas of statistical inference and the applications of probability theory (and especially stochastic processes) in biological and physical science.

The first article (a reprint from *Dialectica*) is a review of the foundations of probability theory and is remarkable for a clear and early statement of the true role in the frequency theory of the strong law of large numbers (forcing on us an interpretation for probabilities $p < 1$, once the interpretation of $p = 1$ has been admitted). The second article (a talk to the Manchester Statistical Society) has some historical interest for its account of the controversy in 1944 when the Royal Statistical Society was criticized in some quarters for its (successful) attempt to institute a diploma examination. The third article, on factor analysis in psychology, is from an Uppsala symposium.

Two articles on stochastic processes follow: one an extremely readable general survey and the other, more technical, concerned with the difficult estimation and testing problems in this area. The book concludes with an address delivered before the third Soviet Mathematical Congress in Moscow, with a study of irreversibility from the statistical point of view, and with a brief comment on the present controversies surrounding the subject of statistical inference. DAVID KENDALL

(*Continued from page 840*)

action of *nor*adrenaline persisted for some time (up to 3 min) after the passage of ions had been stopped (Fig. 1); but this effect was less marked when smaller currents were passed through finer electrodes (Fig. 2).

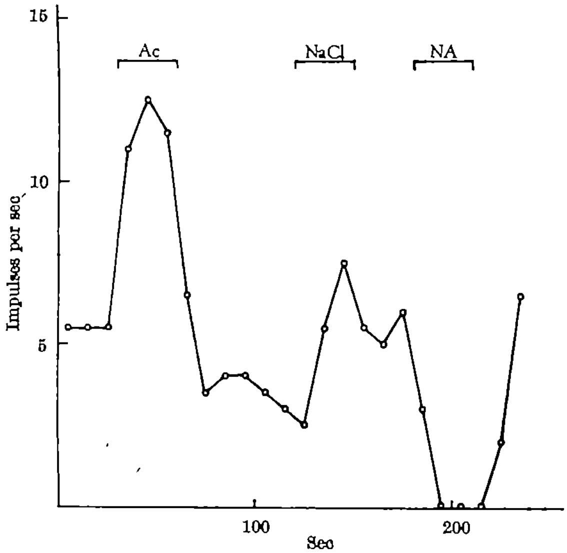

Fig. 2. Acetylcholine and sodium chloride applied with currents of 25 n.amp, *nor*adrenaline with 12 n.amp (electrode tip 2μ)

Acetylcholine was tested on 111 neurones, of which 30 (27 per cent) showed a response, 25 (22 per cent) being excited and 5 (5 per cent) being inhibited. Examples of these effects are shown in Figs. 1 and 2. The action of acetylcholine was usually more rapid in onset than that of *nor*adrenaline and rarely outlasted the period of application by more than 10 sec. These results with acetylcholine thus confirm the findings of Salmoiraghi and Steiner[7].

Interesting results were obtained when both drugs were tested on the same neurone. Ninety-three neurones were tested in this way and 22 (24 per cent) were affected by both *nor*adrenaline and acetylcholine. The majority of these, thirteen, were excited by both, but nine were excited by one drug and inhibited by the other (Figs. 1 and 2). These results are summarized in Table 1. All the results described here could be obtained consistently, that is, repeated applications of the drugs to the same neurone produced the same effects. They thus support the findings of Bradley and Mollica[11] using intracarotid injections.

Table 1. SUMMARY OF THE RESPONSES OF NEURONES TESTED WITH BOTH ACETYLCHOLINE AND *nor*ADRENALINE

		*nor*adrenaline		
		+	−	0
acetylcholine	+	13	5	5
	−	4	0	1
	0	12	0	44

+, Excitation; −, Inhibition, 0, no effect or doubtful effect.

In these experiments no attempt has yet been made to identify the type of neurone physiologically but to judge from the electrode positions and the fact that most of the neurones responded to somatic stimulation over a large area of the body, it is likely that many of them were reticular. It is interesting to note that some of the neurones inhibited by one drug and excited by the other were of the type that are inhibited by some somatic stimuli and excited by others[12].

The relevance of these results to chemical transmission in this region of the brain remains to be examined, although they suggest that cholinergic and adrenergic receptors may be present on the same neurones.

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MUTAGENIC ACTIVITY OF CELLULAR MACROMOLECULES IN *DROSOPHILA MELANOGASTER*

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THE mutagenic activity of deoxyribonucleic acid (DNA) in *Drosophila* has now been conclusively demonstrated[1], especially as regards the induction of small chromosome deletions resulting in the *Minute* phenotype. On the other hand, it was not possible to induce transformation at specific gene loci by DNA extracted from organisms with the wild-type alleles. Clearly, therefore, the extracted DNA lacks the specific organization of metazoan genes and is accordingly unacceptable for incorporation into the functional genome. Mutagenic activity was not restricted to any specific DNA organization: heterologous samples from rat liver as well as from bacteriophage *T*4 were found to be active. This raised the question as to whether the DNA type of mutagenesis could also occur with other biological macromolecules. Genetic tests were accordingly undertaken with deoxyribonucleoproteins (DNP), ribonucleic acid (RNA) and histones, and the results are presented in this communication.

Drosophila DNA has been successfully extracted from developing eggs (pre-larval embryos) by a method[2] devised by Dr. K. S. Kirby involving the use of diethyldithiocarbamate and phenolphthalein diphosphate. Heterologous DNA, from rat liver and bacteriophage *T*4, were prepared by the usual *p*-aminosalicylate/phenol technique[3]. DNP was prepared from embryonic cells (developing eggs) or larval

Table 1. THE *Minutes* AND SEX-LINKED RECESSIVE VISIBLES INDUCED BY VARIOUS BIOLOGICAL MACROMOLECULES DETECTED IN THE F_1 MALES FROM ATTACHED-*X* EXPERIMENTS. THE TIME AFTER TREATMENT IN DAYS INDICATES THE PROGENY FRACTIONS ANALYSED

Agent	Conc. (per cent)	Days (after treatment)	Males observed	*Minutes* No.	*Minutes* Per 10^3	Rec. visibles No.	Rec. visibles Per 10^3
Control	—	21	74,414	71	0·95 ± 0·11	11	0·15 ± 0·05
DNA: *Drosophila*	0·2	21	30,336	75	2·47 ± 0·28	9	0·30 ± 0·10
	0·5	9	55,178	130	2·36 ± 0·21	18	0·24 ± 0·07
	1·0	15	5,227	59	11·29 ± 1·46	4	0·77 ± 0·38
Rat (liver)	0·1	21	48,933	96	1·96 ± 0·20	13	0·27 ± 0·07
Bacteriophage *T4*	0·25	18	17,882	36	2·01 ± 0·33	3	0·17 ± 0·10
DNP: *Drosophila*	0·5	8	7,313	21	2·87 ± 0·63	4	0·55 ± 0·27
	1·0	15	18,028	63	3·49 ± 0·44	3	0·17 ± 0·10
RNA: *Drosophila*	1·0 (*a*)	15	15,165	32	2·11 ± 0·37	2	0·13 ± 0·09
	1·0 (*b*)	21	46,859	114	2·43 ± 0·23	17	0·36 ± 0·09
Histones: Calf thymus	0·15	21	33,207	137	4·13 ± 0·35	10	0·30 ± 0·09

salivary glands. The tissues were homogenized in isotonic saline and their nuclei were separated by centrifugation through a sucrose gradient. The pellet of nuclear material was extracted[4] with molar sodium chloride and the dissolved DNP was then precipitated by dilution. RNA was prepared from the adult flies by a recently developed procedure[5] involving the use of naphthalene-1,5-disulphonate and phenol/8-hydroxyquinoline. This procedure is now being developed[6] for the consecutive separation of RNA and DNA from the same batch of flies. DNA was found to accumulate at the phenol/aqueous interphase and was later isolated by the *p*-amino-salicylate/phenol method. Histones were prepared[4] from calf-thymus by extraction with dilute hydrochloric acid.

All macromolecules were administered in isotonic saline (0·4 per cent sodium chloride) by micro-injection into the hæmocoel of adult males (30 ± 5 h after eclosion). RNA, histones and DNA (at low concentrations) were in solution; high DNA concentrations were highly viscous, and DNP was in suspension. The treated males were subjected to genetic tests for the detection of the induced mutations. Chromosome fragments and visible mutations (autosomal dominants and sex-linked recessives) were tested for by the attached-*X* technique (with *y*, *v*, *f* markers). The sex-linked recessive lethals and visibles were detected by the Muller-5 method. *Minutes* were scored in the male progeny only of the attached-*X* tests, since slight reductions in bristle size may be difficult to detect in the females over the marker *forked* (a bristle effect), which is present in the test stock. In all experiments the treated males were mated to fresh females at 3-day intervals and the mutation-rate was determined separately in each brood. Breeding was undertaken in mass cultures with a maximum of ten pairs per half-pint bottle.

The mutagenic efficiency of the tested macromolecules as regards the induction of *Minutes* is summarized in Table 1. It can be seen that in all experiments the rate of these mutations was raised significantly above the control-level, indicating a decisive activity in the induction of small chromosome deletions. Whenever different concentrations of the same macromolecule were used, there was clear evidence for a dose response: the mutation-rate increased roughly in proportion to the injected concentration. A notable exception is the experiment with a sample of homologous DNA (0·5 per cent; Table 1), where the mutation-rate was almost equal to that induced by a lower concentration (0·2 per cent; Table 1) of another specimen. The sample with the lower potency was fibrous and highly viscous in solution (gel at 1 per cent concentration) indicating a high degree of molecular integrity. A slight degradation of DNA may, therefore, be conducive to mutagenicity, thus suggesting that the active unit may not be the intact macromolecule.

A consideration of the data with DNA revealed that, at the same injected concentration, roughly the same rate of *Minutes* was induced, irrespective of whether the material was homologous or heterologous. This may not be surprising for the DNA samples from *Drosophila* and rat liver, since their average base composition[3] is nearly the same. Phage DNA, on the other hand, is substantially different in molecular size and base composition; cytosine is represented by 5-hydroxymethyl cytosine—with a glucose residue joined to the hydroxymethyl group—and there is a relative excess in the adenine–thymine pairs. It would seem, therefore, that the mutagenicity of DNA is independent not only of its biological organization but also of its gross chemical composition.

Deoxyribonucleoprotein (DNP) was less effective in the induction of *Minutes* than DNA, giving roughly half its mutation-rate for the same concentration. The proportion of DNA in the DNP samples tested was not determined, but is expected to constitute half its amount, since the extracted material was mainly nucleohistones. The activity of DNP, therefore, appeared to be in the proportion of its DNA content. However, this could be fortuitous, and the mutagenicity of DNP might prove to be a function of the integral molecule, rather than its DNA fraction.

Ribonucleic acid (RNA), while undoubtedly mutagenic, proved to be considerably less effective than DNA in the induction of *Minutes*, giving roughly one-fifth its mutation-rate at the same injected concentration. Histones, on the other hand, were more active than DNA, inducing double the rate of *Minutes* for the same dose. The sample of histones tested was heterologous (from calf thymus); but it is probable that the activity of the homologous material will be roughly the same, since histones do not show any species specificity.

In sharp contrast to the activity of the tested macromolecules in the induction of small chromosome deletions (*Minutes*) they proved to be virtually ineffective in the induction of viable chromosome fragments. The hyperploid individuals detected in the female progeny of the attached-*X* experiments with the various macromolecules were as follows: DNA, 2/152,335; DNP, 0/23,103; RNA, 1/57,017; histones, 1/31,741. It would seem certain, therefore, that the tested macromolecules did not induce chromosome breaks to any appreciable degree. The small chromosome deletions they induced, therefore, could not be the outcome of breakage, and must accordingly be due to gene inactivation *in situ*.

Table 2. THE SEX-LINKED RECESSIVE LETHALS AND VISIBLES INDUCED BY VARIOUS BIOLOGICAL MACROMOLECULES DETECTED IN THE F_2 PROGENY FROM MULLER-5 EXPERIMENTS

Agent		Conc. (per cent)	Days (after treatment)	Chromosomes tested	Lethals		Visibles	
					No.	Per 10^3	No.	Per 10^3
Control		—	15	15,807	28	1·77 ± 0·33	2	0·13 ± 0·09
DNA:	*Drosophila*	1·0 (*a*)	18	1,623	8	4·93 ± 1·74	5	3·08 ± 1·37
		1·0 (*b*)	18	2,123	5	2·36 ± 1·05	3	1·41 ± 0·81
	Rat (liver)	0·1	21	3,905	7	1·79 ± 0·68	1	0·26 ± 0·26
	Bacteriophage *T4*	0·25	15	2,478	4	1·61 ± 0·81	3	1·21 ± 0·70
DNP:	*Drosophila*	0·5	3	497	2	4·02 ± 2·84	2	4·02 ± 2·84
RNA:	*Drosophila*	1·0 (*a*)	12	1,334	2	1·50 ± 1·06	3	2·25 ± 1·30
		1·0 (*b*)	18	3,874	6	1·55 ± 0·63	2	0·52 ± 0·37
Histones:	Calf thymus	0·15	21	3,165	5	1·58 ± 0·71	0	0·00

Table 3. THE MUTATION-RATE AS REGARDS *Minutes* IN THE EARLY (0–9 DAYS) AS COMPARED WITH THE LATE PROGENY (> 10 DAYS), INDUCED BY VARIOUS BIOLOGICAL MACROMOLECULES

Agent		0–9 days progeny Post-spermatogonial			> 10 days progeny Spermatogonial			Difference	
		Males	*Minutes*	Per 10^3	Males	*Minutes*	Per 10^3	χ^2	P
DNA:	*Drosophila*	22,222	59	2·66	8,114	16	1·97	1·12	0·28
		3,867	55	14·22	1,360	4	2·94	11·48	0·0007
	Rat (liver)	27,497	60	2·18	21,436	36	1·68	1·55	0·21
	Phage *T4*	14,924	30	2·01	2,958	6	2·03	0·0004	0·98
RNA:	*Drosophila*	14,254	28	1·96	911	4	4·39	2·39	0·12
		28,545	65	2·28	18,314	49	2 68	0·73	0·40
Histones:	Calf thymus	23,559	99	4·20	9,648	38	3·94	0·12	0·73

The induction of recessive 'visible' (morphologically detectable) mutations by macromolecules has also been investigated. The sex-linked recessive visibles scored in the F_1 of attached-X experiments are given in Table 1 and those detected in the F_2 of Muller-5 tests are shown in Table 2. It can be seen that for the same treatment the visible rates in Table 2 are considerably higher than in Table 1. This is entirely due to the superiority of the Muller-5 method in the detection of visibles, since it permits the detection of the less viable and sterile mutants. The extreme rarity of visible mutants and the limited sample of chromosomes that can be tested in a manageable experiment necessarily means that the visible rate is subject to considerable error. This is reflected in the results recorded here in the marked variation in the mutation-rate in replicate experiments and the lack of its association with the administered dose. These results, therefore, do not permit an accurate comparison between macromolecules as regards visible induction, but they are sufficient to indicate which of these molecules are active in this respect. The majority of the experiments with DNA, RNA and DNP showed a significant increase in the visible rate above the control-level, mostly by a factor of 2–5 (Table 1) and occasionally by as much as 10–40 fold (Table 2). Doubtless, therefore, these molecules are effective in the induction of recessive visibles. With histones, however, the attached-X experiment (Table 1) gave a visible rate not significantly above the control-level ($P = 0·15$), and in the Muller-5 test (Table 2) there were no visibles in a sample of well over 3,000 tested X-chromosomes. It is probable, therefore, that histones are ineffective in the induction of point mutation visibles.

The sex-linked recessive lethals occurring in the Muller-5 experiments with the tested macromolecules are given in Table 2. In practically all experiments only the control lethal rate was recovered. The only significant increase in lethal rate occurred with a rather impure sample of homologous DNA (1 per cent; *a*, Table 2), but could not be repeated in a replicate experiment with a pure sample (1 per cent; *b*, Table 2). It would seem, therefore, that biological macromolecules are not appreciably effective in the induction of recessive lethals.

The response of the various stages of spermatogenesis to the mutagenicity of macromolecules was measured by the comparison of the mutation-rates in the early and late progeny of the treated males. The speed of germ line sampling in the successive broods, under the experimental conditions utilized in the present investigation, has already been determined[7]. Post-spermatogonial stages (sperm, spermatids and spermatocytes) are mainly sampled during the first three broods (9 days after treatment). Spermatogonia are partially used, together with early spermatocytes, in the fourth brood (10–12 days), but are mainly recovered in the fifth and later progeny (more than 12 days after treatment). In Table 3 the mutation-rate in the first 9 days progeny is compared with that in later offspring. This gives a rough idea of the relative response of the post-spermatogonial stages to that of spermatogonia. The relative frequencies of mutant to non-mutant males in the two sectors of the germ line in each experiment were compared in a 4-fold contingency table, and a χ^2 for deviation from equality was determined. Where more than one experiment was undertaken with the same macromolecule, the algebraic sum of χ was calculated for the determination of the significance of the general trend in the observed deviations. A glance at Table 3 shows that most of the separate experiments do not manifest a marked difference in the response of the two sectors of the germ line. Only one experiment with homologous DNA showed a lower response for spermatogonia which reached decisive statistical significance. However, a consideration of the data with homologous DNA as compared with RNA reveals a reversal in the deviation trend which may prove significant. In both experiments with DNA, the mutagenicity of spermatogonia was lower than that of the post-spermatogonial stages and the combination χ was decisively significant ($\Sigma\chi/\sqrt{n} = 3·2$; $P = 0·0007$). In contrast, both experiments with RNA showed the opposite trend: the mutation-rate in spermatogonia was higher than in post-spermatogonial stages and the combination χ for this trend was on the verge of significance ($\Sigma\chi/\sqrt{n} = 1·7$; $P = 0·05$). It is therefore probable that RNA mutagenicity is favoured in the metabolically active proliferating spermatogonia.

It has already been pointed out[1] that the mutagenic activity of DNA in metazoan cells is unrelated to the phenomenon of bacterial transformation, in so far as it does not involve a genetic exchange between the exogenous DNA and functional genes. Nevertheless, this does not exclude the possibility that some chemical exchange could have occurred between the administered DNA and that in the genetic material. The DNA incorporated in this way, being physiologically inactive, would afterwards be eliminated from the genome, thus leading to chromosome deletions. If this were the mechanism involved, it would be expected to be more effective the greater is the structural similarity between the administered DNA and that in the recipient cell. In fact, no significant difference was found in the mutagenic activity of homologous DNA as compared to the heterologous material. Furthermore, RNA was also effective in the induction of chromosome deletions, though to a much lower degree. It may still be argued, however, that all these nucleic acids have related structural sub-units (the nucleotides), a situation which might permit chemical exchange on the submolecular level. Indeed, nucleotide exchange may well be the mechanism involved in the induction of recessive visibles, which are assumed to arise through changes in the base order within the genic DNA. Chemical exchange, however, is unlikely to be the mechanism involved in the induction of chromosome deletions (*Minutes*) by nucleic acids, since the phenomenon was shown to occur more effectively with histones. Significantly, these proteins were ineffective in the induction of point mutation visibles, presumably because they cannot affect any nucleotide exchanges.

The demonstration that nucleic acids, nucleoproteins and histones are effective in the induction of small chromosome deletions indicates that the phenomenon is non-specific as regards the inducing molecule. It is highly probable, therefore, that the mechanism involved is physical, operating on the mechanics of chromosome replication. A possible interpretation is that the exogenous molecule becomes 'adsorped' to some locus within the treated chromosome complement. The chromosome template at that locus will accordingly be ineffective, thus resulting in a deletion in the replica at the site corresponding to the 'covered' spot. If this were the mechanism involved, then mutagenic activity (as regards the induction of *Minutes*) would also occur with charged non-biological macromolecules. Experiments are now in progress to test this possibility.

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MALIGNANCY AND TISSUE METABOLISM

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THE characteristic properties of malignant growths, such as their frequent invasiveness, their destructive effects on neighbouring tissues and their proneness to give rise to metastatic lesions in the host, are presumably linked in some yet undefined manner to the metabolic behaviour of these growths. The outstanding metabolic property of tumours is, as is well known, a relatively high aerobic, and anaerobic, glycolytic rate as compared with that in the adult host tissue—a feature clearly seen not only in transplanted tumours in animals but in spontaneous tumours in experimental animals and also in man[1,2]. There is some evidence, too, that the more malignant the cell the greater is its rate of anaerobic glycolysis[3,4]; but it is difficult to establish such a relationship on any quantitative basis.

It would clearly be of service, in further studies of the relationships between tumour growth and metabolism, to throw some light, or to offer some suggestion that would throw light, on the apparent connexion between malignancy and the relatively high glycolytic behaviour of tumour growths.

We propose to demonstrate, in the experimental results recorded here, that a glucose deficiency in the neighbourhood of a normal tissue, which may be caused by a high glycolytic rate in a tumour placed in its vicinity, will bring about an efflux from the normal tissue of amino-acids and possibly other nutrients. Among such substances (depending on the tissue in question) is glutamine, which is readily absorbed and metabolized by the tumour tissue in its neighbourhood[5]. Glutamine is indispensable for optimal protein synthesis[6–8] and growth[9–11] of the tumour. On this view, the high glycolytic rate of a tumour is a means whereby the neoplastic cells bring about an efflux from neighbouring normal cells of nutrients to the detriment of the normal cells but essential for the growth of the tumour itself. Such a view would partly account for the apparent relationship between malignancy and tumour glycolysis.

The general method of investigation adopted in this work has been to expose slices of normal mammalian tissue to the presence of suspensions of Ehrlich ascites carcinoma cells or slices of solid tumours and to observe whether such exposure (by incubation of the tissues together in the conventional Warburg manometric apparatus at 37° for 1 h) results in the loss of amino-acids from the normal tissue and in their accumulation in the tumour tissue. Radioactive glucose is used as the sole organic substrate in these experiments, and analyses are made of the radioactive amino-acids in the normal tissue and in the cancer cells when these tissues are incubated alone or together. The incubations have been carried out in the conventional Warburg manometric apparatus. The normal tissue chosen for investigation has been rat brain cortex slices, as there already exist considerable data concerning the conversion of glucose into amino-acids in this tissue under our experimental conditions[13] and information is also available concerning the factors that influence transport of amino-acids in this tissue[14–16].

Swiss white (CF_1) mice, bearing 6–7 days old Ehrlich ascites cells, were killed by cervical dislocation, and the ascitic fluid was removed with a Pasteur pipette. The peritoneal cavity was washed with ice-cold Krebs–Ringer phosphate medium, *p*H 7·4. The suspension was centrifuged at 800*g* for 20 sec, the supernatant discarded, and the cells were resuspended in cold saline and recentrifuged. The process was repeated until the cell suspension was free of any perceptible blood contamination. The cells were then centrifuged at 800*g* for 2 min to pack them thoroughly, and appropriate dilution of the packed cells was made so as to give 10 mg dry weight of cells in 0·2 ml. of the suspension, which was the quantity used in each manometric vessel.

The solid tumours, namely, Ehrlich carcinoma and Walker 256 carcinosarcoma, were dissected from the animals and chilled in crushed ice. They were then sliced using a Stadie–Riggs slicer. Slices weighing 50–60 wet weight were used in each manometric vessel.

Rats were killed by stunning. The brains were removed and chilled in ice. Slices were taken from the cortex using a Stadie–Riggs slicer. Slices, about 50–60 mg wet weight and not more than 0·3 mm thick, were used in each manometric vessel.

Incubation and chromatographic technique. Incubation media of the following composition were used in all experiments: 145 mM sodium chloride, 1·5 mM potassium dihydrogen phosphate, 1·5 mM magnesium sulphate, 5·8 mM potassium chloride, and 3·6 mM calcium chloride, and sodium phosphate buffer *p*H 7·4 to give a final concentration of 10 mM per vessel. The incubation volume was 1·0 ml. and the final glucose concentration in each vessel was 5 mM. The concentrations of other metabolites are given in the following tables. Radioactive glucose or amino-acids were diluted with the corresponding non-radioactive compounds. Tumour tissues or brain cortex slices were either incubated separately or were put together in the same incubation vessel. The tissues, in the latter case, were separated and assayed separately for their free amino-acid contents by chromatographic techniques. The incubation was carried out for 1 h (except when otherwise stated) in an atmosphere of oxygen, carbon dioxide being absorbed by potassium hydroxide in the centre well of the manometer vessel.

After the incubation period the tissues were washed quickly in ice-cold Krebs–Ringer phosphate medium and placed in 2·0 ml. 95 per cent ethanol. Ehrlich ascites cells, however, were washed by centrifugation and 5·0 ml. 95 per cent ethanol were added to each centrifuge tube. Solid tumours and normal tissues were homogenized and the amino-acids were extracted in 5·0 ml. 95 per cent ethanol for 2 h at 4° C. After the extraction period (30 min), the contents of each tube were centrifuged and the supernatant transferred to a fresh tube. The sediment was extracted twice with 1 ml. 95 per cent ethanol and 1 ml. of distilled water, and all the washings were pooled. The extract was dried at 30° in a current of air. The dried residue was dissolved in 0·5 ml. of 95 per cent ethanol. 0·1 ml. was plated on aluminium planchets and counted in a flow counter to determine the total radioactivity of the extract. The remaining ethanolic extract was spotted on a Whatman No. 1 filter paper for two-dimensional ascending chromatography, using the following solvents: Phase I, *sec* butanol/90 per cent formic acid/water (70 : 11 : 17; v/v/v); Phase II, 90 per cent phenol/water/ammonia (sp. gr. 0·88, 946 ml. : 106 ml. : 1·7 ml.).

After chromatography the papers were dried thoroughly and kept in contact with Kodak Medical ('No Screen') X-ray films for autoradiography, the time of exposure depending on the activity at the origin of the paper. Films were then developed and the radioactive spots were located on the paper. The radioactivity of the spots on the paper were quantitatively measured with a Tracerlab counter with a mica window 28 mm diameter and a thickness of 1·5–1·8 mg/cm². Activities were corrected for background.

All results are expressed as µmg atoms of substrate carbon incorporated into the amino-acid per 100 mg wet weight tissue. The calculations were carried out according to Gonda and Quastel[17]. The values give the means of at least four experimental results and the standard deviations of the means.

Glucose conversion to amino-acids in tumours and normal tissues. Results recorded in Table 1 show that labelled amino-acids are formed from labelled glucose in a variety of tumours incubated aerobically for 1 h at 37°, the labelling being apparent in glutamate, alanine, aspartate and glycine. Among these labelled amino-acids, glutamate is formed in greatest abundance. Solid tumours in the form of slices or Ehrlich ascites carcinoma cells in suspension are almost equally effective. The formation of labelled alanine is, however, largest with the ascites cell suspension under the given experimental conditions. No labelled glutamine is seen. The results are consistent with the observations of Kit and Graham[18] on the biosynthesis of amino-acids from labelled glucose in the ascitic forms of Mecca and Gardner lymphosarcomas, Ehrlich ascites carcinoma, lymphatic leukæmia and various melanomas, and of Busch, Fujiwara and Keer[19] on the formation of alanine and glutamate from labelled glucose in Walker 256 carcinosarcoma. The formation of alanine, aspartate and glutamate may be accounted for by transamination of the corresponding α-ketonic acids during the operation of the citric acid cycle in the tumours[20,21]. The lack of formation of labelled glutamine in the amino-acid patterns of the tumours recorded in Table 1 is also consistent with observations on the lack of free glutamine in Ehrlich ascites cells[6,22,23], with the report[24] on the lower glutamine content in tumours than in corresponding normal tissues, and with the fact that glutamine undergoes rapid destruction in tumours[6,22,23].

Examination of the formation of labelled amino-acids from labelled glucose by normal tissues under similar experimental conditions shows wider differences between the amino-acid patterns of the normal tissues than between those of the various tumours examined. Results recorded in Table 1 show the characteristic pattern given by rat brain cortex slices[12,16,17] and the very different patterns exhibited by rat spleen, kidney cortex or liver slices. Spleen

Table 1. Amino-acids derived from Glucose-u-^{14}C (5 mM) by Neoplasms and Normal Tissues

	Glycine*	Glutamate	Glutamine	γ-Amino-butyrate	Aspartate	Alanine
Tumours						
Ehrlich ascites carcinoma cells	41 ± 4	165 ± 12	0	0	39 ± 2	270 ± 27
Ehrlich solid carcinoma slices	55 ± 5	101 ± 6	0	0	24 ± 2	44 ± 4
Walker 256 carcino-sarcoma slices	78 ± 8	250 ± 15	0	0	66 ± 4	49 ± 8
Novikoff hepatoma slices	77 ± 9	217 ± 20	0	0	100 ± 15	169 ± 18
Melanoma slices	29 ± 3	347 ± 35	0	0	121 ± 12	126 ± 10
Normal tissues						
Rat brain cortex slices	0	1,070 ± 70	341 ± 30	219 ± 20	155 ± 13	161 ± 12
Rat spleen slices	0	259 ± 13	0	0	109 ± 8	82 ± 10
Rat kidney cortex slices	0	205 ± 20	0	0	52 ± 7	66 ± 4
Rat liver slices	0	30 ± 2	0	0	0	0
Regenerating rat liver slices	0	40 ± 2	0	0	0	0
Chick embryo (fifth day; whole)	0	240 ± 25	300 ± 20	80 ± 6	100 ± 12	0

All results are expressed as μmg atoms carbon (derived from glucose) incorporated/100 mg wet weight tissue.

* Including serine.

and kidney cortex slices show some similarities in distribution and magnitude of the labelled amino-acids with those exhibited by the tumours. It is of interest to note that the highly proliferating tissue, chick embryo, shows an amino-acid pattern under the given experimental conditions quite different from that of the tumours, for example, the formation of labelled glutamine and γ-aminobutyric acid and the lack of formation of labelled alanine.

The results support the well-known generalization that tumours as a whole tend to uniformity in metabolism in contrast to normal adult tissues that exhibit wide differences in metabolic behaviour.

Effects of incubation together of normal tissue and neoplasms. On incubating together rat brain cortex slices and Ehrlich ascites carcinoma cells for 1 h under aerobic conditions at 37° in presence of 5 mM-glucose-u-^{14}C, and examining the labelled amino-acid pattern of each tissue after such incubation it is found that the following characteristic changes occur (Table 2): (1) The glutamine content of the brain tissue is lowered, usually to about half its normal value. (2) The glutamate content of the ascites cells is considerably increased, usually to about three-fold its normal. The glutamate content of the brain tissue is but little affected. (3) The alanine content of the brain tissue is increased and there is usually a small (but occasional large) rise of alanine content of the tumour cell.

These changes of amino-acid patterns found on admixture of brain cortex slices and Ehrlich ascites cells are also observed on incubating together rat brain cortex slices with Ehrlich solid carcinoma slices or with Walker 256 carcinosarcoma slices. Results are recorded in Table 2.

Table 2. Effects of Incubation of Mixtures of Tissues (Normal and Neoplasms) on Conversion of 5 mM Glucose-u-^{14}C into Amino-acids in each Tissue

	Glutamate	Glutamine	Alanine
(1) Brain cortex slices	1,070 ± 70	341 ± 30	161 ± 12
Ehrlich ascites cells	165 ± 12	0	270 ± 27
After incubation of mixed tissues			
Brain cortex slices	1,109 ± 70	178 ± 6	517 ± 32
Ehrlich ascites cells	581 ± 15	0	286 ± 19
(2) Brain cortex slices	1,174 ± 70	389 ± 9	200 ± 15
Ehrlich solid carcinoma slices	101 ± 3	0	44 ± 4
After incubation of mixed tissues			
Brain cortex slices	1,037 ± 24	234 ± 6	275 ± 12
Ehrlich solid carcinoma slices	325 + 20	0	60 ± 2
(3) Brain cortex slices	1,056 ± 44	440 ± 21	190 ± 11
Walker 256 carcinoma slices	250 ± 15	0	49 ± 3
After incubation of mixed tissues			
Brain cortex slices	931 ± 10	231 ± 4	239 ± 6
Walker 256 carcino-sarcoma slices	780 ± 28	0	90 ± 12
(4) Brain cortex slices	992 ± 61	400 ± 22	164 ± 14
Kidney cortex slices	205 ± 10	0	34 ± 2
After incubation of mixed tissues			
Brain cortex slices	1,265 ± 65	440 ± 5	436 ± 18
Kidney cortex slices	430 ± 15	0	70 ± 6
(5) Brain cortex slices	1,174 ± 70	389 ± 9	255 ± 35
Spleen slices	259 ± 13	0	82 ± 10
After incubation of mixed tissues			
Brain cortex slices	1,081 ± 10	369 ± 10	376 ± 20
Spleen slices	400 ± 40	0	60 ± 4

Results expressed as μmg atoms carbon (derived from glucose) incorporated into the amino-acid per 100 mg wet weight tissue.

Incubation, together, of rat brain cortex slices with rat kidney cortex slices or with rat spleen slices does not, in contrast to the tumours, bring about a large fall in brain labelled glutamine. However, the labelled glutamate content of the kidney cortex slices, or spleen slices, is increased with relatively small change in the labelled glutamate content of the brain tissue. Moreover, the labelled alanine content of the kidney cortex, brain cortex, and spleen tissues is increased by the mixed incubation procedure.

The characteristic property of the tumours examined is their lowering of labelled glutamine in brain tissue with which they have been incubated.

Further experiments showed that the addition of a suspension of Ehrlich ascites carcinoma cells which had been heated at 100° C for 10 min brings about no change in the labelled amino-acid pattern, derived from labelled glucose, in the presence of rat brain cortex slices.

Effects of changes of glucose concentration on the action of Ehrlich ascites cells on amino-acid formation from glucose in presence of rat brain cortex. Results given in Table 3 show that the percentage fall in the concentration of labelled glutamine in rat brain cortex slices brought about by incubation with Ehrlich ascites carcinoma cells is largest with the smallest concentration of glucose tested, that is, 1 mM, and that it disappears when the glucose concentration is increased to 10 mM or to a higher concentration. The rise in glutamate concentration of the Ehrlich ascites cells, due to incubation with the brain cortex tissue, occurs at all concentrations of glucose tested under the given experimental conditions. An interesting observation is that the concentration of labelled γ-aminobutyric acid in brain is increased by the addition of the tumour cells at the higher glucose concentrations.

The fact that the fall in concentration of brain glutamine, by incubation of the brain tissue with the tumour cells, is suppressed at high glucose concentrations together with the fact that there is a parallel increase in concentration of tumour labelled glutamate suggests that the responsible event is deprivation of the brain tissue of glucose by aerobic tumour glycolysis. Such deprivation, under the given experimental conditions, will only occur at relatively low glucose concentrations and will disappear when the latter is raised.

It is known that transport of amino-acids into isolated brain cortex tissue is energy-dependent[13]; that the presence of glucose under aerobic conditions brings about optimal rates of entry[14,15] and that the absence of glucose even under aerobic conditions

Table 3. INFLUENCE OF VARYING GLUCOSE CONCENTRATIONS ON THE CONVERSION OF GLUCOSE-U-^{14}C INTO AMINO-ACIDS BY RAT BRAIN CORTEX SLICES, EHRLICH ASCITES CARCINOMA CELLS AND MIXTURES OF THESE TISSUES

	Initial glucose conc. (mM)	Glutamate	Glutamine	γ-Amino-butyrate	Aspartate	Alanine
(1) Brain cortex slices	1	406 ± 11	75 ± 5	77 ± 6	112 ± 7	47 ± 5
Ehrlich ascites cells	1	71 ± 2	0	0	13 ± 3	152 ± 20
After incubation of mixed tissues						
Brain cortex slices	1	317 ± 14	14 ± 2	65 ± 4	111 ± 8	83 ± 4
Ehrlich ascites cells		141 ± 3	0	0	17 ± 2	111 ± 8
(2) Brain cortex slices	3	495 ± 15	139 ± 5	61 ± 8	83 ± 4	45 ± 2
Ehrlich ascites cells	3	51 ± 4	0	0	20 ± 3	186 ± 10
After incubation of mixed tissues						
Brain cortex slices	3	381 ± 12	77 ± 4	76 ± 6	58 ± 5	110 ± 3
Ehrlich ascites cells		148 ± 8	0	0	30 ± 1	206 ± 3
(3) Brain cortex slices	5	1,070 ± 70	341 ± 12	219 ± 10	155 ± 15	161 ± 20
Ehrlich ascites cells	5	165 ± 4	0	0	39 ± 2	270 ± 10
After incubation of mixed tissues						
Brain cortex slices	5	1,109 ± 70	178 ± 10	373 ± 20	105 ± 7	517 ± 26
Ehrlich ascites cells		581 ± 15	0	0	50 ± 4	286 ± 11
(4) Brain cortex slices	10	1,267 ± 97	468 ± 30	302 ± 4	166 ± 9	290 ± 15
Ehrlich ascites cells	10	186 ± 8	0	0	35 ± 4	294 ± 21
After incubation of mixed tissues						
Brain cortex slices	10	1,291 ± 101	554 ± 28	574 ± 36	144 ± 3	755 ± 10
Ehrlich ascites cells		543 ± 28	0	0	48 ± 0	344 ± 24
(5) Brain cortex slices	20	1,461 ± 71	594 ± 21	393 ± 54	194 ± 14	340 ± 20
Ehrlich ascites cells	20	245 ± 10	0	0	35 ± 3	532 ± 15
After incubation of mixed tissues						
Brain cortex slices	20	1,441 ± 80	746 ± 40	589 ± 61	147 ± 15	778 ± 71
Ehrlich ascites cells		831 ± 65	0	0	54 ± 9	714 ± 60

Results expressed as μmg atoms carbon (derived from glucose) incorporated into amino-acids per 100 mg wet weight tissue.

greatly diminishes the rate of amino-acid entry into brain[15]. On the other hand, transport of amino-acids into Ehrlich ascites cells is as effective aerobically in the absence of glucose as it is aerobically, or anaerobically, in the presence of glucose[25–27]. It is to be expected, therefore, that brain tissue, in the absence of glucose, will lose some of its accumulated amino-acids by diffusion into the medium, even under aerobic conditions, and that these amino-acids will be taken up by the tumour cells present in the medium. Experiments were carried out to test this conclusion.

Effects of transfer of respiring rat brain cortex slices containing labelled amino-acids into a glucose-free medium. There is normally relatively little leakage of labelled amino-acids from rat brain cortex slices respiring in a radioactive glucose–Ringer medium[16,17], the amount being of the order of 10 per cent of that present in the tissue with the exception of alanine, where a leak of 20 per cent occurs.

The results of experiments devised to observe the effects of incubating brain cortex tissue, which contains radioactive amino-acids, in a glucose-free Ringer medium are shown in Table 4. It will be seen that after 15 min aerobic incubation of the slices in the glucose-free medium, two labelled amino-acids are found in the medium, namely, glutamine and alanine, the former in larger quantity than the latter and amounting to more than 50 per cent of the total labelled glutamine present. After 30 min aerobic incubation, traces of labelled glutamate and aspartate also appear in the medium but none of labelled γ-aminobutyrate. As oxidation of the amino-acids is proceeding in the brain tissue during the incubation period, the total radioactivity in the amino-acids in the tissue and medium is less after the 30 min incubation than after 15 min. It is clear, however, the ratio of the concentration of labelled glutamine in the medium to that in the tissue is not diminished by lengthened incubation of the tissue in a glucose-free medium; on the contrary, under the given experimental conditions, it tends to increase. After 30 min exposure the proportion of the total labelled glutamine that has leaked into the glucose-free medium is 70 per cent.

Effects of glutamine on labelled amino-acid formation from labelled glucose in Ehrlich ascites carcinoma cells. Experiments were carried out to observe the effects of the addition of labelled and unlabelled-glutamine on the labelled amino-acid pattern, derived from glucose-U-^{14}C, in presence of Ehrlich ascites carcinoma cells. Results are recorded in Table 5.

The addition of 1 mM glutamine-U-^{14}C to a suspension of Ehrlich ascites carcinoma cells gives rise to labelled glutamate, aspartate and alanine, the yields of the former labelled amino-acids being approximately the same and much larger (about ten-fold) that of labelled alanine. Coles and Johnstone[28] have reported that the major end-products of glutamine in Ehrlich ascites carcinoma cells are (apart from carbon dioxide) glutamate and aspartate, and it is known[22] that much of the glutamine utilized is converted to glutamate. Interconversions of

Table 4. EFFECTS OF TRANSFER OF RESPIRING RAT BRAIN CORTEX SLICES CONTAINING RADIOACTIVE AMINO-ACIDS INTO A GLUCOSE-FREE MEDIUM

	Brain cortex* slices; initial content of labelled amino-acids	After 15 min aerobic incubation in glucose-free medium		After 30 min aerobic incubation in glucose-free medium	
		Brain slices	Found in medium	Brain slices	Found in medium
Glutamate	811 ± 34	501 ± 30	0	375 ± 38	15 ± 3
Glutamine	148 ± 17	76 ± 8	137 ± 5	43 ± 2	105 ± 10
γ-Amino-butyrate	135 ± 2	170 ± 12	0	114 ± 3	0
Aspartate	138 ± 13	184 ± 6	0	218 ± 17	12 ± 2
Alanine	118 ± 6	52 ± 8	38 ± 6	38 ± 4	28 ± 2

Results expressed as μmg atoms carbon (derived from glucose) incorporated into amino-acids per 100 mg wet weight tissue.

* Brain cortex slices were originally incubated with 5 mM glucose-U-^{14}C for 30 min and the slices were then transferred to a glucose-free Ringer medium.

Table 5. EFFECTS OF LABELLED AND UNLABELLED GLUTAMINE, AND OF GLUTAMATE, ON AMINO-ACID FORMATION FROM GLUCOSE-U-^{14}C IN THE PRESENCE OF EHRLICH ASCITES CARCINOMA CELLS

	Glutamate	Alanine	Aspartate
5 mM Glucose-U-^{14}C	165 ± 12	270 ± 27	39 ± 2
1 mM Glutamine-U-^{14}C	500 ± 9	49 ± 2	566 ± 16
5 mM Glucose-U-^{14}C + 1 mM Glutamine-U-^{14}C	1,957 ± 102	1,324 ± 30	359 ± 14
5 mM Glucose-U-^{14}C + 2 mM Glutamine	255 ± 14	1,710 ± 28	141 ± 1
5 mM Glucose-U-^{14}C + 5 mM Glutamate	225 ± 40	600 ± 65	51 ± 4
5 mM Glutamate-U-^{14}C	547 ± 67	< 10	287 ± 39
5 mM Glutamate-U-^{14}C + 5 mM Glucose	1,397 ± 19	< 10	128 ± 12

Results expressed as μmg atoms carbon incorporated into amino-acids per 100 mg wet weight tissue.

glutamate into aspartate and alanine are to be expected from the operations in the tumour cell of the transaminating systems and the citric acid cycle. Conversion of labelled glutamate into labelled aspartate is seen in Table 5, but the yield of labelled alanine is less than that from a smaller concentration of glutamine. Addition of glucose suppresses labelled aspartate formation from labelled glutamate (Table 5)[28] as it does that from labelled succinate[17].

As it has been demonstrated that in a glucose-free Ringer medium glutamine diffuses from respiring brain tissue into the medium, the effects of the addition of either unlabelled glutamine or labelled glutamine on the amino-acid pattern derived from glucose-U-^{14}C in presence of Ehrlich ascites cells were examined. The results given in Table 5 show that the addition of unlabelled glutamine has little effect on the yield of labelled glutamate, but increases that of alanine and of aspartate, presumably as a result of its ability to transaminate with the corresponding labelled α-ketonic acids derived from glucose-U-^{14}C. In this respect, glutamine is superior to glutamate (Table 5). The addition, however, of labelled glutamine (1 mM) greatly increases the yield of labelled glutamate formed by Ehrlich ascites cells in presence of glucose-U-^{14}C, the amount formed being considerably higher than the sum of the yields formed from the glucose and glutamine individually. This is to be expected if the added labelled glutamine gives rise in the tumour to labelled glutamate and thus increases the steady-state-level of glutamate.

In the light of these results, it is possible to arrive at a reasonable explanation of the results given in Tables 2 and 3. Radioactive glutamine, transferred from brain cortex slices into the extracellular medium, made deficient in glucose by the high aerobic glycolytic activity of the tumour cells, is taken up by the tumour cells and there partly converted into labelled glutamate. The labelled glutamate pools with that derived from labelled glucose in the tumour cells and has the effect of increasing considerably there the steady-state-level of glutamate. This may be due to saturation of the glutamate utilizing systems. There are concomitant small rises in labelled aspartate and labelled alanine-levels in the tumours which may also be due to the absorption of glutamine (Table 5).

Conclusions. These results indicate the importance, for the study of integrated organized systems, of investigations of the interactions of isolated tissues incubated together. An example of such an interaction is the demonstration that the presence of mouse liver slices diminishes the inhibitory activities of L-5-ethylsulphinylmethylhydantoin on glycine incorporation into tumour cell proteins[29].

The results recorded here show that the incubation together of tumour tissue with rat brain cortex tissue in a physiological Ringer medium containing a limited glucose supply brings about deprivation of glutamine from the brain tissue and a parallel (but greater) rise in glutamate in the tumour tissue. This finding is explained by the high aerobic glycolytic behaviour of the tumour, which deprives the normal tissue of glucose. This deprivation causes an efflux of glutamine which is at once utilized by the tumour.

Presumably a similar result would obtain if glutaminase were released from the tumour cells into the medium and there, by destroying completely the glutamine present in the medium, cause an efflux of glutamine from the brain tissue. Some preliminary experiments have, in fact, showed that this phenomenon can, in fact, take place. However, the results (Table 3) show that increase of glucose in the medium prevents the fall in the brain glutamine brought about by the tumour, and it is difficult to understand this phenomenon if glutaminase is solely responsible for the efflux of glutamine from the brain tissue. It may, however, play a contributory part.

If the foregoing explanation concerning the interrelations between tumour tissue and normal tissue is correct, the implications in the consideration of malignancy of tumours are obvious. Deprivation of a normal tissue, adjacent to the tumour, of glucose, by operation of the high aerobic glycolytic rate of the tumour, will lead eventually to the loss by leakage of those tissue constituents, the active uptake of which is controlled by glucose oxidation. Among such constituents is glutamine, which plays an important part in tumour growth and metabolism. It is to be expected that tumour cells will invade, and proliferate in, those regions where nutritional conditions are optimal and where substances, such as glutamine, having a controlling effect on tumour growth will become available as a result of glucose deprivation. The results recorded in this article are suggestive that such a mechanism is at least one of the factors underlying the apparent relation between malignancy and tumour metabolism. Clearly, much more has to be learned about the magnitude of glucose concentrations in the vicinity of tumours and the possible effects of glucose deprivation on the loss of essential cell constituents from a variety of normal tissues before the quantitative aspects of this mechanism on tumour malignancy can be assessed.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Role of Turbulence in Star Formation in Elliptical Galaxies

Dr. G. R. Burbidge has proposed[1] that the energy source for intense radio emitters (largely giant elliptical galaxies) can most reasonably be expected to be derived from a temporarily greatly enhanced rate of supernova activity in a galaxy. He further proposed[1] that this enhancement resulted from a collective stellar catastrophe in which older stars close to a condition of instability could participate in a chain reaction of Type 1 supernova explosions in which one explosion triggers that in a neighbour. As an alternative, it has been proposed[2] that the enhancement resulted from collective stellar formation in which the more massive stars would evolve to the Type II supernova stage in a reasonably short period of time. In the latter proposal it was assumed that the energies associated with gas in an elliptical galaxy are small compared with the magnetic energy, and hence the magnetic field must control the contraction of the gas to form stars, and will prevent fragmentation until a late stage in which conservation of angular momentum has caused the formation of a disk.

Dr. Burbidge[3] has pointed out that elliptical galaxies often contain highly turbulent gas at their centres, and the energy per unit mass of such gas greatly exceeds the probable associated magnetic energy. He therefore criticizes the neglect of the energy of turbulence in this calculation.

Osterbrock[4], who analysed the turbulence in the galactic centres, notes that the turbulent energy should be quickly dissipated. He suggests that the energy input necessary to maintain the turbulence comes from ionizing radiation supplied by faint hot stars in advanced stages of evolution as they pass through the galactic centre, where their motions will be most rapid because the centres represent deep gravitational potential holes. He shows that the gas density in the galactic centre is several orders of magnitude greater than assumed by me for the galactic volume generally. He notes that the much thinner gas in the outer parts of *NGC* 4278 seems not to be turbulent.

Thus it seems, in agreement with Dr. Burbidge, that turbulent energy must dominate conditions in the gas in such galactic centres. Indeed, it seems likely that this turbulence must effectively disconnect the magnetic field in the centre from that in the rest of the galaxy. However, there seems to be no necessity that turbulence must similarly dominate the gas in the rest of the galaxy. Not only is the energy input to such turbulence much smaller but also, because the gas is much thinner relative to the assumed magnetic field, such a magnetic field is likely to dominate the gas if its strength is 10^{-6} gauss or greater.

In these circumstances, I do not believe that the neglect of turbulent energy invalidates the foregoing mechanism of collective star formation. Although the turbulent gas in galactic centres will not participate in the collective condensation, none the less this gas occupies only a very small part of the galactic volume, and its mass is very small compared with that which was assumed for a typical elliptical galaxy as a whole.

Dr. Burbidge[3] also has raised the very important question as to whether it is proper to apply the virial theorem to the small gas component of a galaxy which acts as a deep potential hole in which the gas is immersed. It was assumed that there is little interaction throughout most of the galaxy between stars and gas, and also, that, owing to the small degree of flattening in elliptical galaxies, the gas has little angular momentum, being mostly supported by the magnetic field. It is clear that the presence of a steep gravitational potential gradient must strongly affect the condensation of a rapidly rotating gas. It is also evident that since the gas is 'trapped' in a potential hole there is an effective external pressure applied to it, which should make a condensation process easier.

In any event, it was assumed that the use of the virial theorem should give the general order of magnitude for the conditions in which the gas can condense. During the condensation, the principal way in which angular momentum can be conserved is through the centre-of-mass of the condensing gas taking up an orbit about the centre of the galaxy. This is another reason for not expecting the gas in the deep potential hole at the centre to participate in the condensation. The visible evidence for this off-central condensation should be the not-too-uncommon presence of visible 'jets' off-centre in intense radio sources; these should represent regions near the centre of the condensation where the magnetic field is sufficiently highly compressed that high-energy electrons in the field will emit visible synchrotron radiation.

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It appears that we are now in agreement that star formation in the central regions of elliptical galaxies under the conditions originally postulated by Dr. Cameron is highly improbable. Cameron still believes that such processes may go on outside the nuclear regions of such galaxies. To this I would offer the following brief comments:

(1) We have no evidence at present that any appreciable amount of gas exists in an elliptical galaxy outside the central region. So far no elliptical galaxy has been detected by 21-cm techniques.

(2) If we accept the idea that a low-density gas pervades the outer parts of a galaxy, then the situation is similar to that in the halo of our own Galaxy. Here for material to be supported above the plane we must either suppose that we have a cold but turbulent gas[1,2] ($T \approx 100°$ K, $\rho \approx 10^{-26}$ g/cm^3, turbulent velocity ≈ 200 km/sec) or a hot gas[3] ($\rho \approx 10^{-27}$ g/cm^3, $T \approx 10^{6}°$). The magnetic energy also contributes significantly to the support provided that the field is of the order of 10^{-6} gauss or greater. Such a situation would appear from a theoretical point of view to be

very unfavourable for star formation. This is borne out by the fact that in our own Galaxy no young stars are seen in the halo regions.

(3) If low-density gas is continuously being fed into the volume of an elliptical galaxy by, for example, the ejection of gas from evolving stars, it will initially have the velocity dispersion associated with these stars, that is, a dispersion of several hundred kilometres per second. For such a gas to recondense into new stars implies that star formation can go on under similar conditions to those in the centre, which we are agreed makes it very improbable. If the turbulence decays through collisions, the gas will fall into the centre, or into a thin rotating disk, where again the conditions are unfavourable for star formation.

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GEOPHYSICS

Tentative Correlation of Samoan and Hawaiian Islands using 'Reversals' of Magnetization

PALÆOMAGNETIC studies in the oceanic islands of the Pacific were started in March 1960 when a collection of 564 oriented rock samples was made from various islands in the South and South-West Pacific. A further 164 samples were collected from most of the major islands of the Hawaiian Group during June–September 1961. Palæomagnetic results from a collection made by Doell somewhat earlier in 1961 on the Island of Hawaii (that is, one island within the Hawaiian Group) have already been reported[1]. No samples were taken from this Island to avoid duplication of Doell's sampling. This investigation of the magnetic properties of the rocks in these Islands was undertaken for several reasons, one of which was to investigate the possibility of using the occurrence of 'reversals' of magnetization as a basis for geological correlation both within and between the island groups. Previous correlations have been difficult or impossible to establish because of the absence of fossil or other marker horizons.

Roche[2] has examined the polarity of samples in the well-dated Quaternary sequence of France, and has observed that the directions of magnetization of the rocks show two distinct groupings, the younger rocks having directions parallel to the present Earth's magnetic field, and the older rocks having antiparallel directions. These two groups are referred to as 'normal' and 'reversed' respectively. The change from 'reversed' to 'normal' takes place in the middle of the Lower Pleistocene. If this level of reversal can be identified in the Pacific Islands then it could be a marker horizon of considerable utility. This communication describes the polarities observed in surveys in the Samoan and Hawaiian Islands, and indicates the potentialities of the method.

Two or three samples were collected at each of numerous collecting sites, and two specimens were cut from each of these, and their directions of magnetization measured. All specimens were subjected to magnetic cleaning[3] by partial demagnetization in alternating fields; this treatment reduces the possibility that the observed polarities could be due to systematic partial magnetic instability. The samples from the Hawaiian Islands in particular showed a high magnetic stability, most of the directions in test specimens remaining little changed in alternating fields as high as 800 oersteds (peak).

The formations sampled, the number of sites and their polarity are listed in Table 1. Formations containing only 'normal' sites are termed 'normal', and formations with only 'reversed' sites are termed 'reversed', although the polarity only strictly applies to the actual sites sampled. Formations containing sites of both polarity are called 'mixed'. In the Samoan Islands, a reversal of magnetization occurs between the Leone and Taputapu Formations on Tutuila, and within the Fagaloa Formation on Upolu. All samples from formations on Savai'i are 'normally' magnetized and include two sites in the Fagaloa Formation. In the Hawaiian Islands, a 'reversal' occurs in the Honolua, Honomanu and Wailuku Formations on Maui, and the Koloa Formation on Kauai, while it occurs between the Koolau

Table 1. TENTATIVE CORRELATION TABLE OF SAMPLED HAWAIIAN AND SAMOAN FORMATIONS

(*a*) Samoan Islands

	Tutuila	Upolu	Savai'i
Historic and recent	Leone (2*N*)	Puapua (1*N*)	Aopo (5*N*) Puapua (5*N*)
Pleistocene		Mulifanua (6*N*) Salani (3*N*) Fagaloa (14*M*)	Fagaloa (2*N*)
Pliocene and older	Taputapu (5*R*) Pago (13*R*) Alofau (5*R*) Olomoana (3*R*)		

(*b*) Hawaiian Islands

	Kauai	Oahu	Molokai	Lanai	Maui		Hawaii (ref. 1)
					(W.)	(E.)	
Historic and recent	Koloa (2*M*)	Honolulu (1*N*)				Hana (3*N*)	Puna and Historic (ref. 1) (27*N*) Kahuku (ref. 1) (29*N*) Hamakua (ref. 1) (27*N*)
Pleistocene		Koolau (8*R*) Waianae (14*M*)	East (7*R*) West (4*R*)	Lanai (3*R*)	Honolua (1*R*) Wailuku (6*M*)	Kula (6*N*) Honomanu (1*R*)	Pololu (ref. 1) (50*N*) Ninole (ref. 1) (25*N*)
Pliocene and older	Napali (3*N*)						

The number of sites and their polarity (*N* = 'normal', *R* = 'reversed', *M* = 'mixed') is given with each formation. The formations are in stratigraphic sequence within each island, but no attempt has been made to indicate either the stratigraphic extent or the detailed relationships of individual formations.

and Honolulu Formations on Oahu. Samples from Molokai and Lanai are all 'reversed' and Doell's samples from the Island of Hawaii are all 'normal'[1]. The 'normal' sites which occur beneath this level (for example, the Napali Formation on Kauai, and some sites in the Waianae Formation of Oahu) may belong to a 'normal' zone which occurred below this 'reversed' zone.

The level of the most recent reversal in both island groups is consistent with the geological estimates of age[4] in all cases, with the exception of the two oldest formations on the island of Hawaii (Pololu and Ninole) which show 'normal' polarity and which have been attributed, on geological evidence, to the Tertiary. The reason for this exception is not clear, but it is possible that the formations are younger than has been thought (that is, late Lower Pleistocene or younger), but sampling inadequacy or a geological non-sequence could equally be the cause.

Although it is tempting to suggest a correlation between the level of reversal in the Fagaloa Formation and the most recent reversal in the Hawaiian Islands, more work will be needed to clarify this. Nevertheless the results outlined here, particularly those from Samoa, make clear the potentialities of this method as an aid in geological correlation within and between the Pacific Islands over very large distances.

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Some High-pressure Transformations in Pyroxenes

We have recently investigated the effects of high pressures on the following germanates, which were found to possess pyroxene structures, namely, $FeGeO_3$, $CoGeO_3$, $(Mg_{0.75}Ni_{0.25})GeO_3$, $MgGeO_3$ and $MnGeO_3$. These compounds were synthesized by heating tablets formed from intimately mixed oxide components at various suitable temperatures between 900° C and 1,400° C for times sufficient to reach equilibrium. In the case of $FeGeO_3$, the tablet was formed from the required proportions of iron powder, Fe_2O_3 and GeO_2. This was wrapped in platinum foil, sealed in an evacuated silica tube, and then heated at 900° C for 24 h. X-ray diffraction photographs of the resultant products revealed that $FeGeO_3$, $CoGeO_3$ and $(Mg_{0.75}Ni_{0.25})GeO_3$ possessed clinopyroxene structures whereas $MgGeO_3$ and $MnGeO_3$ displayed orthopyroxene structures. We were unable to synthesize a compound with the composition $NiGeO_3$.

The behaviour of these pyroxenes at high pressures was next investigated in a squeezer apparatus using techniques which we have previously described[1]. The pressure-range covered was 0–60 kilobars at a temperature of 700° C. Runs were of 1 h duration, after which the sample was quickly cooled, and then subjected to X-ray diffraction and optical examination. Results of these runs are summarized in Table 1. In each case, the germanate pyroxene was found to display phase transformation. We have previously published results of the investigation on $MgGeO_3$ (ref. 2).

At pressures less than those which were necessary to form the ilmenite polymorphs, $MgGeO_3$ and $MnGeO_3$ were observed to transform completely to clinopyroxenes.

The results in Table 1 clearly suggest two principal modes of transformation for germanate pyroxenes under high pressures. The pyroxenes may either break down to a denser mixture of orthogermanate plus germanium dioxide, or they may transform directly to a closer packed ilmenite structure. These transformations are relevant to the behaviour of silicate pyroxenes such as $MgSiO_3$ under high pressures.

The crystal chemistry of germanates and silicates is very closely related, and corresponding silicate and germanate compounds are usually isostructural. As demonstrated by the olivine–spinel and quartz–rutile transitions, germanates generally require smaller pressures to undergo given phase transformations than silicates. In one sense, therefore, germanates may be regarded as high-pressure models for silicates. The reason for this behaviour has been pointed out by Bernal[3] and Wentorf[4]. When a silicate is subjected to high pressure the large oxygen ions tend to contract relatively more than the small silicon ions, hence the radius ratio $\left(\frac{R_{Si}}{R_O}\right)$ increases. Now the radius ratios of constituent ions largely determine the relative stabilities of different structures. Since the zero pressure radius of Ge^{++++} (0·48 Å) is already slightly larger than that of Si^{++++} (0·42 Å), germanates therefore require smaller pressures to achieve the critical radius ratios required for given transitions than do the corresponding silicates.

This reasoning suggests that silicate pyroxenes such as $MgSiO_3$ may behave analogously to germanate pyroxenes under high pressure. The following transformations would therefore appear possible:

$$(1)\ 2\,MgSiO_3\ (\text{pyroxene}) \rightarrow Mg_2SiO_4\ (\text{olivine}) + SiO_2 \left(\begin{matrix}\text{stishovite}\\ \text{rutile struct.}\end{matrix}\right) \Delta v = -4{\cdot}9\ \text{c.c.}$$

$$(2)\ 2MgSiO_3\ (\text{pyroxene}) \rightarrow Mg_2SiO_4\ (\text{spinel}) + SiO_2\ (\text{coesite})\ \Delta v = -3{\cdot}3\ \text{c.c.}$$

$$(3)\ MgSiO_3\ (\text{pyroxene}) \rightarrow MgSiO_3\ (\text{ilmenite})\ \Delta v = -5{\cdot}1\ \text{c.c.}$$

The conditions required for reactions (1) and (2) here have previously been discussed by Ringwood[5,6]. Because of the very small free energy of formation of enstatite from olivine + quartz at zero pressure, the directions of the equilibria at high pressure are dominated by the $P.\Delta v$ term in the free energy, where P is pressure and Δv is the volume change associated

Table 1. Results of High-pressure Experiments on Germanate Pyroxenes at 700 ± 10° C

Compound	Initial structure	Transformation pressure (kilobars)	Reaction products	Percentage increase in density due to phase transformation (approx.)
$FeGeO_3$	Clinopyroxene	10 ± 3	Fe_2GeO_4 (spinel) + GeO_2 (rutile)	10
$CoGeO_3$	Clinopyroxene	10 ± 3	Co_2GeO_4 (spinel) + GeO_2 (rutile)	10
$(Mg_{0.75}Ni_{0.25})GeO_3$	Clinopyroxene	22 ± 3	$(Mg_{0.75}Ni_{0.25})_2GeO_4$ (spinel) + GeO_2 (rutile)	10
$MgGeO_3$	Orthopyroxene	28 ± 3	$MgGeO_3$ (ilmenite structure)	15
$MnGeO_3$	Orthopyroxene	25 ± 5	$MnGeO_3$ (ilmenite structure)	15

with the reaction. For reactions (1) and (2) the Δv's are large and negative. It can be shown that reactions (1) and (2) will therefore proceed to the right at pressures which are only slightly higher than those required to form the rutile polymorph of SiO_2 or the spinel polymorph of Mg_2SiO_4. Whether enstatite will break down according to reaction (1) or reaction (2) depends on the relative pressures required for the olivine–spinel and quartz–rutile transitions.

We have previously investigated the olivine–spinel transition in Mg_2SiO_4 and concluded that it will occur in the range 130 ± 20 kilobars at 600° C, with a somewhat greater probability of values in the higher part of this range[1]. Stishov and Popova[7] have placed the coesite–stishovite transition at 160 kilobars at 1,200° C. Making an allowance for the revised pressure calibrations of Kennedy and La Mori[8] would decrease this to 120 kilobars. We have investigated the stability of stishovite at 530° C using silicic acid as starting material. At 90 kilobars, the silicic acid was converted entirely to coesite. At 100 kilobars, coesite plus a trace of stishovite was found. Finally, at 110 kilobars, the sample contained more than 50 per cent of stishovite. From these preliminary determinations, it would appear that the coesite–stishovite transition pressure is around 105 kilobars at 530° C. It seems clear that the pressure required for the coesite–stishovite transition is substantially smaller than that required for the olivine–spinel transition, accordingly reaction (1) will be preferred to (2).

The breakdown of enstatite to olivine plus stishovite according to reaction (1) serves as an upper limit to the stability of enstatite. The pressure required would probably not be substantially higher than 120 kilobars at 600° C. It is just possible that this reaction might be averted if the enstatite transformed directly to an ilmenite structure (reaction (3)) at some pressure below 120 kilobars. An investigation of this possibility is at present in progress. Preliminary evidence suggests that reaction (1) will occur first, and hence enstatite will not transform directly to an ilmenite structure. It is emphasized that these results are tentative and that more work is needed to confirm them.

The foregoing conclusion does not affect the question of the ultimate stability of an ilmenite polymorph of $MgSiO_3$. Ringwood[6] has previously discussed this question and believes that the $MgSiO_3$ (ilmenite) may be formed in the deep mantle according to the reaction:

$$Mg_2SiO_4 \text{ (spinel)} \rightarrow MgSiO_3 \text{ (ilmenite)} + MgO \text{ (periclase)}$$

We have also carried out preliminary investigations of the stabilities of the pyroxenoids $MnSiO_3$ and $CaSiO_3$ at high pressures. In each case, transitions to new structures have been found.

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PHYSICS

Doppler Frequency Multiplication by a Semiconductor Depletion Layer

Reflexion of a plane electromagnetic wave from a 'mirror' moving in the direction of the wave source can be used for frequency multiplication via the Doppler effect. A moving plasma boundary[1,2] can be used to provide such a mirror at frequencies less than or equal to the plasma frequency:

$$f_p = \frac{1}{2\pi}\left(\frac{q_e^2 N}{m\varepsilon}\right)^{1/2}$$

where q_e and m are the charge and mass of the electron respectively, N the electron number density in the plasma and ε the plasma-free dielectric constant of the space containing the plasma. For generation of sub-millimetre waves by this means, very high density plasmas (electron densities of 10^{15}–10^{17} per cm^3) are necessary. The plasma formed by the electrons and holes in an impurity doped semiconductor readily offers such high densities.

Frequency multiplication to sub-millimetre waves by the foregoing scheme is also possible, if the slow electromagnetic waves propagated through the semiconductor plasma are reflected at the moving plasma boundary. Such a reflecting boundary is provided by the depletion (plasma-free) region in a semiconductor. The semi-conductor plasma, which has an extremely small relaxation time ($\tau \simeq 0{\cdot}5 \times 10^{-12}$ sec) at room temperature, can be made fairly non-dissipative ($\omega\tau \gg 1$) at low temperatures (for example, $\tau \simeq 10^{-10}$–10^{-9} sec for germanium at 4° K). At very low temperatures, germanium and silicon are essentially insulators with carriers frozen into impurity-levels or into bands, and not free to contribute to the conduction process.

Various methods that can be used for producing free carriers are: breakdown of impurity-levels by a microwave field; breakdown of impurity-levels by an applied d.-c. electric field, and irradiation with photons of frequency ε_i/h, where ε_i is the energy gap between the impurity-levels and the conduction band and h is Planck's constant. The low collision loss semiconductor plasma, thus obtained at low temperatures, should be capable of propagating slow electromagnetic wave modes similar to those possible in gaseous plasmas.

The depletion layer boundary can be moved with velocities of the order of 10^7 cm/sec by microwave pump modulation, and can reflect a pulsed wave incident on it in the slow plasma-wave mode of the non-depleted regions of the semiconductor. During the time that the depletion layer boundary is moving towards the source of the incident signal, a higher frequency reflected signal results. The frequency and power relations of the reflected and incident signals in such a dispersive medium can be derived by a method similar to that of Pierce[3] for non-dispersive media, and are given by:

$$\frac{\omega_2}{\omega_1} = \frac{1 + v/v_{p1}}{1 - v/v_{p2}}$$

and (1)

$$\frac{P_2}{P_1} = \frac{\omega_2}{\omega_1}\ \frac{1 + v/v_{g1}}{1 - v/v_{g2}}$$

where v = velocity of the reflector (depletion layer) during the time of the incident signal (assumed constant); P_1, P_2 = the incident and reflected powers; v_{p1}, v_{g1} = phase and group velocities respectively at the incident frequency ω_1; v_{p2}, v_{g2} = phase and group velocities respectively at the reflected frequency ω_2. In order to generate sub-millimetre waves from incident microwaves, it is necessary that $v \simeq v_{p2}$. Considerable slowing of the waves by means of a magnetoplasma can be achieved at a frequency $\omega_2 \simeq \omega_p$. The electromagnetic waves in plasmas can be slowed down to velocities of the order of the root mean square thermal velocities of electrons $\left(\frac{3kT}{m}\right)^{1/2}$ (assuming a Maxwellian distribution) before Landau damping inhibits the wave propagation.

An exact parallel of the foregoing scheme may be used for frequency multiplication of the r-f waves in piezoelectrics. One end of such a medium is provided with a piezoelectric depletion layer[4] which provides a strain boundary and can act as a reflector to the incident sonic waves. Such a depletion layer is obtained in semiconductive piezoelectrics such as cadmium sulphide, zinc oxide, etc., which are known to be extrinsic *n*-type semiconductors. The sonic wave velocity v_d in piezoelectrics is known to be extremely slow ($\simeq 2$ to 6×10^5 cm/sec) and fairly constant over a wide frequency-range. The frequency and power of the reflected signal in such a non-dispersive medium can be degenerated from relations in equation 1 to the following:

$$\frac{\omega_2}{\omega_1} = \frac{1 + v/v_d}{1 - v/v_d}$$

and (2)

$$\frac{P_2}{P_1} = \left(\frac{\omega_2}{\omega_1}\right)^2$$

By pump-varying the junction bias, the depletion layer is moved at a velocity corresponding to that of the sonic wave, which, during the time that it is moving towards the source of the incident pulses, results in the increased frequency of the reflected wave. For microwave applications, low-temperature operation is necessary to reduce the losses suffered by sonic waves due to interaction with thermal phonons in the material.

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Random Loose Packing of Binary Mixtures of Spheres

THE attention which J. D. Bernal[1] has directed to the importance of geometrical models for representing the structure of liquids has elicited considerable recent interest in the packing of spheres[2–7]. While previous investigations were often concerned with the dense random state[8,9], some of the more recent work has been devoted to looser states[3,4,6]. Both Scott[3] and MacRae and Gray[4] obtained loose deposits of spheres by a rolling procedure, while Rutgers[6], in common with Leva *et al.*[10] some years earlier, obtained a loose packing by dumping or pouring. Porosities thus reported, though significantly higher than those found in the dense random state, are nevertheless still lower than those obtained either by rapidly inverting a cylinder[11] or by gradually diminishing to zero the gas (or liquid) flow through a fluidized bed[12,13]. It is the latter porosities which correspond to those observed in both moving packed[14] and incipiently fluidized[13] beds.

The method adopted in the present work for reproducing the 'random loose' condition was the rapid inversion procedure of Oman and Watson[11]. Glass spheres of two different sizes were well mixed in a stoppered glass cylinder 7·77 cm in diameter and some 50 cm long. The cylinder was inverted, then quickly righted, and the bed height measured. Knowing the weights and densities of both components, the bed porosity (void volume fraction, ε) could be computed. Bed heights varied from 12 to 25 cm, over which range experiments on individual particle sizes had indicated that neither height of fall nor bottom effect was a significant variable.

Spheres investigated were glass beads $2{\cdot}19 \pm 0{\cdot}20_0$, $2{\cdot}92 \pm 0{\cdot}08_7$, $3{\cdot}97 \pm 0{\cdot}09_5$, $4{\cdot}94 \pm 0{\cdot}13_6$, and $5{\cdot}87 \pm 0{\cdot}17_5$ mm in diameter, and $16{\cdot}40 \pm 0{\cdot}32_6$ mm glass marbles. Fourteen of the fifteen possible binary combinations of these sizes were tested, each binary at eleven different mixture compositions, ranging from 0 to 100 per cent of the larger component in increments of 10 per cent by weight. In addition, one binary combination consisting of sieved micro-beads averaging 79·7 and 702μ was also tested at these compositions, in a 3·46-cm diameter cylinder. For these conditions, only those binaries which included the glass marbles had an appreciable wall effect, according to prior experiments on single sizes. Each test was performed five times, the maximum deviation between them averaging 0·005 void fraction.

For each binary, the results were compared with values calculated on the assumption of linear variation of porosity with volume fraction (void-free basis) of the larger component. For most pairs, a volume contraction was observed over the entire composition range. The only exceptions were three of the smaller size ratios, in which volume expansion occurred over part of the composition range along with contraction in the remainder, and one pair (diam. ratio $= 1{\cdot}24_4$) in which expansion actually occurred over the entire composition range. The maximum observed contractions in each case are recorded in Table 1.

Table 1

Larger diam. (mm)	Porosity, larger alone	Smaller diam. (mm)	Porosity, smaller alone	Maximum contraction ($\Delta\varepsilon_{max}$)	Vol. % larger (in solids) at $\Delta\varepsilon_{max}$
0·702	0·449	0·0797	0·432	0·152	70
2·92	0·405	2·19	0·413	0·008	49
3·97	0·413	2·19	0·413	0·015	49
3·97	0·413	2·92	0·405	0·007	50
4·94	0·419	2·19	0·413	0·041	42
4·94	0·419	2·92	0·405	0·024	68
4·94	0·419	3·97	0·413	0·000	100,0
5·87	0·427	2·19	0·413	0·055	72
5·87	0·427	2·92	0·405	0·030	64
5·87	0·427	3·97	0·413	0·018	63
5·87	0·427	4·94	0·419	0·017	71
16·40	0·471	2·19	0·413	0·136	52
16·40	0·471	2·92	0·405	0·123	63
16·40	0·471	3·97	0·413	0·088	63
16·40	0·471	4·94	0·419	0·069	50

The maximum contraction correlated well with the diameter ratio (larger/smaller)ν. The linear regression line between $\Delta\varepsilon_{max}$ and log ν is given by:

$$\Delta\varepsilon_{max} = 0{\cdot}1737 \log \frac{\nu}{1{\cdot}239} \pm 0{\cdot}0080$$

This equation is inapplicable below $\nu = 1\cdot24$. Below this diameter ratio, in any event, volume contractions can no longer be relied on to occur.

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Re-entrant Motion in Special Relativity

A RECENT communication[1] discusses the uniform rotation of a rigid circular disk as a problem in special relativity. Phipps is in error in supposing that there is more than one way to measure the peripheral velocity in a Lorentz frame in which the centre of the disk is at rest. He will see that the velocities v and $r\omega$ are equal (if it is not at once obvious from the kinematics in S) by drawing a Minkowski diagram showing the approximately parallel world lines of adjacent markers. These move with velocity v in S, and are at rest in S^*. S^* ascribes a spatial displacement Δx^* to the pair of events consisting of successive transits of a point fixed in S. Since the two markers are at rest in S^*, Δx^* may instead be determined from a pair of events in their histories simultaneous in S^*. But then Δx^* is just the rest displacement, namely, $2\pi r/n\sqrt{1-v^2/c^2}$. This reflects the well-known fact that in a rigid rotating (but non-holonomic) co-ordinate system the ratio circumference : radius is given by $2\pi : \sqrt{1-v^2/c^2}$. Substituting the above value for Δx^* into the Lorentz formula, we find that the 'round-trip' velocity $r\omega$ and the relative velocity v are in fact equal.

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[1] Phipps, T. E., *Nature*, 195, 67 (1962).

I BELIEVE that Dr. Phipps is mistaken in his assertions (1) that the round trip velocity $v_{r.t.}$ of a point on the edge of a rotating disk, defined by $v_{r.t.} = \omega r = 2\pi r/T$, where T is the period, is not equal to the instantaneous edge velocity, v, and (2) that ωr can become greater than c, the velocity of light.

Dr. Phipps's proof of these assertions depends on his assumption that a rapidly rotating disk remains rigid; whereas, independently of the strength of the disk, this cannot occur. Dr. Phipps's subsequent conclusions concerning time dilatation also depend on his incorrect proof. Therefore, there is no reason to reject the usual result of special relativity that a person riding on the edge of a rotating disk will age more slowly than his stationary twin.

Let us follow Dr. Phipps's derivation in detail. He considers a disk the outer edge of which is marked with point markers which divide it into n segments, each of rest-length s_0. Let n be sufficiently large so that the curvature of a segment can be neglected. Now consider a Lorentz frame S^* in which a particular edge-segment of the disk is momentarily at rest as the segment passes a fiducial point in the laboratory system S.

Define two 'events' as the passage opposite the fiducial point in S of two adjacent markers on the edge of the disk. These two events are separated by an interval $(\Delta x, \Delta t)$ in S and $(\Delta x^*, \Delta t^*)$ in S^*, where, by special relativity:

$$\Delta x^* = \frac{\Delta x - v\,\Delta t}{\sqrt{1-v^2/c^2}} \qquad (1)$$

Now, as noted by Dr. Phipps, $\Delta x = 0$ and $\Delta t = T/n$. Then, to obtain the instantaneous velocity v from equation (1), it is only necessary to obtain Δx^*, which Dr. Phipps assumes is given by $\Delta x^* = s_0$. However, if the circumference of the disk is $2\pi r$ when measured in the laboratory system S, then by symmetry, the length of any segment measured in S is $s_0 = 2\pi r/n$. Therefore, in the frame S^*, which is at rest with respect to one of the segments, this segment has the greater length $s_0/(\sqrt{1-v^2/c^2})$. This follows from special relativity, according to which any object appears shortened if it is moving; that is, it appears longest in its own rest system. Substituting:

$$\begin{aligned}&\Delta x^* = s_0/\sqrt{1-v^2/c^2} = 2\pi r/(n\sqrt{1-v^2/c^2}),\\ &\Delta x = 0,\ \Delta t = T/n\end{aligned} \qquad (2)$$

in equation (1), we obtain the usual result:

$$v = 2\pi r/T = \omega r \qquad (3)$$

in disagreement with the result obtained by Dr. Phipps.

The validity of this derivation depends on the circumference of the rotating disk remaining $2\pi r$ when measured in S, rather than appearing foreshortened to $2\pi r\sqrt{1-v^2/c^2}$ as it would be if the circumference were rigid. Thus, we have assumed that the circumference stretches as a result of the motion, each segment attaining the length $s_0/\sqrt{1-v^2/c^2}$ in its own rest system. If we instead force the circumference to be rigid, then the radius of the disk must shrink. It is impossible kinematically for the radius and circumference to remain rigid and for the disk to stay in one piece. If the disk were infinitely strong as well as infinitely rigid, it could not be made to rotate, as work could not be done against its infinite cohesive forces.

I conclude that the round-trip velocity and instantaneous velocity are equal and that both remain less than c. Also, to attain edge speeds approaching c, rather than using an ideal rigid disk, we should use one of ideal rubber with one-way stretch.

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[1] Phipps, T. E., *Nature*, 195, 67 (1962).

OBJECTIONS to my analysis of re-entrant motion generally fall into one of two categories, (1) criticisms that accept the analysis within its terms of reference (namely, those of ordinary special relativity), but view the result as sufficiently ludicrous to refute one or more of the idealizations (for example, linear mathematics, rigid bodies) embodied in those terms of reference; (2) criticisms of the analysis as false and incompatible with special relativity.

Dr. Lichtenberg's replacement of the 'ideal rigid disk' by an 'ideal rubber' one puts his objection in the first category. While this criticism has undeniable merit, its rigorous consistency with the relativity principle is in doubt. To begin, let us fix in mind the fact that the locus of points in the laboratory frame S traced out by any point (hence all points) on the disk edge is a circle of radius r; rest-radius and moving-radius being always the same, by the assumption of special relativity theory that transverse motion of a measuring rod does not affect its length. Therefore any departure from 2π of the ratio of disk circumference (interpreted as a locus of points) to radius must be detectable only by an observer who participates in the motion of the disk. Considering such an observer, we note two alternative possibilities:

(1) He himself and his measuring rods are affected by motional influences in exactly the same way the disk-edge-marker segment lengths (of rest-length s_0) are affected. (To make this plausible we may suppose measuring rods and disk edge to be made of the same material.) In this case, no matter what the speed of the disk, it is impossible for the observer in question to measure any motional influence on the disk-edge segment lengths; and $\Delta x^* = s_0$, as asserted in my original communication. This disk-riding observer will of course find, by laying down measuring rods along the circumference and radius, the conventional ratio 2π, in agreement with the conclusion of the observer in S. Neither observer will encounter any occasion to introduce non-linear mathematics or a non-Euclidean spatial metric.

(2) The observer and his measuring rods are Lorentz-contracted, while the disk-edge-marker segment lengths are not. In this case the disk-edge-fixed observer measures an expansion of the segment lengths from their rest-length s_0 to a moving length $s_0/\sqrt{1-(v^2/c^2)}$, as asserted by both Dr. Lichtenberg and Dr. Boyer. If we define the 'ether' as that co-ordinate system in which the centre of the disk is at rest, our observer now has a simple way (by determining segment expansion) to measure his absolute speed with respect to the ether. This is true for an instantaneous measurement of disk-edge segment length, so that it applies not only to the disk-edge-fixed observer but also to an observer in a Lorentz frame S^* in which the disk-edge segment is momentarily at rest. Therefore we have provided a Lorentz observer with a method of measuring material-body speed with respect to an 'ether', in presumptive violation of the relativity principle.

Theories of 'one-way stretch' non-rigid disks (Lichtenberg) or of 'rigid but non-holonomic' co-ordinate systems (Boyer) are thus not relativity theories. They lead to circumference-to-radius ratios unequal to 2π in a moving sub-space of the same dimensionality as the parent flat space, a circumstance that has in the literature been variously characterized as 'well-known' (Boyer) or 'absurd'[1] depending on the context and point of view adopted. The foregoing discussion prompts the generalization that all motion-dependent-metric theories are incompatible with a strict interpretation of the relativity principle.

The second type of objection is raised by critics who detect an illegitimacy in the method of velocity measurement I used; compare Dr. Boyer's remark that "Phipps is in error in supposing that there is more than one way to measure the peripheral velocity in a Lorentz frame in which the centre of the disk is at rest". There are in the case of uniform rotary motion not one but three ways of disk-edge velocity measurement that are legitimate in the sense of being: (1) classically equivalent; (2) operationally well defined. The first two apply to rectilinear motion as well.

Method A. Two close-spaced fiducial marks are made on the disk-edge a known distance apart. A single fiducial mark is made in the laboratory system. A clock is located at the laboratory fiducial mark. The disk-edge speed v_A is obtained by dividing the pre-determined disk-edge-marker separation by the time-interval recorded by the clock between fiducial mark coincidences.

Method B. Two close-spaced fiducial marks are made in the laboratory system a known distance apart. A single fiducial mark is made on the disk-edge. Two clocks are introduced, each placed at one of the fiducial-mark locations in the laboratory system, and synchronized by some convention, universally (but arbitrarily) chosen as the Einstein convention. The disk-edge speed v_B is obtained by dividing the pre-determined laboratory-system marker separation by the time interval recorded by the clocks between fiducial-mark coincidences.

Method C. A single fiducial mark is made on the disk-edge. A single fiducial mark is made in the laboratory system. A clock is located at the laboratory mark. The disk-edge speed v_C is obtained by dividing the pre-determined distance travelled in the laboratory system by the disk-edge marker (equal to disk circumference, $2\pi r$, as previously established) by the time-interval recorded by the clock between successive fiducial-mark coincidences.

The substantive content of my previous communication was that $v_C = v_B/\sqrt{1-(v_B^2/c^2)}$. It is easily shown that, because of the Lorentz contraction, $v_A = v_B/\sqrt{1-(v_B^2/c^2)}$. Hence, $v_A = v_C$. Several critics have noted this equality and regarded it as a refutation of my previous analysis, because it is known that Method A is the 'wrong' way to measure velocity; hence any result that agrees with Method A must be wrong. Perhaps the reader can be prevailed on to postpone making a value judgment among the three velocity-measuring methods.

It should not be surprising that Methods A and C yield the same result, which is at variance with the result of Method B. Methods A and C make use only of proper time (measured by a single clock, without appeal to any definition of 'simultaneity' of spatially separated events); while Method B is unique in using 'co-ordinate' time (measured by a definition of simultaneity based on the Einstein convention).

The question as to whether an alternative clock-synchronization convention applied in Method B could yield a velocity v_B' in agreement with Methods A and C (so that $v_A = v_B' = v_C$ in relativistic as well as classical physics) is easily answered in the affirmative. The necessary and sufficient condition is as follows: a light pulse is sent out at time zero from the first clock and the event of its arrival at the second clock is defined as occurring at time zero there. In this way the two clocks read proper time ($\Delta\tau = 0$ on the light cone, so the light propagates in zero proper time at infinite 'proper speed'), and it is easily confirmed that the proper-time measurements so obtained by Method B agree with the proper-time measurements of Methods A and C and that the concomitant velocities agree. But, of course, none of these 'velocities' has anything to do with the v_B appearing in the Lorentz-transformation formulæ; for, as remarked above, v_B is operationally defined in a quite different way, with a different clock-synchronization convention.

Although the classical equivalence of the three velocity-measuring methods provides us with no *a priori* basis of preference, those who believe the Lorentz-invariant aspects of experience to be more closely correlated with objective 'reality' than the non-Lorentz-invariant aspects may be tempted to attach more fundamental significance to velocity-measurement methods (*A*, *C*, and the modified form of *B* just discussed) that invoke only invariant proper time than to methods (such as *B*) that invoke arbitrary simultaneity conventions of non-invariant significance.

It should now be apparent that customary analyses of circular motion, based on the assumption that $v_C = v_B$, cannot easily be supported by analysis consistent with the relativity principle; moreover, that the non-equality of v_B and v_C should be no more disturbing than the non-equality of v_B and v_A, in view of the differences in operational definition of the various kinds of 'velocity'. While doubts naturally remain, there would appear to be nothing so ludicrous in the result of my previous communication as to disprove the applicability of special relativity (with all its traditional idealizations) to problems of re-entrant motion.

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[1] Landau, L., and Lifshitz, E., *The Classical Theory of Fields*, 24 (Addison-Wesley, 1951). These authors, as a result of failing to define 'circumference' as a locus, unfortunately confuse the metric of a stationary observer with the metric that would be attributed by such an observer to a moving observer. Thus, the particular 'absurdity' they expose has a definitional origin and is unconnected with the rigid-body property to which they ascribe it. (All reference to the matter has been eliminated from the second revised edition, 1962.)

CHEMISTRY

Vibrational Energy Transfer between $N_2X^1\Sigma_g^+$ ($v = 0$) and NO $A^2\Sigma^+$($v = 3, 2$ and 1)

A GREAT deal of experimental data on vibration-translation energy transfer in gases has been accumulated by means of ultra-sonic and shock-wave techniques; very little information exists, however, on the rates of intermolecular exchange of vibrational energy. Basco, Callear and Norrish[1] have emphasized the probable importance of this effect in the relaxation of NO $X^2\Pi$ with CO even though the energy discrepancy is as high as 266 cm⁻¹. Lambert *et al.*[2] have pointed out the importance of such processes in the vibrational relaxation of mixtures of hydrocarbons and concluded that transfer occurs at practically every collision. Similar conclusions were reached by Valley and Legvold[3].

We have recently investigated this problem by exciting the γ-band system of nitric oxide in the presence of argon and nitrogen. We have minimized the effects of pressure broadening for which Terenin and Kleinberg[4,5] had to introduce large corrections and have measured the rates of relaxation of NO $A^2\Sigma^+$ in argon and nitrogen. Rapid exchange of vibrational energy occurs in nitrogen due to the process:

$$\text{NO A}^2\Sigma^+(v=x) + \text{N}_2X^1\Sigma_g^+(v=0) = \text{NO A}^2\Sigma^+(v=x-1) + \text{N}_2\,X^1\Sigma_g^+(v=1)$$

for which the energy discrepancy is only about 12 cm⁻¹. The rate of this exchange and its variation with quantum number have been measured and converted to the number of gas kinetic collisions required to remove one quantum of vibrational energy, using a collision diameter[6] of 3·8 Å for NO $A^2\Sigma^+$ and a radiative lifetime of $2{\cdot}2 \times 10^{-7}$ sec calculated from the *f*-value[7].

A 500-W xenon arc lamp was used as a high-intensity light source to excite the γ-band system of nitric oxide. Spectra were recorded on 'Selochrome' or *Q*3 plates after dispersion of the fluorescence with a Hilger medium quartz spectrograph. A large slit opening of 1·1 mm produced broad bands on the plates and densities were recorded on a Joyce–Loeble recording micro-densitometer. Plate densities were converted into arbitrary intensity units by calibration of the plates with a hydrogen discharge lamp. The experiments were carried out at 20° ± 2° C.

Vibrational relaxation due to added gases was detected by means of an ammonia filter which cut off all radiation below 2200 Å and thus removed all but the $v' = 0$ progression from the fluorescence. If relaxation in the excited state did not occur the intensity of the $v' = 0$ progression was the same whether or not the light was filtered through ammonia; if vibrational relaxation did occur, the intensity of this progression increased with unfiltered light. With pure nitric oxide a slight enhancement was observed but this was very slightly decreased rather than increased on adding argon, showing that relaxation with argon is a very slow process. Strong enhancement occurred in the presence of nitrogen. From measurements on the absorption spectrum it was confirmed that argon and nitrogen pressure broadened the γ-bands to the same extent; thus the number of molecules excited to any vibrational level is the same in the presence of the same total pressure of either of these gases. By changing the partial pressure of nitrogen the rate of relaxation was varied and was followed by means of plate photometry. The rates of transfer from $v' = 1$ to $v' = 0$ and from $v' = 2$ to $v' = 1$ were measured; a preliminary measurement has been made on the rate of transfer from $v' = 3$ to $v' = 2$. An isobutene filter removed all but the $v' = 1$ and 0 progressions and propylene removed all but $v' = 2$, 1 and 0 progressions.

In order to determine the number of collisions required to remove one vibrational quantum, it is essential to know accurately the rates of quenching of the electronically excited molecules by gases present in the system since they affect the mean lifetime. Self-quenching by NO $X^2\Pi$ is particularly important. Quenching half-pressures of carbon dioxide in the presence of different pressures of nitric oxide and argon were determined from conventional Stern–Volmer plots. From the variation of the carbon dioxide quenching half-pressures under different conditions it was possible to calculate the quenching half-pressures of all three gases at zero pressure of the other two. It may be noted that the carbon dioxide half-pressure is in fair agreement with that of Young and Sharpless[8] and that carbon dioxide is about three times more effective than NO itself in quenching NO $A^2\Sigma^+$.

The variation in intensity of the γ-band system with nitric oxide pressure was measured at pressures less than 1 mm. Applying a correction for light absorption, assuming a Doppler shape for the rotational lines and negligible spin doubling in NO $A^2\Sigma^+$ it was possible to arrive at a value for the self-quenching half-pressure in agreement with that quoted in Table 1. Moreover, it was shown that any difference in the quenching-rates by nitrogen and argon could be neglected under the conditions in which the relaxation measurements were made. It must be emphasized that when a system is strongly self-quenched the measured quenching half-pressures of other gases will depend considerably on the pressure of the fluorescing gas present. This undoubtedly accounts for certain discrepancies in the values of quenching half-pressures found in the literature.

Table 1. QUENCHING HALF-PRESSURES

Carbon dioxide	0·31 mm
Nitric oxide	0·91 mm
Argon	~ 1,400 mm

It is now possible to predict the mean lifetime of a molecule excited into the $A^2\Sigma^+$ state in any mixture of nitric oxide, argon and nitrogen and therefore possible to determine Z, the number of collisions required to remove one vibrational quantum. These results are shown in Table 2 and are compared with Z calculated from the theory of Herzfeld, Schwartz and Slawsky[9].

Table 2

	$Z_{3,2;0,1}$	$Z_{2,1;0,1}$	$Z_{1,0;0,1}$	$Z_{1,0;0,1}$ (calculated)
Nitrogen	240	470	830	3,000
Argon	—	—	> 5,000	10^{10}

These results are in good agreement with the theory, and it is unnecessary to postulate chemical interaction. The adiabatic nature of the interaction is consistent with the low quenching efficiency of nitrogen. The ratio $Z_{3,2;0,1} : Z_{2,1;0,1} : Z_{0,1;0,1}$ agrees with the predicted ratio of 1 : 1·5 : 3 and our results are consistent with the prediction that collisions in which more than one quantum is exchanged are rare.

The enhancement of the $v' = 0$ progression when unfiltered light is used with pure nitric oxide may be due either to abnormally fast relaxation by NO $X^2\Pi$ which has been suggested by Carrington[10], or to cascade radiation or quenching from the $C^2\Pi$ state reported by Young and Sharpless[8]. It also appears probable that some of the enhancement of the $v' = 0$ bands on adding nitrogen is due to collision-induced transitions from the $C^2\Pi\,(v = 0)$ state. The δ bands are quenched much more strongly by nitrogen than are the γ-bands. This work is being extended to investigate the quenching of the δ bands in more detail and to measure accurately the rate of the exchange of NO $A^2\Sigma^+(v = 3)$ with nitrogen.

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Hydrogen Bonding in Gaseous Mixtures: Amine–Alcohol Systems

EARLIER infra-red detection of hydrogen bonding in gas-phase mixtures of strong acids and weakly basic materials[1] has now been extended to cover mixtures of weakly acidic and more strongly basic compounds. In each case it has been found possible to choose substances which hydrogen bond, and at the same time to avoid condensation, or precipitation of a salt. We now report the infra-red detection of hydrogen bonding in gaseous mixtures of methanol and various amines, ethylenimine and ammonia.

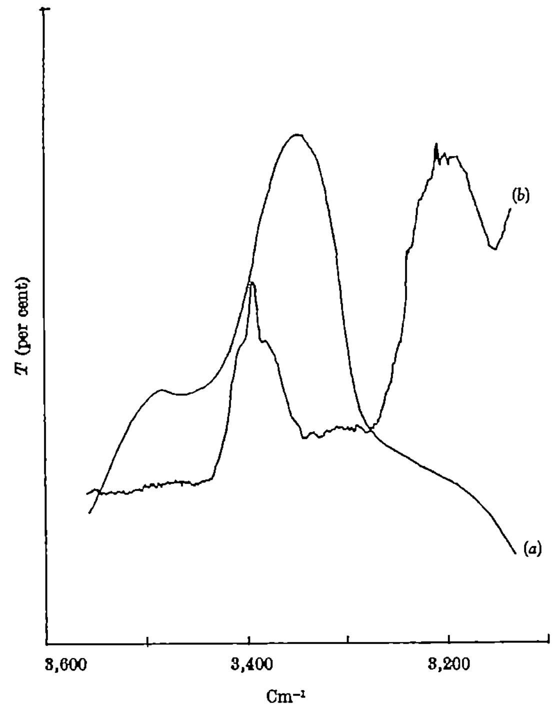

Fig. 1. *a*, Methanol–trimethylamine complex; *b*, trimethylamine

In Fig. 1 the infra-red absorption spectrum in the region 3,100–3,600 cm⁻¹ is shown for the hydrogen-bonded complex formed between methanol and trimethylamine. In obtaining the spectrum, allowance has been made for the absorption of the free amine, shown in curve *B*; methanol does not absorb appreciably in this region. The dependence on pressure of the band-intensity shows that it arises from a 1 : 1 complex.

The band contour shows a strong qualitative resemblance to those of the complexes formed by ethers with hydrogen fluoride or hydrogen chloride. There is a strong central peak accompanied by weaker bands on each side. It appears that this form of band contour, with variations in the frequency displacements and intensities of the side bands, may be fairly general in simple hydrogen-bonded complexes, and it is natural to offer a general interpretation. In the particular case of trimethylamine, the central peak at 3,350 cm⁻¹ may be assigned as ν_3, essentially the O–H stretching vibration in the complex. This frequency shifts to 2,500 cm⁻¹ when methanol-*d* is used. The weaker band at about 3,495 cm⁻¹ may then be attributed to the sum band $\nu_3 + \nu_1$

$$\leftarrow R_3\text{N} \cdots\cdots \underset{\nu_1}{\text{H}} \rightarrow\!\!-\text{O}R\rightarrow \qquad \text{R}_3\text{N}\!-\!\!\leftarrow \underset{\nu_3}{\text{H}}-\text{O}R\rightarrow$$

and the weaker absorption in the region 3,200 cm^{-1} interpreted as the difference band $\nu_3 - \nu_1$. The assignment of a frequency of about 145 cm^{-1} to ν_1 leads to a hydrogen bond stretching force constant of approximately 0·25 *md/A*.

There is an alternative possible explanation of the band at 3,495 cm^{-1} which has to be considered, since this type of explanation has been suggested for other hydrogen-bonded complexes. A moderately weak band appears in the spectrum of the free amine, as shown in Fig. 1 (curve *B*), at about 3,395 cm^{-1}. The corresponding combination-level in the complex might be supposed to enter into Fermi resonance with the $\nu = 1$ state of the O–H stretching vibration and the stolen intensity to appear in the band at 3,495 cm^{-1}. Such an interpretation would have to be regarded as specific to the methanol–trimethylamine complex for it has been shown that Fermi resonance cannot account for the qualitatively similar band contours for complexes formed between ethers and hydrogen fluoride or chloride. On the other hand, the interpretation based on the appearance of sum-and-difference bands is a general one allowing for quantitative differences in the band contour because of different possible values for the low-frequency stretching vibration ν_1 and the relative intensities of the combination bands for the different complexes.

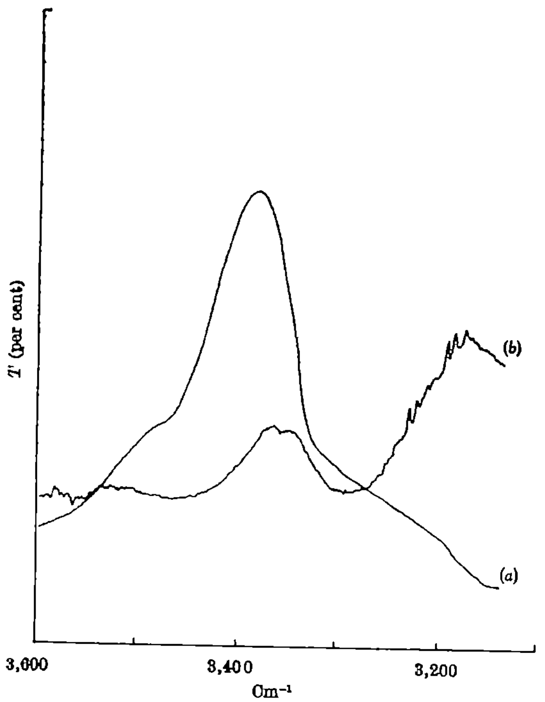

Fig. 2. *a*, Methanol–dimethylamine complex; *b*, dimethylamine

The analogous part of the spectrum of the complex formed by dimethylamine and methanol is shown in Fig. 2 (curve *A*). In this case the O–H stretching frequency for the complex falls very close to the N–H stretching frequency of the amine (about 3,350 cm^{-1}, curve *B*) but there are no indications of interaction between the two vibrations in the complex. The shoulders on the high- and low-frequency sides of the central peak may be assigned as sum-and-difference bands as before. The difference between the contours for the two complexes may be accounted for if it is supposed that ν_1 has a lower value for the dimethylamine complex, and so the sum band is not resolved as a separate peak in this case, but appears as a shoulder. The suggestion that the hydrogen bond is weaker in this complex is in accord with the usual assumption that the displacement, $\Delta\nu$, of the hydrogen stretching frequency of the complex from that of the free molecule is an indication of hydrogen bond strength. The frequency displacements for the various complexes are as follows:

Base	$(CH_3)_3N$	$(CH_3)_2NH$	CH_3NH_2	NH_3
Δ (cm^{-1})	330	300	235	170

The band contours for the complexes formed by methanol with methylamine and with ammonia qualitatively resemble the band shown in Fig. 2, and are interpreted in the same way.

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Conception and Significance of the Parachor

As a part of a broader scheme dealing with empirical equations in organic chemistry, the parachor was also revised. Parachor is defined[1] in equation (1), where γ is the surface tension, d denotes the density and M the molecular weight:

$$P = \gamma^{1/4} d^{-1} M = \Sigma P_i \tag{1}$$

This expression is considered to be an additive quantity, that is, can be approximately expressed as a sum of empirical increments P_i corresponding to the single atoms or groups in the molecule. As an additive quantity the parachor has been used in solving various structural problems.

The present revision of equation (1) was carried out from a purely empirical point of view, namely, the accordance of the calculated and found values was taken as the only criterion of its correctness, regardless of the eventual physical meaning[2]. Since the parachor is composed of three quantities which are *a priori* independent, it is necessary to examine their influence on the final result and their eventual statistical dependence *a posteriori*. Especially important is the influence of the molecular weight which itself can cause the approximately additive character of the whole expression provided that the remaining quantities are changed relatively to a small extent. Therefore, it is not possible to compare directly the calculated and found values according to equation (1), since on both sides of the equation the molecular weight is included, on the right-hand side implicitly in the atomic increments. The statistically correct examination was carried out in several series of homologous compounds in which the influence of the molecular weight could be eliminated, since according to equation (1) the following expression should apply:

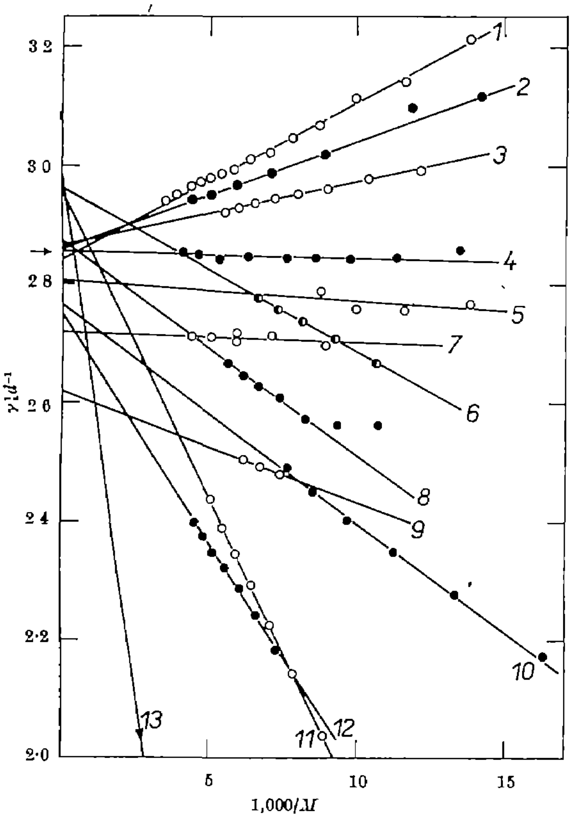

Fig. 1. The statistically correct examination of the additivity of the parachor by plotting $\gamma^{1/4}d^{-1}$ versus $1/M$ according to equation (2). The individual lines represent the following homologous series. 1, *n*-alkanes; 2, alkenes-1; 3, alkines-1; 4, ethers, 5, aldehydes; 6, α,ω-dinitriles; 7, ketazines; 8, 4-alkylpyridines; 9, alkyl benzyl ketones; 10, 1-nitro compounds, 11, α,ω-dichlorides, 12, alkyl nicotinates; 13, α,ω-dibromides. The arrow denotes the point of intersection claimed for the value 40 0 of the CH_2-increment. The majority of experimental values were taken from ref. 3

$$\gamma^{1/4}D^{-1} = \frac{C}{M} + \frac{P(CH_2)}{14{,}026} \qquad (2)$$

where C is a constant characteristic for each homologue series and $P(CH_2)$ is the increment of the methylene group. From a graphical representation (Fig. 1), it is seen that equation (2) is not fulfilled even for homologue series of relatively simple derivatives. Since the straight-lines do not intersect in one point, the increment of the group CH_2 cannot be determined common for all homologue series so that contentions concerning its right value[1-3] are ungrounded. It follows that the generally used formula, equation (1), is not well founded; the additivity is only approximately pretended by the additivity of the molecular weight or of the molar volume.

From both measured quantities the density exerts a far greater influence on the result. The influence of the surface tension, weakened still by the empirical exponent 1/4, represents only a smaller correction which does not contribute to the additive character of the over-all expression and, on the contrary, causes deviations from the additive behaviour; for example, at least with simpler hydrocarbon derivatives the principle of additivity is fulfilled more exactly for the molar volume than for the parachor. Now since measured values of the surface tension have been used almost exclusively for calculation of the parachor and discussed in this form, very little is known about the dependence of the surface tension on the structure.

Both quantities under consideration are markedly statistically dependent (substances with a greater specific weight have a higher surface tension) and their influence on the parachor is mutually compensated. Therefore, in the unfavourable case, up to 1/4 of all organic compounds can yield experimental values acceptable for a chosen structure; at the same time especially the surface tension can achieve even more than one half of the possible values, an acceptable accuracy of the result being maintained.

Conclusions concerning the structure, made on the basis of the parachor, are therefore dubious. Some authors[2,3] attempt to improve this method by a detailed specialization of the increments and by introducing corrections for interaction of the groups. This leads to a certain arbitrariness on one hand, and to a successive loss of the additive character on the other. In a similar way, the dependence of every physical quantity on structure can be grasped; instead of the parachor it would be more simple and suitable to discuss in this way the molar volume.

It can be concluded that the conception of the parachor has no material significance and its liquidation can be suggested.

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Free Radicals formed during the Oxidation and Reduction of Peroxides

The reduction of hydrogen peroxide by transition metal ions is thought to involve hydroxyl radicals as intermediates[1]. We have found that dilute solutions of titanous ion and hydrogen peroxide (about 10^{-3} M) react together to form a free radical of short life. This has been observed by using a flow system in which the reactants are mixed just before entering the cavity of a Varian electron spin resonance spectrometer. The spectrum is a single line near $g = 2$, width about 3 gauss, the intensity of which at maximum flow-rate is proportional to $[Ti^{3+}]$ and $[H_2O_2]$ when these are low.

The radical may be $\cdot OH$ or $\cdot O_2H$, according to the reactions[1]:

$$Ti^{3+} + H_2O_2 \rightarrow Ti^{4+} + OH^- + \cdot OH$$
$$\cdot OH + H_2O_2 \rightarrow H_2O + \cdot O_2H$$

There is evidence, however, that the perhydroxyl radical gives a much broader signal because, when hydrogen peroxide[2], *t*-butyl hydroperoxide, and cumyl hydroperoxide are oxidized by ceric ion, single lines of width 27, 14, and 6 gauss, respectively, are observed, presumably corresponding to peroxy radicals:

$$Ce^{4+} + RO_2H \rightarrow Ce^{3+} + H^+ + RO_2^{\cdot}$$

An attempt to observe the *t*-butoxy radical in the reaction between *t*-butyl hydroperoxide and titanous ion resulted in the appearance of a quartet of intensities 1 : 3 : 3 : 1, the distance between adjacent peaks being about 24 gauss. This is apparently due to the methyl radical[3] arising from the reactions:

$$Ti^{3+} + Me_3COOH \rightarrow Ti^{4+} + OH^- + Me_3CO\cdot$$
$$Me_3CO\cdot \rightarrow Me_2CO + CH_3\cdot$$

These results suggest that the radical observed in the reaction between hydrogen peroxide and titanous ion is hydroxyl.

The signal corresponding to this radical disappears when the reactant solutions contain various aliphatic or aromatic compounds and is replaced by spectra of new radical intermediates. For example, 2-propanol gives a septet together with a weaker quartet, peak-to-peak distances being about 20 and 24 gauss, respectively. These are similar to spectra previously ascribed to $\mathrm{Me_2\dot{C}{-}OH}$ and $\mathrm{\dot{C}H_3}$ which were observed in the solid state at low temperatures[3,4] except that the hyperfine line-widths which we observe are narrower (approximately 1·5 and 2·5 gauss, respectively).

These results confirm that the hydroxyl radical is very reactive, may be obtained in aqueous solution at room temperature, and can be used to generate other free radicals. A discussion of all the spectra and a description of the flow system will be published.

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BIOCHEMISTRY

An Examination of HeLa Cancer Cells for Production of Gamma-Globulin

A METHOD has been described for detecting and measuring the autonomous production of well-defined proteins by cells in tissue culture[1,2]. The method is exceedingly sensitive and is applicable even when the cells are cultivated in a 'natural' nutrient medium, that is, a medium also containing serum; such media have an enormous excess of pre-existing proteins. The tissue is incubated in presence of a radioactive amino-acid and the whole of the soluble protein is extracted. Thereafter individual proteins are isolated from the mixture and burnt, and the carbon dioxide obtained tested for radioactivity with a gas counter (efficiency nearly 100 per cent)[3,4]. The identity of a fraction can be confirmed by treatment with specific antiserum, followed by determination of the distribution of the radiocarbon between precipitate and supernatant. Blank experiments show that after incubation of natural medium with radioactive amino-acid without cells practically no radiocarbon is found in protein.

Now we have tested HeLa human cervix carcinoma cells, grown as described before[5], for the production of γ-globulin. Pieces of cultivated HeLa tissue were trypsinized, and aliquots of the suspension obtained were introduced into plasma-coated roller tubes. The resulting monolayers were incubated several days at 37° C with a natural medium. Then the medium was changed for a protein-free medium of exactly known composition ('synthetic' medium[6,7]), also containing 0·5 μc. of 1-^{14}C-D,L-leucine per tube. The incubation was continued for 4 days, 1-ml. human cord serum was added as a carrier to each roller tube, the cells were broken by repeated freezing and thawing and the cell debris removed by centrifugation. The supernatant was freed from low-molecular substances, including the radioleucine, by ultra-filtration under pressure, followed by repeated washing with physiological saline solution[2].

Table 1. RADIOACTIVITY IN THE γ-GLOBULIN FRACTION FROM HELA CELLS

Exp. No.	Final cell No.	Activity of total soluble protein (d.p.m.)	Activity of γ-globulin (d.p.m.)	Part of protein activity in γ-globulin (per cent)
1	352,000	72,000	1,330	1·8
2	352,000	72,000	1,940	2·7
3	429,000	84,000	2,340	2·8
4	429,000	84,000	2,930	3·5

Thereafter, the γ-globulin was separated from the mixture of the soluble proteins by precipitation in the cold with ethanol[8]. Between 3·6 and 5·6 mg γ-globulin—of course, mostly from the carrier—were recovered. The purity of the preparation was checked by electrophoresis in starch gel[9] and staining, and no protein found elsewhere than in the γ-globulin band. The activities per roller tube found in four separate experiments and calculated to the total amount of γ-globulin present in the carrier are given in Table 1. The cell numbers were obtained from counts, after trypsinization, of the cells in parallel tubes, that is, in roller tubes which had been charged with equal numbers of cells and afterwards kept in the same conditions as the tubes used for radiochemical analysis.

In further experiments the soluble proteins obtained by ultra-filtration were subjected to electrophoreses in agar gel. In each case, two parallel samples (0·2 ml. of 7 per cent protein solution—the protein again mostly derived from the serum carrier) were used. After electrophoresis, one sample was stained to indicate the position of the protein bands. The other sample was cut in strips at right angles to the field, the strips were burnt, and the radioactivities per unit strip width determined. Only 3·3 per cent of the total radiocarbon found in the electropherogram was contained in the γ-globulin fraction, in reasonable agreement with the results given in Table 1. The percentage is, however, less than that given previously[1,2]. Essentially the same result (3·7 per cent) was obtained after incubation with radioleucine in a natural instead of a synthetic medium; in this case no protein carrier was added after incubation. Incidentally, it was confirmed in the last-mentioned experiments that the protein of the medium remains practically non-radioactive on incubation with active amino-acid in the absence of cells.

Finally, a γ-globulin preparation obtained from HeLa cells incubated with radioleucine by the ethanol fractionation method and afterwards purified by electrophoresis in starch gel was tested radio-immunologically. 1·2 mg γ-globulin were mixed in the optimal ratio with antiserum against human γ-globulin from rabbits. The antiserum had been tested for specificity by the Ouchterlony method[10]. The total volume was 6 ml. The precipitate formed and the supernatant were burnt separately, and the radioactivities measured. The activity of the precipitate was found to be (1·2 ± 1·9) disintegrations per minute (d.p.m.), that of the supernatant (270 ± 10) d.p.m. Thus the protein fraction from HeLa cells which behaves in respect to precipitation with alcohol as well as in respect to electrophoresis in starch like γ-globulin cannot be identified as normal human γ-globulin by immunology.

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Analysis of Fatty Acids of Continuously Cultured Mammalian Cells by Gas-Liquid Chromatography

SINCE the introduction of gas-liquid chromatography by James and Martin[1], a variety of animal tissues have been analysed for their fatty acid composition. The present report describes the application of the technique to the analysis of the fatty acid content of mammalian cells in continuous cultivation. Because of the small quantity of sample required by this method, it seemed well suited to the examination of fatty acids in cultured mammalian cells. Furthermore, because of the advantages inherent in the technique, analyses of the fatty acids of the cells could be made periodically with ease. Inasmuch as regular trypsinization and re-seeding of cells are necessary for the maintenance of a continuously cultured line of cells, the effect of this treatment on the fatty-acid content of the culture was examined at intervals over about twelve months.

The two cell cultures used in this work were strain HeLa and bovine lymphosarcoma (obtained from the Department of Veterinary Science, Pennsylvania State University, University Park, Pennsylvania). The cells were propagated in 1-l. Blake bottles (Corning Glass). The bottles were seeded with about 1·5 × 10⁷ cells in 50 ml. of lactalbumin-bovine serum (10 per cent) medium and incubated at 37° C. The cells were extracted at the end of a 48-h growth period. One lot of lactalbumin hydrolysate and a single pool of bovine serum were used to prepare growth medium throughout the study.

Cultures to be analysed were washed with Hanks's balanced salt solution after removal of the growth medium and freed from the glass surface with 0·3 per cent trypsin solution and washed again in balanced salt solution. Hot ethanol was used to extract the lipids from the cells. The lipids were saponified and the non-saponifiable material removed with petroleum ether (30°–60° C). The fatty acids were freed from their salts using a sufficient quantity of 5 N sulphuric acid and afterwards extracted with petroleum ether. The fatty acids were esterified using a solution of 1 per cent sulphuric acid in methanol and refluxing for 2 h. The methyl esters were extracted from this solution with petroleum ether. The solvent was evaporated under nitrogen and 30λ of benzene or petroleum ether were added to the methyl ester samples.

The gas chromatograph (Barber-Colman, model 20) used an ionization chamber as detector (tritium, 0·1 c.) and argon as the carrier gas at 20 lb. pressure. The stationary phase for the chromatography columns was ethylene glycol succinate polyester (17 per cent) coated on 'Celite', 80–100 mesh. The column-length was 10 ft. and its temperature was maintained at 180° C.

The series of peaks resolved on the chromatogram range from lauric to linoleic acids. Little or no evidence was obtained for C_8 or C_{10} compounds. The lower-boiling-point compounds, if present, may have been somewhat obscured by the extended peak of the solvent. Calculating the peak areas of this range of compounds and using the total as 100 per cent, the individual compounds are calculated as the ratio of the individual peak areas to the total, giving the weight per cent as reported in gas chromatographic analysis of other lipid-containing substances[2,3]. The compounds were identified by comparison of their retention times with those of known standards[3]. In Tables 1 and 2 are given the results of the analyses of HeLa and lymphosarcoma cells over a period of approximately one year. The transfer numbers represent the serial trypsinizations and reseedings of the cell cultures.

The fatty acids below lauric (C_{12}) and above linoleic ($C_{18\cdot2}$) are not included in this communication because they are present in small quantities compared to the compounds included in the tables, or absent altogether. These fatty acids are now being investigated.

From the analyses reported here, it is evident that the lipids of cultured mammalian cells may readily be

Table 1. QUANTITATIVE ANALYSIS BY PEAK AREAS FROM GAS CHROMATOGRAMS* OF FATTY ACID METHYL ESTERS TAKEN FROM VARIOUS CULTURE TRANSFERS OF HELA CELLS

Methyl ester C	Culture transfer No.							
No.	3†	5	12	17	32	40	45	49
12	1·7‡	1·4	1 6	1·3	1·8	1·7	1·8	1·2
14	1·7	2 0	1·6	1·6	1·1	1·4	1·4	1·7
16	16·8	17·2	17·0	16·6	17·1	16·7	16·9	16·9
16·1	3·7	3·4	3·3	3·6	3 6	3·9	3·7	3·7
18	15·4	15·8	15·1	15 4	15·8	16·0	15·8	15·2
18·1	27·8	28·4	28·0	27·6	27·9	28·5	27·5	27·8
18·2	10·0	9·6	10·2	9·9	9·9	10 3	10·4	10·0
Total								
Saturated	35·6	36·4	35·3	34·9	35·8	35·8	35·9	35·0
Unsaturated	41·5	41·4	41 5	41·1	41·4	42·7	41·6	41·5
Unidentified	22·9	22·2	23·2	24·0	22·8	21 5	22·5	23·5

* Samples run on polyester succinate packed column at a temperature of 180° C.
† Transfers in this Laboratory.
‡ Expressed as weight per cent.

Table 2. QUANTITATIVE ANALYSIS BY PEAK AREAS FROM GAS CHROMATOGRAMS* OF FATTY ACID METHYL ESTERS TAKEN FROM VARIOUS CULTURE TRANSFERS OF BOVINE LYMPHOSARCOMA CELLS

Methyl ester C	Culture transfer No.						
No.	121†	128	137	138	139	141	142
12	0·8‡	0·9	0·8	1·2	1·0	0·9	1·0
14	2 4	2·9	2 4	2·2	2·5	2·4	2·1
16	16·6	16·9	17 8	16·4	16·2	16·6	16 5
16 1	5 0	4·1	4 9	4·1	4·3	4·0	4·4
18	9·7	10·7	9 3	9·9	10·1	10·2	10·3
18·1	24 8	25 5	26·1	26·0	25·1	25·8	26·1
18·2	12·7	12·4	12·1	12·9	12 7	11·9	12·9
Total							
Saturated	29·5	31·4	30·3	29 7	29 8	30·1	29·9
Unsaturated	42·5	42·0	43·1	43 0	42·1	41·7	43·4
Unidentified	28·0	26·6	26·6	27·3	28·1	28·2	26·7

* Samples run on polyester succinate packed column at a temperature of 180° C.
† Transfers in this Laboratory.
‡ Expressed as weight per cent.

studied using gas-liquid chromatography. Further, it is of interest to note that in a well-established cell culture, propagated continuously, the fatty acids of the cells remain essentially constant, qualitatively and quantitatively, through a considerable number of culture transfers and over an extended period of time, at least if the growth medium constituents are not altered.

Additional investigations are in progress which compare the lipids of a variety of continuously cultured cell lines at varying times during the growth cycle of the cells, and the effects of virus infection on the lipid metabolism of the cells.

We are indebted to Dr. Stuart Patton for advice and help.

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Demonstration of Peroxidase Activity in the Rat Kidney and Liver: Peroxidase Effect of Cytochrome c

Earlier investigations[1,2] of peroxidase activity in homogenates of rat kidney and liver have led to the conclusion that the total activity of the soluble fraction can be accounted for by its hæmoglobin content. Several objections can, however, be raised against this. As hæmoglobin (as well as its derivatives) and catalase are the two main complicating factors in the determination of endogenous peroxidase activity of tissue homogenates, the ideal estimation procedure is that which completely removes the hæmoglobin and the catalase from the homogenates. Complete removal of hæmoglobin is impossible. However, a method is described here for preparing a tissue homogenate free from catalase and containing only minimal amounts of hæmoglobin.

The kidneys of adult albino rats were perfused each with 100 ml. of physiological saline at room temperature through the aorte, removed and trimmed of visible fat and connective tissue at the renal hilus. Thereafter, they were cut in small pieces and an equal volume of physiological saline added before homogenization in a Lourdes 'Multimixer' at 0° C (ice water). The supernatant after high-speed centrifugation (105,000*g*) for 0·5 h, containing the soluble material of the homogenate, was dialysed against running tap water and physiological saline, respectively. Electrophoresis on paper (Fig. 1) demonstrated (using benzidine–barium peroxide as location reagent) one component with strong peroxidase activity migrating towards the cathode (a tissue component) and one component with weak peroxidase activity migrating towards the anode (free hæmoglobin). On heating the supernatant for 10 min at +65° C a heavy precipitate of protein was formed. In the supernatant thus obtained no catalase activity could be demonstrated using a modification of the perborate method of Feinstein[3], and the electrophoresis demonstrated only the peroxidase active tissue component on benzidine staining, almost as strong as before heating. The peroxidase activity of this component was also tested using pyrogallol as hydrogen donor and hydrogen peroxide as oxidizing substrate. The type reaction (Fig. 2, 1) described initially a zero-order course; later on, the rate fell off into a more or less first-order reaction curve. The *p*H optimum was found to be 3·65 using citrate-phosphate buffers prepared according to McIlvaine[4]. The activity increased exponentially with the concentration of pyrogallol and hydrogen peroxide until the limiting concentrations (in the final reagent solution) of about 0·13 M and 0·08 M were reached, respectively. At concentrations of hydrogen peroxide above 0·08 M a slight inhibition was observed. The

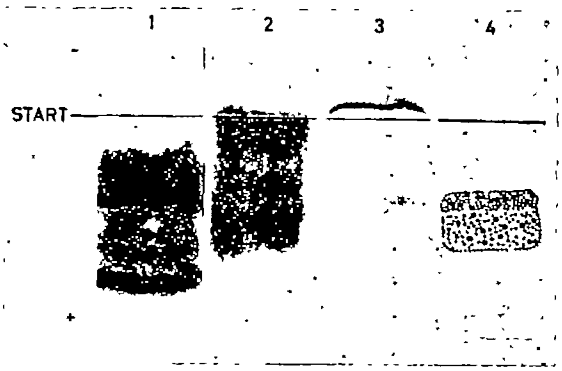

Fig. 1. Migration pattern on paper electrophoresis of the proteins in the soluble fraction of the kidney homogenate. 1, Normal rat serum stained with amido black, as a reference. 2, 3 and 4, The homogenate. 2, Amido black staining shows no distinct lines, but only a 'diffuse' staining (mostly tissue proteins). 3, Benzidine–barium peroxide staining shows two lines giving the colour of benzidine blue, one migrating towards the anode (free hæmoglobin) and one migrating towards the cathode (the tissue component). 4, Site for catalase reaction. The dotted area corresponds to the development of gas bubbles on the filter paper. The size of the dots corresponds to that of the gas bubbles. Michaelis veronal buffer *p*H 8·6. 10 mm³ of the material was applied on each paper strip

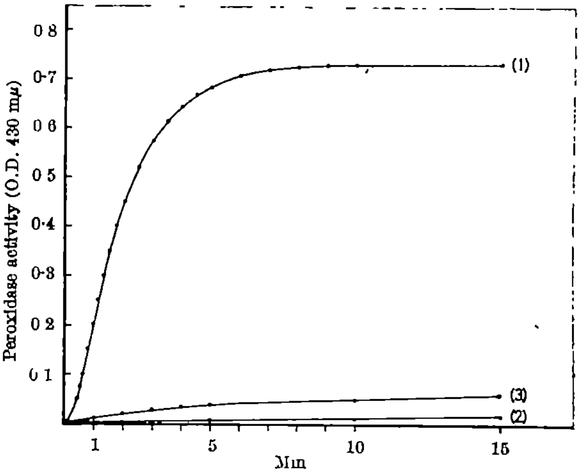

Fig. 2. Typical progress curves obtained when estimating the peroxidase activity: (1) of the kidney homogenate (the soluble fraction); (2) of a 4 mg per cent solution of crystalline rat hæmoglobin (ref. 7) (this concentration was equivalent to the content of hæm in the soluble fraction before heating, measured as pyridine hæmochromogen (ref. 8)); (3) of a 4 mg per cent solution of crystalline rat hæmoglobin saturated with human haptoglobin. Test system: 2·5 ml. of a 0·16 M solution of pyrogallol in citrate–phosphate buffer (ref. 4) + 0·05 ml. of the sample to be tested + 0·5 ml. of a 0·5 M aqueous solution of hydrogen peroxide. The final *p*H of this mixture was 3·65 and the reaction temperature + 20° C. A blank solution was prepared in the same way but with distilled water instead of hydrogen peroxide. The formation of purpurogallin was determined by the optical density at 430 mμ

Km values were calculated to be $9\cdot5 \times 10^{-3}$ M (pyrogallol) and $5\cdot6 \times 10^{-3}$ M (hydrogen peroxide). The activity was proportional to (a linear function of) the concentration of the homogenate added. Thus, the kinetics of this reaction fulfils the criteria of an enzymatic reaction and the tissue component must be referred to as a peroxidase.

Electrophoresis on paper demonstrated the peroxidase-active tissue component to be a cationic (basic) protein which, due to adsorption, was difficult to elute from the filter paper on preparative electrophoresis. However, the elution succeeded when the electrophoresis was performed in the following way: an acid-cleaned Whetman No. 31 filter paper and a *tris*-ethylenediamine tetraacetic acid–boric acid buffer of *p*H 9·0 (ref. 5) were used. 1 ml. of the homogenate supernatant (neither dialysed nor heated) was applied on a dry sheet (18 × 42 cm) by drawing a fine capillary pipette repeatedly along the starting line. The electrophoresis was run for 16 h at a potential gradient of 4·5 V/cm. Using this procedure a distinct pink-coloured line appeared on the cathodic side well separated from the other protein fractions. The material of this line was eluted with distilled water when the paper was still wet, and the spectrophotometric analysis demonstrated the characteristic absorption maxima of reduced cytochrome *c* ($CyFe^{++}$) with the α-band at 550 mμ, the β-band at 520–521 mμ and the Soret peak at 415 mμ.

Testing the liver homogenate, prepared in the same manner, identical results were obtained. Exactly the same *p*H optimum was found, but the peroxidase activity of the kidney homogenate relative to that of the liver was about 2·8 : 1; this is of the same order as reported earlier on the relative content of cytochrome *c* in these organs[6]. The peroxidase reaction described appears to be a useful method for quantitative measurement of cytochrome *c* in tissue homogenates of perfused organs.

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Inhibited Incorporation of Ammonium Chloride labelled with Nitrogen-15 in Chick Embryos treated with β-Aminopropionitrile

WE have described experiments on the incorporation of ammonium chloride labelled with nitrogen-15 into the collagen of β-aminopropionitrile-treated (lathyritic) chick embryos[1]. After 3–4 days continued hatching the specific concentration in atom per cent excess was higher in collagen of the lathyritic embryos. In 4-h experiments with carrageenin granuloma slices the incorporation of radioactive proline was decreased by addition of β-aminopropionitrile. We believe that the discrepancy depended on the time factor. The primary effect is probably an inhibition in the utilization of the amino-acids so that the labelled constituents remain longer in the amino-acid pool and cause an apparently increased incorporation at later stages.

Table 1. NITROGEN-15 (PER CENT ATOM EXCESS) IN THE DIFFUSIBLE AMINO-ACID* POOL OF WHOLE CHICK EMBRYOS

Experiment	Control	β-Aminopropionitrile-treated
1	0·066	0 211
2	0·177	0 345

* The amide nitrogen was removed by steam distillation after hydrolysis on boiling water bath in N sulphuric acid for 3 h and subsequent alkalization (ref. 2). The samples were pooled from 10–20 embryos.

The retarded removal of nitrogen-15 from the amino-acid pool was proved by 4-h experiments using 12-days-old chick embryos (Table 1). The other conditions were the same as previously. Similar but smaller difference was observed after 24 h. Work is in progress to decide whether the distribution of the labelled nitrogen among the amino-acids is normal in lathyrism.

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Catalytic Activity of Sub-units of Glutamic Dehydrogenase

L-GLUTAMIC dehydrogenase (GDH) has been shown to dissociate into sub-units under a variety of experimental conditions, such as dilution[1], high or low *p*H[2,3], and presence of DPNH[4], urea[5], diethylstilbœstrol[6]. or bicarbonate ions[7]. The relationship between the state of aggregation of the enzyme and its catalytic activity has been the subject of some controversy. Several investigators have attempted to relate the state of aggregation of the enzyme, as studied at high protein concentrations, to catalytic activity measured at protein concentrations several orders lower in magnitude[4,8,9]. Recent light scatter studies in this laboratory have shown that if the degree of splitting of the enzyme is plotted against *p*H and enzyme concentration, a quite irregular mathematical surface results[2]. Consideration of such surfaces plotted from experimental results obtained in the presence of various substrates, coenzymes, and other compounds suggested to us that it is, in general, quite invalid to relate catalytic activity measured under one set of conditions to degree of aggregation of the protein measured under different conditions. We therefore sought to measure both catalytic activity and the degree of aggregation of the protein in identical solutions so that these two properties might be related to each other in a valid manner.

Since we have obtained evidence for at least four different levels of organization of sub-units of the GDH molecule[2] (and ultracentrifuge studies by Frieden[7] reveal at least two such levels), it seemed advisable to confine the observations to a set of conditions under which only a single transition between levels of organization occurs. For this initial investigation of the relation between structure and function of sub-units, we chose the process of splitting by dilution, described originally by Olson and Anfinsen[1].

To render this account compatible with a scheme of nomenclature to be described in full elsewhere, we define the 'normal' aggregated form of the molecule as the α form, and the sub-units into which the α form splits on dilution as the β units. This process (which is reversible) may be referred to as the 'α, β-dissociation'.

Having selected the process we wished to examine, two requirements remain: (1) a set of conditions under which this process alone occurs; (2) a way of reducing the reaction-rates under these conditions to values that can be measured accurately throughout the necessary range of protein concentration. Both requirements must be satisfied simultaneously. Requirement (1) was met by reference to the mathematical dissociation surfaces referred to here; in phosphate buffer from *p*H 5·8 to at least 7·4 in the presence of DPN and L-glutamate only the α,β-dissociation occurs. Requirement (2) was satisfied by a combination of devices. (*a*) That *p*H was selected which provided reasonably low rates of reaction but still remained within the range satisfying condition (1). (*b*) Both substrate and coenzyme concentrations were adjusted to the lowest values at which linear reaction-rates could be obtained. (*c*) A competitive inhibitor was used. Glutaric acid has been shown to be a strictly competitive inhibitor for L-glutamate in the GDH catalysed reaction[10]; in the presence of L-glutamate, glutaric acid has almost no effect on the state of aggregation of the enzyme.

Having found conditions which satisfy requirements (1) and (2) simultaneously, we were able to make valid measurements of the catalytic activity of the α and β units of GDH.

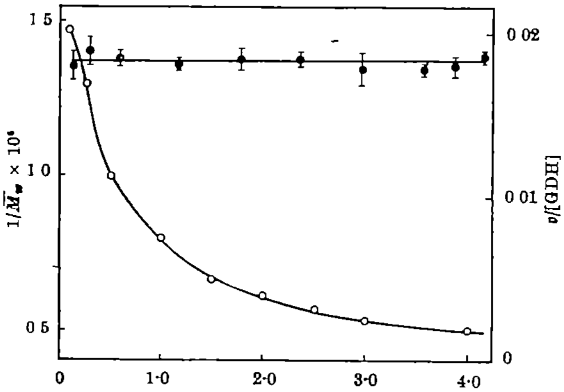

Fig. 1

The effect of dilution on the dissociation of GDH and on its catalytic activity is shown in Fig. 1, which shows the dependence of $1/\overline{M}_w$ (○) and v/mg GDH per ml. (●) on protein concentration. Average deviations from the mean values of v for each point are indicated by the length of the vertical dashes through the closed circles. Both measurements were carried out on solutions containing 0·167 mM DPN, 0·333 mM L-glutamic acid, 66·7 mM glutaric acid, and GDH at the concentration indicated, in 0·2 M potassium phosphate buffer, adjusted to *p*H 7·18 ± 0·07 with KOH. The solution, containing all components except substrate, was incubated at 25° C for 5 min. The substrate was then added and either $\overline{M}_w$ or v measured immediately thereafter. $\overline{M}_w$ was determined as described in reference 3. The specific refractive index increment was found to be 0·1951. v was measured as described in ref. 11, using a Beckman *DU* monochromator equipped with a Gilford model 200 optical density converter. The slit-width was 0·06 mm; the chart speed was 12 in./min. Protein concentration was measured spectrophotometrically using light scatter corrections as described in ref. 3.

As the protein concentration is lowered from 4 mg per ml. to 0·1 mg per ml., the weight average molecular weight ($\overline{M}_w$) decreases three-fold (as observed previously by Kubo *et al.*[5]). The specific catalytic activity (v/mg GDH/ml.), however, undergoes no change whatsoever. It must be emphasized that, for any given protein concentration, $\overline{M}_w$ and v were measured in identical solutions.

It is clear, then, that with L-glutamate as a substrate both α and β units of GDH have full and identical catalytic activities.

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PHYSIOLOGY

Effect of Morphine on Rats bearing Walker Carcinosarcoma 256

It has been shown that morphine will inhibit release of ACTH following exposure to stressors[1,2]. On the other hand, animals bearing tumours exhibit evidence of increased adrenal cortical secretion[3,4]. In view of the wide use of morphine and its derivatives in control of pain in persons with cancer, it was of interest to determine the effect of morphine administration on tumour-bearing animals. Because of the subsequent finding that carcass nitrogen in tumour-bearing rats was further decreased when morphine was administered, the effect of growth hormone, which has been found to reverse the anti-anabolic action of cortisone[5], was investigated.

Male albino rats, weighing 195–220 g, were inoculated subcutaneously with Walker carcinosarcoma 256. One group, along with its control, received daily doses of saline (0·15 c.c./100 g body-weight) subcutaneously; while the other group and its control received 2·5 mg morphine in the same volume of saline daily. The experiment was terminated after 14 days. The adrenals, tumours, and right femurs were removed. Femur-length was measured with a vernier caliper after defatting and cleaning. The carcass consisted of the residue. Total nitrogen was

Table 1. *A*, Effect of Morphine on Adrenal Weight and Carcass Nitrogen of Tumour-bearing Rats

	Initial wt. (g)	Final wt. (g)	Femur length (cm)	Adrenal wt. (g)	Tumour wt. (g)	Tumour nitrogen (g)	Carcass nitrogen (g)
N	204·8	—	2·98	—	—	—	5·83 ± 0 08
N_{sal}	204·6	222 7	3·17	49·1	—	—	6·33 ± 0·07
N_{mor}	204·4	201·5	3·15	48·4	—	—	5·77 ± 0·05*
T_{sal}	203·9	241·9	3·16	114·5 ± 12	30·5	0·48 ± 0·06	5·94 ± 0·03*
T_{mor}	204·1	225·9	3·15	98·3 ± 8	31·5	0·50 ± 0·03	5·24 ± 0·07†

B, Effect of Growth Hormone on Carcass Nitrogen of Tumour-bearing Rats

	Weight (g)	Femur length (cm)	Tumour wt. (g)	Tumour nitrogen (g)	Carcass nitrogen (g)
N_{sal}	293	3·23	—	—	8·31 ± 0·06
T_{sal}	316	3·18	18·2 ± 1·4	0·28 ± 0 03	7 80 ± 0 18*
T_{GH}	321	3·20	25·0 ± 2·1	0·40 ± 0·04‡	7·72 ± 0·15
T_{mor}	269	3·18	24·8 ± 1·3	0·39 ± 0·03‡	6·49 ± 0 15†
$T_{\mathrm{mor\,+\,GH}}$	281	3·18	25·5 ± 2 3	0·39 ± 0 04‡	6 94 ± 0·12§

N, Normal animals; N_{sal}, normal animals receiving 0 15 c c. saline/100 g daily; N_{mor}, normal animals receiving 2·5 mg morphine in the same saline dose; T_{sal}, tumour-bearing animals receiving saline, T_{mor}, tumour-bearing animals receiving morphine; T_{GH}, tumour-bearing animals receiving growth hormone.

* Significantly less than N_{sal} value. † Significantly less than T_{sal} value. ‡ Significantly more than T_{sal} value. § Significantly more than T_{mor} value.

determined by dissolving, overnight at 60° C, in concentrated sulphuric acid (500 c.c. per 150 g tissue) and taking an aliquot for Kjeldahl analysis.

In a second experiment, male rats, weighing 182–186 g, were similarly treated with the exception that bovine growth hormone was injected subcutaneously into tumour-bearing animals with and without the simultaneous administration of morphine. The following dose schedule was used: 100 µg on the first and second day after inoculation; an increment of 100 µg was added every second day until a maximum dose of 600 µg was reached. This was continued until completion of injections on the fourteenth day.

Normal rats receiving saline injections gained 8·5 per cent in weight and carcass nitrogen and grew, as judged by increase in femur-length, during the fourteen-day period (Table 1*A*). The morphine-treated animals, during this period, did not gain in weight or carcass nitrogen content; however, growth was not impaired. The weights of the adrenals were similar in the two groups. The tumour-bearing animals grew to the same extent as did their controls. Weight and nitrogen content of the tumours were alike in those which were injected with saline or morphine. Adrenal weight was increased 2·3 times in the saline-treated tumour-bearing animals and 2·0 times in the morphine-treated group. Nitrogen content of the tumour-free carcass of the saline-treated group was 2 per cent above what it was at the time of tumour inoculation while it was 10 per cent below this in the morphine-treated group.

In the second experiment (Table 1*B*), morphine reduced carcass nitrogen in tumour-bearing animals by 20 per cent. Unlike the first experiment, a statistically significant increase in tumour nitrogen resulted. Growth hormone did not influence carcass nitrogen in the tumour-bearing rats although tumour growth was greater than in the untreated rats. Under the influence of growth hormone, carcass nitrogen of morphine-treated animals was increased by 0·45 g, however.

Morphine-treated animals were found to be extremely sensitive to insulin. Such animals, bearing tumours, appeared to be particularly susceptible, and mortality was 100 per cent after the fifth day of administration of insulin although the dose of insulin which was used did not kill any of the animals bearing tumours which did not receive morphine.

The mechanism by which morphine inhibited gain in weight and deposition of nitrogen is not clear although it may be related to the well-known effects of this substance on appetite. However, the possibility of a suppressive effect on the elaboration of a growth-enhancing substance may be considered. The suppressive action of morphine on ACTH elaboration was demonstrated in experiment 1. Assuming that adrenal weight is related to function, morphine at the dose used induced a modest (but statistically insignificant) decrease in corticosteroid production in tumour-bearing animals. This would not be expected to result in failure to gain carcass nitrogen as compared with tumour-bearing animals not receiving morphine. The favourable action of growth hormone suggests that the elaboration of this substance was suppressed in the morphine-treated animals.

The findings leave unsettled the role which increased adrenal function may have in cachexia; however, they do suggest that morphine may contribute to it. Perhaps attention should be given to the clinical aspects of this problem and to the possibility of administering anabolic agents when morphine is used clinically.

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Rapid Inhibition by Cortisol of Incorporation of Glucose *in vivo* into the Thymus of the Rat

THE hypothesis that the effects of glucocorticoids on certain extrahepatic tissues result from inhibition of glucose utilization was proposed in 1942 by Drury[1] and Ingle[2] in connexion with the protein-catabolic effects of these hormones. It was later brought up by Bullough[3] to explain mitotic inhibition in skin, and related phenomena such as involution of lymphatic tissue. Support for the hypothesis has recently come from several observations[4–7], including demonstrations that glucocorticoids *in vitro* at concentrations approaching physiological levels inhibit glucose uptake by skin[4] and adipose tissue[5], and *in vivo* reduce glucose uptake by adipose tissue isolated 30 min after injection[5]. A number of arguments have been advanced for the view that this action represents the primary metabolic effect of the hormones on adipose tissue[5].

Since the thymus is one of the organs in the rat which is most strikingly affected by glucocorticoids, we have endeavoured to see whether here, too, a rapid effect on glucose utilization might occur. To this end we have measured the effect of cortisol on incorporation *in vivo* of radioactivity from labelled glucose into various fractions of the thymus. The interval investigated was that 2–3·5 h after the injection of cortisol.

Male Sprague–Dawley rats, weighing 300–360 g in Exp. 1 and 200–270 g in Exp. 2, were used following an overnight fast on the fourth day after adrenalectomy. Except for the differences noted, the two experiments described were conducted in essentially the same fashion. A trace amount of uniformly labelled ^{14}C-D-glucose (California Corporation for Biochemical Research; specific activity = 30 mc./m.mole) in 0·5 ml. 1 per cent sodium chloride solution was injected into rats which 2·0 h previously had been injected subcutaneously with either 2·5 mg cortisol (California Corporation for Biochemical Research) in 0·5 ml. suspending medium (Upjohn, No. 98 'Sterile Vehicle'), or the suspending medium alone in the case of the controls. The rats in Exp. 1 received 10^7 counts/min subcutaneously, without anæsthesia; those in Exp. 2 received 7 × 10^6 counts/min by femoral vein injection under ether anæsthesia. 1·5 h after the injection of radioactive glucose (for one rat in each group of Exp. 2 this interval was inadvertently extended by 10 min) the rats were killed by decapitation and the thymus gland was removed, rinsed, weighed and immediately frozen. The assay for 'whole tissue' was obtained by homogenizing a piece of thymus in water, boiling the homogenate almost to dryness and plating the sample from a 15 per cent acetone suspension. This fraction represents all non-volatile tissue components. Protein and lipid fractions were prepared and assayed for radioactivity as described previously[7]. The protein fractions also contain nucleic acids. Radioactivity was measured in a Nuclear-Chicago end-window counter. It was ascertained in preliminary experiments using albumin labelled with iodine-131 that only negligible errors would result from not taking into account the amount of blood and associated radioactivity remaining in the tissue.

Table 1 INCORPORATION OF CARBON-14 FROM ^{14}C-GLUCOSE (UNIFORMLY LABELLED) INTO VARIOUS FRACTIONS OF THE THYMUS GLAND

	Whole tissue		Protein		Lipid	
	Counts/min	Ratio	Counts/min	Ratio	Counts/min	Ratio
Exp. 1						
Cortisol (8)	1,557 ± 58	0·74	513 ± 15	0·66		
Controls (8)	2,100 ± 65		772 ± 32			
Exp. 2						
Cortisol (6)	940 ± 62	0·77	419 ± 15	0·69	330 ± 36	0·71
Controls (4)	1,224 ± 101		604 ± 39		463 ± 44	

Results are given as counts per min at infinite thickness, mean ± *S.E.* Figures in parentheses indicate the number of rats per group. The ratio given is for cortisol-treated to control rats.

Results are given in Table 1. In both experiments, and in all the fractions assayed, incorporation was decreased by about 30 per cent in glands from the cortisol-treated rats. These differences are all statistically significant at $P < 0·05$. We found no significant differences in the mean wet weights of the thymus glands, nor in the protein nitrogen contents (mg/g wet weight) of the glands. The mean level of radioactivity in blood taken at the end and plated directly without fractionation was found to be somewhat higher in samples from cortisol-injected rats; blood glucose concentrations were also higher in these rats, as would be expected from earlier work[7].

While interpretation of these results must at this stage be tentative, the simplest explanation for the decreased incorporation of radioactivity into all fractions of the thymus is that cortisol leads to a decrease in the rate of some early step in glucose metabolism. This explanation is consistent with observations that the rate of oxidation of glucose by thymus cell suspensions from rats or mice is reduced by prior injection of the donor animal with cortisol or cortisone[8,9]. Moreover, since the effects we report are, so far as we know, the most immediate metabolic effects of cortisol on the thymus that have been described, the explanation is also in accord with the hypothesis that inhibition of glucose utilization is the primary metabolic alteration produced by glucocorticoids.

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Retinotectal Connexions after Retinal Regeneration

IT has been shown by Sperry[1] that in adult amphibians regeneration of the optic nerve fibres after rotation of the eyes through 180° about the optic axis leads to reversed visuomotor responses. It has therefore been suggested[2] that there exists a cellular specificity which determines that for any given area of the retina there will be a fixed area of the optic tectum with which it will make connexion. In the experiments reported here this hypothesis was tested by examining the pattern of the retinotectal connexions after only the peripheral portion of the eye had been rotated.

The eyeball in adult newts was transected in a plane parallel to the corneoscleral junction approximately around the equator of the bulb. The part of the eye posterior to the plane of section contained an area of retina corresponding approximately to a solid angle of

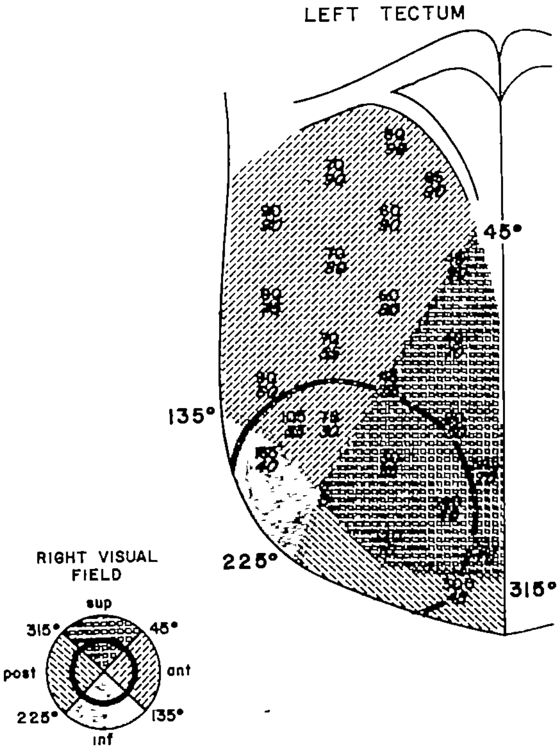
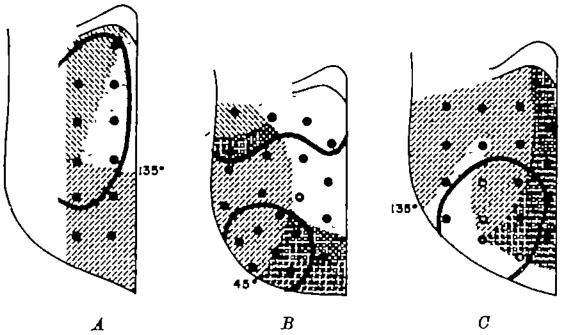


Fig. 1. Diagram of left optic tectum in normal newt, showing the representation of the quadrants of the visual field, designated as in the inset diagram. At each tectal recording point the angular co-ordinates of the centre of its receptive field are given. The upper number of each pair indicates in degrees the angular distance from the vertical axis of the visual field, the lower number (in italics) the angular distance from the centre. The heavy line marks the 50° parallel. The parallel rows of recording points are 200μ apart

60° around the visual axis. Most of the retina was removed from both parts of the eye by irrigation, and the remainder was removed with fine forceps. The front part of the eye was then replaced either in its original orientation or was rotated through 90°–180° with respect to the axis of the back part. Regeneration of the retina, presumably from the pigment layer[3], occurred, with return of vision in 2–3 months as indicated by optokinetic responses to vertical stripes moving in a horizontal plane. The projection of the retina on the optic tectum was determined by plotting the tectal evoked responses to a small flashing light placed at various positions in the visual field.

In the normal unoperated animal evoked potentials of 200–800 μV were obtained and a regular projection of the retina on to the tectum (Fig. 1) was seen, similar to that which has been found in the frog[4,5]. In 4 operated animals in which the axis of the front half of the eye had not been rotated, the projection was substantially normal. In 12 animals in which the front half of the eye had been rotated, several types of abnormalities were found. In 3 of 9 experiments in which a good representation of the central part of the retina was found, this area was located more anteriorly on the tectum than in the normal (Fig. 2*A*), and, in two instances, two areas representing the central retina were found (Fig. 2*B*). The axis of the representation of the central retina was in some instances rotated through approximately 180°, corresponding to the rotation of the front of the eye, despite the fact that the back part of the eye containing the cells from which central retina is believed to regenerate had not been rotated (Fig. 2*A*). The axis of the representation of the peripheral retina conformed with that of the central retina in most cases, so that the whole pattern of the projection was rotated through approximately 180° in 3 animals (Fig. 2*A*), and unrotated in 3 others (Fig. 2*C*). In some of the other animals there were areas the orientation of which did not correspond with that of either the front or back part of the eye.



A *B* *C*

Fig 2. Diagrams of different patterns of retinal projection, observed after retinal regeneration following rotation of the front part of the eye through 180°. The quadrants of the visual field are indicated as in Fig. 1. The heavy line in each case marks the 50° parallel, which has been taken as a conservative estimate of the outer limit of the 'central' retina, that is, that part of the retina overlying the portion of the pigment layer that was left intact at the back of the eye. The black dots indicate the recording points on the tectum. *A*, Representation of central retina abnormally located, orientation of whole representation rotated (only part of tectum explored). *B*, dual representation of central retina, orientation of part of representation rotated, of part unrotated; *C*, representation of central retina normally located, orientation of whole representation unrotated

The results are not consistent with the idea that the re-establishment of the visual pathway is governed by a fixed correspondence between each retinal locus and its projection on the tectum. On the other hand, it does suggest that some property of either the central or peripheral part of the retina may be dominant in determining the axis of the whole retinal projection.

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HÆMATOLOGY

Recovery and Transfusion of Human Erythrocytes after freezing in Polyglycol Solutions

It has been demonstrated that it is possible to preserve erythrocytes in viable condition for long periods of time by storing them at a temperature which is low enough practically to arrest the metabolic activity of the cells[1]. The temperature required for prolonged preservation is below −70° C, and glycerol has generally been used to prevent the lysis due to freezing and thawing[2]. Such mixtures of erythrocytes and glycerol are strongly hypertonic and cannot be used directly for transfusion because the sudden decrease in osmotic pressure would cause lysis of the cells.

A controlled dilution procedure[3] or a special centrifuge[4] have been successfully used for preparing an erythrocyte–glycerol mixture for transfusion. The finding[5] that polymers which do not penetrate the cell can protect the erythrocytes from lysis on freezing suggested the possibility that the processing after thawing might be eliminated or greatly simplified since the osmolar concentrations required are much lower than when penetrating solutes are used[6]. We previously reported that both rabbit and human erythrocytes could be recovered in viable condition after freezing in solutions of polyethylene glycols[7]. More recently the successful transfusion in the rabbit of erythrocytes previously frozen in polyvinylpyrrolidone solutions has been reported[8]. In the present communication we wish to report that human erythrocytes have been recovered after storage at − 79° C in polyethylene glycol solutions and that the post-transfusion survival of these cells in man is good.

Human blood collected by ordinary blood-bank procedure using acid citrate–dextrose solution (ACD) anticoagulant was mixed with an equal volume of a polyglycol solution, 100 ml. of which contained 40 g of polyethylene glycol *E500M* (Dow Chemical Co., average molecular weight of 635), 2·5 g sodium citrate dihydrate and 0·25 g citric acid monohydrate. This solution was sterilized by autoclaving. The blood and the polyglycol solution were at 4° C when mixed and after 5 min at this temperature they were frozen. For the transfusion experiments, 100 ml. of the mixture in a 200 ml. capacity blood-bottle was shell frozen by rotation on motor-driven rollers in a − 79° C bath (solid carbon dioxide–alcohol). After storage at − 79° C, the bottle was immersed in a 40° C bath and shaken until thawing was just completed. To the thawed mixture was added 100 ml. of cold 10 per cent sucrose solution and the cells were separated by centrifuging and removal of supernatant fluid. The cells were washed once with 100 ml. of 10 per cent sucrose solution and then once with 0·9 per cent sodium chloride solution. The per cent of the cells originally present which was recovered was calculated from measurements of the total quantity of hæmoglobin in the cell suspension and in the combined washings. These measurements were made photometrically by the cyanmethæmoglobin method. We found that the polyglycols and some other polymers interfere with the oxyhæmoglobin method and its use may cause serious errors in recovery values.

To the suspension of washed cells was added a solution of radioactive sodium chromate containing 80 μc. of chromium-51. After standing for 45 min the cells were washed with cold saline solution and the suspension of the labelled cells was injected without further delay into the recipient. The recipients were hospital patients who were free of hæmolytic disease and who had neither experienced significant loss of blood nor received a blood transfusion within a week prior to the experiment. All the operations for preparing the cells for transfusion which are described here were carried out in the original blood bottle in which the cells were frozen, using ordinary aseptic precautions.

The erythrocyte suspension labelled with chromium-51 was injected with a syringe into the rubber tubing of a previously initiated intravenous infusion of saline which was allowed to run several minutes after the injection. For the determination of the recipient's blood volume, a suspension of his own erythrocytes previously labelled with phosphorus-32 was also injected. Blood samples were drawn from the recipient, the cells were lysed by the addition of powdered saponin and the amounts of the two radioactive isotopes present were measured and calculated by a method similar to that described by Donahue *et al.*[9]. The chromium-51 was measured with a well-type crystal scintillation counter and the phosphorus-32 with an *M*-6 liquid-sample Geiger tube (Twentieth Century Electronics), appropriate correction being made for the small interference of each isotope in the measurement of the other one.

Table 1. RECOVERY AND POST-TRANSFUSION SURVIVAL OF HUMAN ERYTHROCYTES AFTER STORAGE AT —79° C IN POLYETHYLENE GLYCOL SOLUTIONS

Days of storage	Recovery (per cent)	Survival 24 h after transfusion (per cent)
2	83	78
5	74	85
25	66	75
61	59	82
100	50	71
147	41	67

The results of these experiments are shown in Table 1. It is evident that the recovery of cells decreases as the time of storage increases. The loss of cells occurs mainly during the washing process after the mixture has been thawed. If the thawed mixture is washed directly with saline solution instead of with sucrose solution, the loss of cells is considerably larger. This suggests that the cells have become more than normally permeable to cations and that the lysis may be due to osmotic forces. The post-transfusion survival of the recovered cells is relatively good. In general, preserved blood which gives a post-transfusion survival for its erythrocytes of 70 per cent or more is considered suitable for transfusion. In the present series, only the erythrocytes stored for the longest time (147 days) gave a post-transfusion survival less than 70 per cent. It seems likely in the case of these cells (which seem to have a diminished osmotic resistance) that some intravascular hæmolysis of the transfused cells occurred and that the preservation of metabolic function may be better than the post-transfusion survival value indicates. We believe that the results presented here demonstrate the feasibility of preserving human erythrocytes in viable condition at low temperatures in polyglycol solutions. The practical usefulness of this method of preservation depends on the development of techniques which yield a greater recovery of cells after prolonged storage.

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Change in A^3 Hæmoglobin due to β-Chain

SEVERAL authors[1-3] have shown that the minor anodic fraction of hæmoglobin obtained by electrophoresis at pH 8·6 is part of the hæmoglobin modified during the ageing of the red cells. Proof is submitted here that the modification of the electrical charge of hæmoglobin A^3 (Hb^{A_3}) is due only to the modification of the β-chain. This is proved by comparison of the electrophoretic migration of the hybrids obtained between canine hæmoglobin Hb^{CAN} and Hb^{A} and between Hb^{CAN} and Hb^{A_3}.

Hybridization between Hb^{A} and Hb^{CAN} has already been described[4]. If it is carried out with equimolar hæmoglobins[5] the results are as follows:

25 per cent of slow hybrid $\alpha^{A}\ \beta^{CAN}$
25 per cent of fast hybrid $\beta^{A}\ \alpha^{CAN}$

and reconstitution of 25 per cent of human hæmoglobin $\alpha^{A}\ \beta^{A}$ and 25 per cent of canine hæmoglobin $\alpha^{CAN}\ \beta^{CAN}$.

In our work Hb^{A_3} was obtained by chromatography on 'Amberlite'[6] with No. 5 developer, followed by electrophoresis on starch blocks[7] to eliminate non-hæm proteins.

Hybridization was performed in equimolar ratio as described previously[5].

Examination of the hybrids was accomplished by starch-gel electrophoresis at pH 8·6 in borate buffer[8].

In these conditions (Fig. 1), Hb^{A_3} gave two hybrids with Hb^{CAN}: a slow hybrid $\alpha^{A_3}\ \beta^{CAN}$ reacts in the same way as the $\alpha^{A}\ \beta^{CAN}$ hybrid. On the contrary a fast hybrid $\beta^{A_3}\ \alpha^{CAN}$ is faster than $\beta^{A}\ \alpha^{CAN}$.

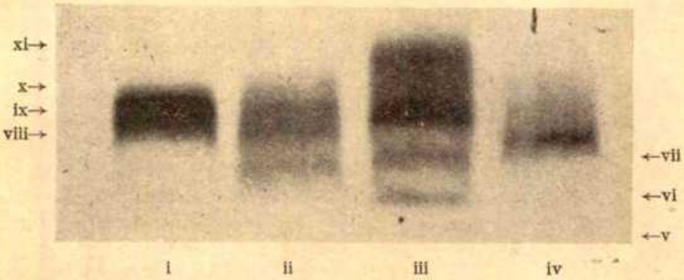

Fig. 1. Hybridization of Hb^{A_3} with Hb^{CAN}. Analysis in starch-gel borate/borate system pH 8·7. i, Mixture of Hb^{A} and Hb^{CAN} marker; ii, hybridization $\mathrm{Hb}^{A} \times \mathrm{Hb}^{CAN}$; iii, hybridization $\mathrm{Hb}^{A_3} \times \mathrm{Hb}^{CAN}$; iv, mixture of Hb^{A_3} and Hb^{CAN} marker; v, commencing line; vi, non-hæm protein; vii, hybrid $\alpha^{A}\ \beta^{CAN}$; viii, Hb^{CAN}; ix, Hb^{A}; x, hybrid $\beta^{A}\ \alpha^{CAN}$; xi, hybrid $\beta^{A_3}\ \alpha^{CAN}$

It seems that the modification of Hb^{A_3} is essentially localized on the β-chain.

Moreover, it has been observed[9] that incubation of Hb^{A} with glutathione modifies its electrophoretic mobility with augmentation of the ratio of Hb^{A_3}.

We have effected hybridization between Hb^{CAN} and Hb^{A_3} artificially induced by glutathione. Results were identical to those already mentioned.

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PATHOLOGY

Optic Neuritis and Encephalomyelitis in Monkey produced with Human Optic Nerve

OPTIC neuritis is an integral part of the encephalomyelitis which rarely follows anti-rabies vaccination with brain preparations[1,6]. This condition in man is an accidental allergic encephalomyelitis and appears to be identical to the experimental allergic encephalomyelitis (EAE) produced in animals by injection of brain. Optic neuritis also occurs in other forms of encephalomyelitis and in multiple sclerosis[2]. The lesions in these demyelinating diseases may be due to an immunological process[3]. If so, an encephalitogenic antigen common to the developmentally related optic nerve, brain and spinal cord may account for the simultaneous appearance of the lesions in these structures. Suggestive evidence of such an antigen has been provided by the demonstration of optic nerve lesions in animals in which EAE was produced by injection of brain[3].

In the investigation reported here we attempted to determine whether the adult human optic nerve contains an antigen common to the brain, cerebellum and spinal cord. Since the brain antigen which causes experimental allergic encephalomyelitis is tissue and not species specific[4], we felt justified in testing in monkeys the encephalitogenic property of human optic nerve.

Optic nerves were obtained from refrigerated cadavers of patients who had no infectious or neurological illness and had been dead not longer than 24 h. Pieces about 1 cm long of the retrobulbar portion of the optic nerves were used. The tissue was ground in distilled water containing 0·25 per cent phenol to make a 33 per cent suspension. Equal amounts of this material and Freund's adjuvant were emulsified with a syringe. The adjuvant was a mixture of equal parts of heavy mineral oil and aquaphor and contained 15 mg heat-killed mycobacteria (*BCG*) per ml. Although the adjuvant is not essential for the production of EAE, it enhances the incidence and severity of the illness[5]. Previous experience has shown that the injection of adjuvant alone or in combination with non-neural tissue does not cause neurological lesions[4,7].

Each of five adult squirrel monkeys received five 0·1-c.c. intracutaneous injections into the abdominal wall. The animals were examined at weekly intervals for two weeks and daily afterwards. The pupils of the monkeys were dilated with atropine.

The 24th day after injection one of the monkeys had early papillitis in the left eye characterized by a moderately swollen, congested and red disk. On the 26th day this animal developed flaccid paralysis of the hind legs, cerebellar ataxia of the upper extremities, nystagmus and body rolling. At the same time, three other monkeys manifested severe signs of brain stem and spinal cord lesions. These four animals were killed *in extremis* 4–6 days after the onset of their illness, that is, about 30 days after the injection. The remaining animal showed no neurological signs and was killed at the same time as the others. Random histological sections of the brain, spinal cord and cerebellum and semi-serial sections of the optic nerves were stained with Nissl and hæmatoxylin and eosin. The spinal cord and the brain of the four sick animals revealed the usual features of acute allergic encephalomyelitis: perivascular infiltration, meningitis and periventricular mononuclear infiltration[3,8]. Both optic nerves of the animal which had papillitis in the left eye showed patches of mononuclear inflammation (Fig. 1). In one other sick animal there were less marked signs of optic nerve inflammation. The nervous system of the animal with no neurological signs contained no lesions. Two adult guinea pigs were also injected with the same dosage of the remaining emulsion of optic nerve and they developed EAE as evidenced by the clinical and histological signs.

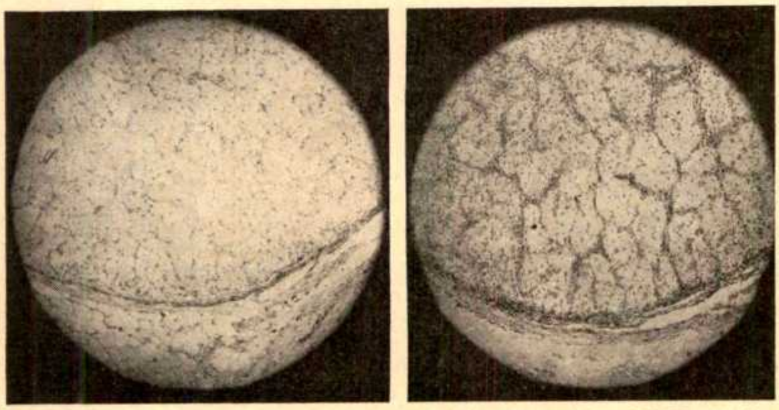

Fig. 1. Optic nerves of monkey. Left, normal; right, optic neuritis and EAE. (Nissl stain; × *c*. 50)

These results demonstrate that the adult human optic nerve shares an encephalitogenic antigen common to the brain and spinal cord. It is also evident that the encephalitogenic antigen(s) is tissue specific since human material produced optic neuritis and EAE in monkeys and EAE in guinea pigs.

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RADIOBIOLOGY

Lethal Effects in Tadpoles of *Xenopus laevis* (Daudin) after Irradiation with High-Energy Electrons

SINCE 1957 in our department, an increasing number of patients—up to now about 1,200—have been treated with high-energy electrons, using a 31-MeV *BBC* betatron. Some observations which we made during this period, are the same as reported also elsewhere[1–4]; but at other points we made divergent observations.

We therefore began investigation concerning: influence of (1) dose-rate; (2) different radiations; (3) site of the irradiated animals in a phantome, using 30-MeV electrons (equal doses being given), on the lethality of tadpoles of *Xenopus laevis* (Daudin). These animals are excellent material for laboratory experiment series—many animals from the same litter can be obtained with the same radiosensitivity and can be kept, for irradiation, in a relative small volume and the irradiation takes place in water; moreover, there are no great problems in maintenance.

For our purpose, the tadpoles were irradiated in a small water-phantome of 15 × 10 × 1 cm, which was surrounded with 'Perspex' plates (dimensions 20 × 20 × 0·5–2 cm). The results reported here are based on investigations, which were repeated many times during 1960 and 1961. In every experiment (point of the curves, showing the lethality) 50–100 animals were irradiated simultaneously.

In the first series, the animals received 500 r. (dose-measurements were made with a Victoreen dosimeter and a Siemens dosimeter with a thimble-chamber in the volume, where the tadpoles were irradiated), given with dose-rates of 50 and 300 r./min. In the series 2 and 3 : 500–1,000–1,500–2,000–2,500 r. were given in one single session.

(1) A considerably higher lethality can be observed after irradiation with a dose-rate of 300 r./min than with 50 r./min.

(2) The reason for these experiments was to show differences in lethality in tadpoles after irradiation with 250-kV, 31-MeV photons, cobalt-60 rays and electron-rays of 30 MeV. In this series, the irradiations were carried out under 'treatment conditions' with dose-rates as used in treatment of tumour-patients. As already mentioned, we used only animals of the same litter for this experiment with a

Table 1. LETHALITY AFTER A SINGLE DOSE OF 500 r.

		Dose-rate	
		50	300 r./min
		Lethality	
(a) 10	days after irradiation	2 %	13 %
(b) 20		14	29
(c) 60		18	42·5

Table 2. ONE SINGLE DOSE, DIFFERENT TYPES OF RADIATION

	Dose for 50 per cent lethality (r.)	Dose-rate (r./min)	Site of the animals, with reference to the depth–dose curve
250 kV	1,800	100	Dose maximum
31 MeV photons	1,860	20	,,
Cobalt-60	1,900	40	,,
Electrons			
30 MeV (a)	2,140	150	,,
(b)	1,570	150	30 per cent

well-defined length as sign for their stage of development.

Comparison of a 50 per cent lethality is given in Table 2.

Calculating RBE factors for tadpoles (RBE factor = dose for a 50 per cent lethality with 250 kV/dose for the same lethality of another type of irradiation) we get: 250 kV, 1; 31-MeV photons, 0·97; cobalt-60, 0·95; 30-MeV electrons at dose maximum, 0·84; 30-MeV electrons at 30 per cent level, 1·15.

In this series, the results of (2) were controlled one year later, using the same arrangements and comparing 30-MeV electrons with the tadpoles once at the maximum dose and secondly at the 15 per cent level of the transition-curve. The dose-rate was constant 50 r./min. In general, the same results were observed (Table 3).

Table 3. DIFFERENCES IN LETHALITY AFTER 30-MEV ELECTRON IRRADIATION, ONE SINGLE DOSE. ANIMALS AT THE DOSE-MAXIMUM AND AT THE 15 PER CENT LEVEL

	Lethality					
	25 per cent			50 per cent		
	15	20	60	15	20	60 days after irradiation
Difference in dose between 15 per cent and dose maximum	340 r.	300 r.	200 r.	450 r.*	260 r.	170 r.
†Ditto, 1960 experiments: Observation-time: 30 days after irradiation		—			550 r.	

* Extrapolated, true value ~ 400–450 r.

Comparing the results of the investigations of 1960 and 1961, the difference in dose for the LD_{50} and the difference in dose, in the relation 15 per cent level: the maximum dose might be caused by the different dose-rate, once the animals were placed at the 30 per cent level; in 1961 it was decided to irradiate the animals at the 15 per cent level, and on the other hand, there might be differences in the general radiosensitivity of the tadpoles.

Additional observations. Measurements of the half-value layer in lead for 30-MeV electrons were as follows:

	Dose maximum	15 per cent level
Half-value layer	2 mm	1 mm
Build up	about 15 per cent in a zone of the first 3·5 mm lead	no build up

† Film-blackening curves of graphical films with and without intensifying screens showed a higher efficiency of electrons (also 30 MeV) at the 30–10 per cent level compared with those at the dose-maximum. The intensifying factor of these screens increased with the depths (in 'Perspex', the dose-rate was kept equal).

Comparing lethal effects in tadpoles of *Xenopus laevis* (Daudin) after irradiations we found:

(*a*) After a single dose of 500 r. the lethality of the tadpoles is considerably higher, using a dose-rate of 300 r., than with 50 r./min.

(*b*) The lethality of the animals increases with the depth in the phantome, also if dose and dose-rate are constant (about 40 per cent increased lethality).

This phenomenon was calculated by Wideroe in advance and might be caused by rapid changes in linear-energy-transfer with increasing depth in water (~tissue). These observations might have some influence concerning tumour therapy with high-energy electrons.

I thank Prof. Lehmann, Zoological Institute, University of Berne, who supplied the tadpoles; it was also in his institute where we were able to keep and follow up the development of the animals after irradiation. I also thank Prof. Zuppinger for his interest and great help and Prof. Minder, Prof. Mayneord and Dr. Wideroe for discussions.

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BIOLOGY

A New Method for grafting Sweet Potato

Moscov[1], Melchers[2], Melchers and Lang[3], Kuyper and Wiersum[4], Hamner and Bonner[5] and Sagaidac[6] have used grafting in plants to examine movement of substances across the graft union. Using this technique, the movement of nicotine across the graft union in *Nicotiana tabacum* has been reported[7]. The movement of the flower-inducing substances in *Xanthium*[8] and substances transmitting the photoperiodic response generally in plants has been reported[9]. Other uses of grafting in sweet potatoes are for investigating virus diseases[10], flower formation of hard-blooming varieties[11] and movement of ascorbic acid[12].

Until recently, cleft grafting (stem-to-stem) and core grafting (root-to-root) were the only two methods used in sweet potato grafting. However, these conventional methods of grafting have the disadvantage that an interfering area, that is, a piece of stem or a portion of root, must, of necessity, be left on the grafted plant. Thus results purporting to demonstrate the passage of substances from the aerial parts across the graft to the root may not be altogether unequivocal. The grafting technique recently evolved by me[13] obviates this cause of uncertainty due to the fact that the stem is directly grafted on the root.

The purpose of this work is to present a new technique of grafting, based on Gaafar's method of sweet potato grafting.

Three sweet potato varieties, namely, Mabrouka, Mangawy and Selecta, were used.

This method as shown in Fig. 1 consists of using a knife to make a suitable deep cut in the root and inserting a suitable number (4–8) of already prepared terminal vine cuttings (scions) into the cut. Up to twenty cuts can be made on the same root. Grafting wax was used to avoid drying of wounds. The roots were potted up and care was taken to provide sufficient water and avoid severe drying out.

Under the condition of this experiment more than 90 per cent success was obtained. The growth of the grafted vines was rapid and normal.

More than a hundred shoots can be fitted on the same root. As shown in Fig. 1 three varieties did take on the same root. This technique enables us to test for resistance for internal cork disease. The normal method is to grow different strains of sweet potato cuttings in infected soil and select plants showing no disease symptoms. By means of the present technique cuttings of different strains can be grafted on the same infected root and thus the passage of the

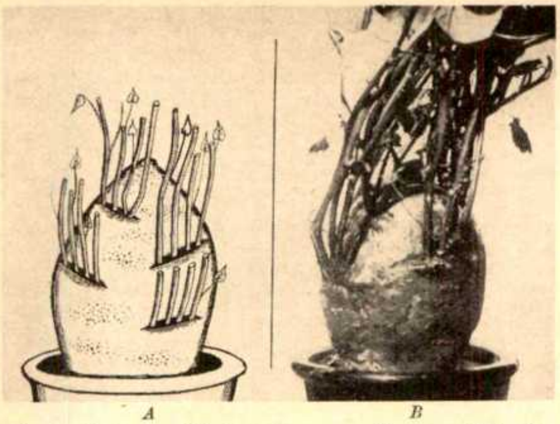

Fig. 1. Stock, var. Selecta; scions, vars. Mangawy, Mabrouka and Selecta. Four shoots to each cut. *A*, Diagrammatic representation; *B*, photograph

virus into shoots is more reliable than the soil test. In other words the present technique, besides its numerous uses, is an efficient and reliable technique for selection for resistance to virus diseases.

This work was carried out at the Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, during the tenure of an International Atomic Energy Agency fellowship.

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Vegetative Propagation in the Coconut

Gangolly and Pandalai[1], in their communication on vegetative propagation of the coconut palm (*Cocos nucifera* L.), mention only one method, namely, the induction of suckers, but state that "there was no sign of suckering seen in any of the treatments so far". They state that they induced root formation from the stem near ground-level with certain hormones and hence conclude that "inducing meristematic activity in the coconut is possible".

But the induction of roots, especially near the base of the coconut stem, has never been difficult. The lowest part of the stem, generally called the bole, which is conical and buried in the soil, is the main root-producing region. Root production begins at the lowest point, the apex of the cone. When the palm attains middle age the bole surface is usually covered with roots, and later on roots are frequently produced from the aerial stem. According to Menon *et al.*[2], about 40 per cent of the palms seen by them produced aerial roots, which could be at any height of the stem. Water-logging raised the percentage to 62·6 per cent and 'root disease' to 52·8 per cent. Root production is greatly favoured by heaping moist river sand and/or coir dust around the stem base. Using these materials and no hormones, I have induced thousands of roots from the aerial stems of 15 coconut palms and one *Areca catechu*[3] at the Central Coconut Research Station, Kayangulam, Kerala (India). But it is striking that, though millions of coconut palms have produced aerial roots, none has developed suckers in consequence.

As Gangolly and Pandalai seem to be unaware of the published work on clonal reproduction, I review it briefly. By air-layering[4], or marcottage, that is to say the induction of roots at a height on the stem, followed by sawing through the stem below them and replanting, one can prolong the life of a valuable tree, but one cannot, of course, multiply it. (Gangolly and Pandalai have misquoted me as describing this rejuvenation as suckering.) Exceptional palms produce aerial ramifications[5–7], and most of these branches may be air-layered[8], and a small clone thus produced. The inflorescences[9–11] and flowers[12–14] of coconut palms sometimes develop into 'bulbil-shoots', and it should be possible to layer these. The most hopeful method, though still a difficult one, is to reverse the flowers into vegetative shoots, and then get them back to the seeding habit, after they have been successfully layered and propagated as individuals. Such shoots have reversed to the fruiting phase in the coconut[15], and *Elaeis guineensis*[16]. Schwarzenbach[17] has achieved the same result in the grass *Poa alpina* by simple physiological treatments.

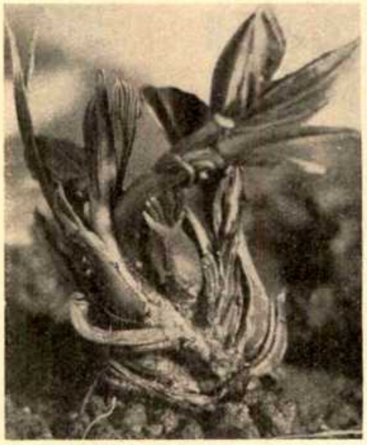

Fig. 1. Mechanical splitting of growing point yields two suckers in coconut

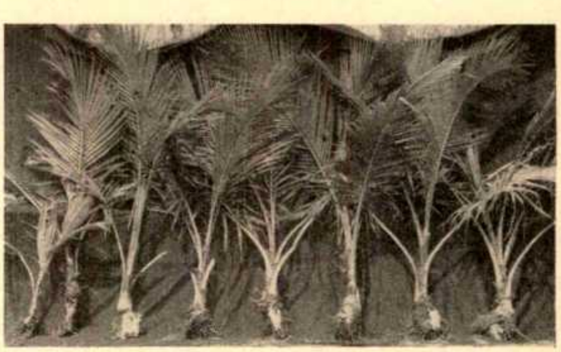

Fig. 2. Individual shoots (8 in all) of a young suckering coconut. Six of these have now become young coconut palms at Kayangulam, Kerala

Another and perhaps easier method is to split the growing point mechanically, by which I have induced suckers in the coconut[15] (Fig. 1) and *Areca catechu*. Stimulation of the axillary buds of young seedlings is also hopeful. I have reported several instances of suckering in young coconut palms[16]. The shoots of two clumps have been separated (eight shoots of one of the clumps can be seen in Fig. 2), and these clones, now growing at Kayangulam, may be valuable for future studies. Tissue culture methods[20] may also prove successful.

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It was not our intention, nor was it considered necessary or feasible, to review the whole aspect of clonal propagation in such a short communication as that referred to[1]. Our main object was to bring to the notice of interested workers the observation made by us on one aspect of root production in the course of work in progress on the crop with hormones and other growth-promoting substances. Nevertheless, the points relevant to our work have been fully covered in our communication, and the other references given in Mr. Davis's communication[2] were felt quite irrelevant in our preliminary account.

The point made by Mr. Davis that aerial rooting in coconuts occurs in Nature is not controverted, but as he himself admits, the phenomenon is observed rather more in the diseased condition of the palms or in the case of palms growing under unfavourable environmental conditions. Observations and experience from our work at this Research Station go to show that, under normal conditions of growth, aerial rooting occurs only very rarely. It was just this aspect that interested us in studying the effect of hormonal applications on root production also, in an experiment designed primarily to see whether coconuts can be made to produce suckers. It was seen that in the trees treated with certain hormonal combinations referred to by us, root production was general and profuse, quite in contrast to the results obtained in untreated trees.

It is wrong to say that we have misquoted Mr. Davis, although we admit that the second reference tagged to ref. 6 in our communication could also have gone along with ref. 4, which is co-authored by Davis. The subject-matter of both ref. 4 and the second one of ref. 6 is the same, the latter being purely a duplication of the former, and there was, indeed, no need at all to quote the latter or other publications of Mr. Davis on the same subject[3,4]. In ref. 5 and the first one under six, mention has been made by us about reports on natural suckering and it is surprising how it can be misinterpreted as confusing between suckering and rejuvenation, especially when we have covered the citation of the literature on rejuvenation under ref. 4.

In regard to the methods of 'clonal reproduction' referred to by Mr. Davis[2], such as layering 'bulbil shoots' and getting them back to the seeding habit, splitting growing points by mechanical means, tissue culture and perhaps others, we feel that at the present stage of knowledge these are nothing more than pure hypothetical possibilities as Mr. Davis himself conscientiously points out and as every reader can see, or chance successes, the practical utility of which has never been demonstrated. Suffice it to say that these methods do not seem to offer at present prospects of any utility in practical coconut growing.

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Endemic-Exotic Earthworm Competition in the American Midwest

It is commonly believed that in areas of European colonization introduced lumbricids tend to supplant the endemic earthworm fauna. Stephenson[1] gives several examples. More recently the spread of *Allolobophora caliginosa* in New Zealand has been documented[2,3], and G. E. Gates[4] has described the distribution of European species in India.

Replacement of the native earthworms of the genus *Diplocardia* in the central United States by introduced lumbricids was described by Smith[5], whose observations were supported by Goff[6], and has been commonly accepted, despite the very limited number of observations. More recent observations in the United States have clouded the issue: W. J. Harman[7] without comment has listed *D. singularis* as the most common earthworm in Lincoln Parish, Louisiana. Causey[8] in Arkansas apparently accepted Smith's views. *D. riparia* has been reported to remain very common in the blacklands prairie soil of Oklahoma and Texas[9,10]. However, the prairie chernozem soils of eastern South Dakota have been reported to have had their zonal horizonation almost completely destroyed by *Lumbricus terrestris*[11,12]. The original earthworm fauna, if any, is not known. Shackleford[13] did find diplocardias in an Illinois prairie. A diplocardian has been found to be common in the uplands of southern Michigan[14], and *D. riparia* has been found to form part of the earthworm population of an agricultural soil in Nebraska[15].

It has been recently suggested that changes in land-use, resulting in a changed habitat and food supply, are the causes of the replacement of endemic by exotic forms[14,16]. This, too, remains a hypothesis, albeit a reasonable one. Some exotic forms seem to occupy previously unoccupied niches.

During the spring of 1960 field reconnaissance was undertaken near St. Louis, Missouri, to determine the extent of replacement of diplocardias by lumbricids in forest soils. Four sites, several miles apart, were selected, each in a different drainage basin. Precise locations are given in a previous publication[17]. The soils on the first three sites were loessial, that on the fourth a deeply leached residual loam, stony (25–95 per cent), developed on limestone. The vegetation of the forested slopes was dominantly oak on site four, a mixture of oak with elm and hickory on sites one and two, and sugar maple with elm, hickory, and oak on site three. These forests are typical of large portions of the central United States. Each of the sites was selected to include a stream large enough to have a cultivated floodplain. Except in site three, some of the hilltops had been in cultivation. All the slope forests had been cut and, probably, grazed in the past. An effort was made to observe several acres on each site, and each site was visited several times. When specimens were preserved, all worms in the sample area were collected. About 800 worms were preserved.

European earthworms were found at each site. *Allolobophora trapezoides* was present in all the bottoms, and *Octolasium lacteum* was found in the forest soils on sites one to three. However, the proportion of lumbricids in the total population on these sites was but 1·7, 18, and 34 per cent, almost all *O. lacteum*. The other worms present were *D. ornata* and *D. conoyeri*. It was noted that *O. lacteum*, when active, was found in the humus layers of the soil, while the diplocardias were found throughout the *A* horizon. This may imply a lack of competition for food. On site four no European lumbricids were to be found on the slopes, and only a small number of the native lumbricid *Bimastos zeteki*, although *D. ornata* and *D. smithii* were abundant, even on gravelly areas of the slope where the humus cover was discontinuous. The pattern of continued diplocardian abundance, even dominance, in the slope forest soils was consistent. Other scattered observations in the St. Louis area, and also in the Catoctin Mt. area of Maryland, also fit this pattern.

The European *A. trapezoides* was generally abundant in the bottomlands : the pattern of dominance in the higher cultivated bottoms is not clear, but directly along the streams of sites one through three diplocardias (mostly *D. riparia riparia*) forms 70–80 per cent of the population. The raw alluvium directly beside a stream, usually composed of a layer of silt and clay over sand and gravel, is exclusively colonized by the larger *D. riparia riparia* (the 'red gravel worm' of colloquial speech). This niche is apparently not occupied north of the range of *D. riparia*, that is, the Sangamon River[8]. *D. riparia* has also been found to be abundant on the floodplain of the Mississippi even in an urban area.

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Action of the Rete Mirabile in the Secretion of Gases by Blood-acidification in the Swim-bladder of the Eel

SEVERAL theories have been advanced to explain how gases are secreted into the swim-bladder of fishes. Haldane[1] suggested that oxygen is dissociated from oxyhæmoglobin by acidification of the blood and then diffuses into the bladder. Ball *et al.*[2], Hogben[3], Fange[4] and others presented evidence in favour of this; Scholander *et al.*[5], however, investigating the effect of acidification of blood from deep sea fishes, concluded that the acidification theory in its present form cannot explain gas secretion in these species at great depth. Scholander *et al.*[6] later showed by experiments with oxygen-18 that all the oxygen which is secreted is derived from that dissolved in the water.

This communication summarizes results from an investigation on the gas secretory mechanism in the swim-bladder of the common eel (*Anguilla vulgaris*). The blood supply to the eel's bladder passes through two parallel counter-current capillary bundles (rete mirabile) and blood samples can be taken from the arteries and veins on both sides of this rete. Such samples were analysed for oxygen[7], carbon dioxide[8], pH (determined within 0 04 unit with a Metrohm pH meter and a micro-blood electrode) and lactic acid (determined by a micro-modification by P. F. Scholander of the Conway[9] method).

The results presented here were all obtained on eels which secreted gas into a closed bladder where the pO_2 never exceeded 1 atmosphere.

During secretion, the pO_2 and pCO_2 in blood taken from the veins draining the secretory epithelium, but before these enter the rete, were always equal to or greater than in the gas inside the bladder. The pH of the blood from the same veins was found to be about one unit lower than in the blood from the artery entering the rete from the dorsal aorta (6·5 as against 7·5). During secretion the blood in the arteries leading from the rete to the secretory epithelium had a lower pO_2 and pCO_2 and a higher pH than had the venous blood just mentioned.

The acid blood from secreting glands contained 115–140 mg per cent lactic acid and 25–35 vol. per cent carbon dioxide as against 10–50 mg per cent and 5–10 vol. per cent respectively in blood from the artery entering the rete from the dorsal aorta. *In vitro* experiments on the buffer capacity of blood of the eel showed that the lowered pH in the blood from the active glands was quantitatively accounted for by its content of the acids mentioned. It was also shown that the rete allowed a certain leakage of lactic acid. The blood showed a marked Root effect.

The results support the following hypothesis: when the swim-bladder is stimulated to secrete, lactic acid appears in the blood stream going through the secreting epithelium. Due to the properties of the rete, part of the acid is recirculated. The resultant local acidification causes the pCO_2 and pO_2 in the blood to increase so that oxygen and carbon dioxide enter the swim-bladder by diffusion.

The important point seems to be that the rete acts as a counter-current multiplier, the acids inside the rete always causing a slight increase in pO_2 and pCO_2.

Kuhn and Kuhn[10] have investigated the efficiency of the rete of the eel as a counter-current multiplier and find, as Scholander[11] did earlier, that it was very efficient. According to their calculations this rete is able to build up, inside the bladder, pressures of nitrogen and argon which exceed those actually measured in any fish if the solubility to these gases in the blood of the bladder were reduced by 1 per cent. They point out that such a reduction would take an addition of some 20 mM electrolyte. Similarly 20 mM lactic acid would suffice (via the Root effect) to explain the observed secretion of oxygen. The increase in lactic acid and carbon dioxide measured in my own investigation represents an increase of some 20–30 mM and should thus be sufficient to explain the observed secretion of all the three mentioned gases.

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ENTOMOLOGY

Anopheles gambiae Complex

THE existence of two fresh-water forms (*A* and *B*) of *Anopheles gambiae* Giles which, when crossed, produce sterile F_1 males has been established by Davidson and Jackson[1]. A salt-water tolerant strain of the same species from near Tanga, Tanganyika, has been shown to be partially incompatible with two strains of the group *A* fresh-water form[2,3], while the variety *melas* has been shown to be incompatible with a fresh-water form (group unknown) from a nearby area in Liberia[4].

Now, the Tanga salt-water form and the *A. melas* from Liberia have been crossed with each other and with each of the two fresh-water forms. Table 1 enumerates these crosses and gives the proportion of male to female offspring in the F_1 generation.

All the crosses produced sterile F_1 males. Sterility was established in three ways: (1) by microscopic examination of the testes; (2) by allowing interbreeding of F_1 males and females in cages and observing the eggs laid by the females; (3) by artificially mating F_1 males and females after the method of Baker *et al.*[5].

Table 1. CROSSES BETWEEN FRESH-WATER AND SALT-WATER FORMS OF *Anopheles gambiae*

Parents			F_1 generation	
Male	Female	Total	Percentage male	Percentage female
Lagos (*A*)	TSW	66	61	39
Kisumu (*A*)	TSW	250	56	44
Pare (*B*)	TSW	156	56	44
Bobo (*B*)	TSW	138	60	40
TSW	Lagos (*A*)	1,010	42	58
TSW	Pare (*B*)	998	36	64
Kisumu (*A*)	*melas*	113	76	24
Liberia (*A*)	*melas*	326	99	1
Pare (*B*)	*melas*	122	81	19
melas	Diggi (*A*)	94	51	49
melas	Pare (*B*)	88	60	40
melas	TSW	62	45	55
TSW	*melas*	193	57	43

Lagos, Lagos, Nigeria; Diggi, Diggi, W. Sokoto, N. Nigeria; Kisumu, Kisumu, Kenya; Pare, Pare, Tanganyika; Bobo, Bobo Dioulasso, Upper Volta; Liberia, Kpain, Liberia, TSW, Tanganyika salt-water form; *melas*, *Anopheles melas*, Liberia.

A—group *A*, *B*—group *B*, see Davidson and Jackson (ref. 1).

The degree of atrophy of the testes varied considerably. In some, the testes were reduced to an organ barely distinguishable from the vas deferens and in which no sign of spermatogenesis could be distinguished. In others the testes appeared normal in size and showed all stages of spermatogenesis up to what appeared to be normal, tailed spermatozoa. These, however, showed no sign of movement in physiological saline. All F_1 adults appeared normal in size and vigour and F_2 eggs were obtained from all but two of the crosses. These eggs were almost invariably sterile and microscopic examination showed no signs of embryonic development. Only on two occasions did a very few larvæ hatch, and these were from the reciprocal crosses between the Pare fresh-water form and the Tanga salt-water form.

An excess of males was always produced from crosses involving fresh-water form males and salt-water form females (both the Tanga strain and *A. melas*). This was most marked in the fresh-water form male × *melas* female cross. The two crosses between fresh-water form females and Tanga salt-water form males produced a slight excess of females in the F_1 generation. Similar crosses between fresh-water females and *melas* males gave a slight excess of males. The reciprocal crosses between the Tanga salt-water form and *A. melas* resulted in near-normal sex-ratios.

That some at least of the hybrid females were reproductively normal was shown by the production of viable offspring from back-crosses. These offspring all showed near-normal sex-ratios (Table 2). In addition to those back-crosses given in this table, the back-cross Tanga salt-water form male × hybrid Tanga male/Pare female was also successful, but no sex-ratio was recorded.

Table 2. BACK-CROSSES BETWEEN FRESH-WATER AND SALT-WATER FORMS OF *Anopheles gambiae*

Parents			F_1 generation	
Male	Female hybrid	Total	Percentage male	Percentage female
TSW	*melas* ♂/TSW ♀	184	47	53
melas	*melas* ♂/TSW ♀	20	45	55
Diggi	*melas* ♂/Diggi ♀	805	46	54
melas	*melas* ♂/Pare ♀	19	37	63
melas	Pare ♂/*melas* ♀	21	57	43

Footnotes of Table 1 apply here.

It would thus appear that *Anopheles gambiae* is a complex of at least four partially incompatible forms. Whether any or all of these deserve rank as separate species remains to be seen. If complete absence of gene-flow constitutes a criterion of specific rank, then these forms cannot be considered as separate species. A restricted flow is possible through back-crossing, at least in the artificial conditions of the laboratory.

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Flour Mite, *Acarus siro* L., 1758, as a Species Complex

FOR more than forty years *Acarus siro* L., 1758, a pest of harvested cereals and cereal products, has been considered a very variable species consisting of several forms. Zachvatkin[1] concluded that these forms had no taxonomic significance since intermediate forms existed. Variation has been attributed both to the adult stage and to the hypopus stage (deutonymph), which sometimes occurs in the life-cycle, generally in response to certain unfavourable conditions. Schulze[2] described a motile migratory hypopus, capable of rapid movement but becoming passive once it has attached itself to another arthropod, and an inert form incapable of movement or attachment but resistant to desiccation. She ascribed both forms to *A. siro*, stating that both could be obtained from single pair matings.

A four-year study on hybridization, hypopus formation and the external morphology of populations of *A. siro* from stored products or outdoor habitats has shown there is involved a species complex made up of three species. Each species is capable of producing only one kind of hypopus, either motile or inert. After an extensive research into the literature, it is considered that the complex contains *A. siro* L., 1758; *A. farris*[3] and a new species.

When *siro* was crossed with the other two species only a small percentage of initial crosses succeeded. In these, gamete mortality occurred since each fertile pair yielded only a few offspring. F_1 hybrid sibling crosses did not produce offspring. Back-crosses to parent forms showed that F_1 females were sterile, whereas the majority of F_1 males were fertile. Initial crosses between *farris* and the third species were more successful; about 80 per cent of crosses produced some viable eggs. Both sexes of F_1 hybrids were fertile but only 25 per cent of F_2 and first back-cross eggs hatched. Fertility of F_2 sibling crosses was further reduced in that only 11 per cent of F_3 eggs hatched.

A. farris produces a motile hypopus and the new species an inert hypopus, quite frequently. Experiments revealed that these two hypopi differ physiologically, particularly in respect to resistance to desiccation. Also, an examination of the genetics of the hybrid hypopi has shown that polygenic inheritance occurs. A few out of many populations of *siro* have produced a motile hypopus which is morphologically different from that of *farris*. However, hypopus production in *siro* is rare.

Following examination of material collected from all over Britain and from parts of Europe, Canada, United States, Chile, Kenya and Egypt, it is concluded that the form designated here as *A. siro* L. is a stored product mite. Since the type no longer exists, arrangements are being made to erect a neotype. Oudemans's syntypes of *A. farris* have been examined and I select as lectotype the single male specimen bearing the following data. Left label (black ink in Oudemans's handwriting and crossed through twice in pencil): *Tyroglyphus farris* Oudemans ♂ type. Right label (black ink): in Kaas Arnhem 6. 2. 1902 Oudemans; below this is a small printed label stating: "Museum Leiden Verz. A.C. Oudemans collection Cat. No. 45". This species appears to occupy outdoor habitats.

A full account of this work is being prepared for publication elsewhere in which the new species will be fully described and named. Meanwhile, in view of the economic importance of this species complex, a simplified key for the separation of the adults is appended. It is based on chætotaxy of tarsus II of either sex, when the tarsus is viewed laterally. The nomenclature of the setæ follows Grandjean[4].

(1) Spine S at ventral apex of tarsus II large, about equal in length to tarsal claw; width of base equal to length of spines' shortest edge; tip of spine directed backwards. Solenidion omega$_1$, on dorsal basal third of tarsus, recumbent with a distinct 'goose-neck' preceding terminal 'head'. *A. siro* L.

Spine S fairly small, about half length of tarsal claw; width at base less than half the length of spines' shortest edge; tip not directed backwards. Omega$_1$ not recumbent, not 'goose-necked'. 2.

(2) Omega$_1$ with sides almost parallel; an indistinct 'neck' expands into a well-formed egg-shaped terminal 'head'. Widest part of 'head' exceeds widest part of stem. *Acarus* sp. n.

Omega$_1$ with sides diverging slightly from base, narrowing imperceptibly to an indistinct 'neck' then expanding slightly into terminal 'head'. Widest part of 'head' never wider than widest part of stem. *A. ferris* Ouds.

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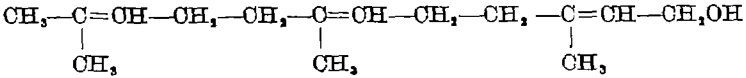
Juvenile Hormone Activity of Isomers of Farnesol

SINCE the juvenile hormone of insects was first extracted from the abdomens of male cecropia moths[1], its presence or the presence of juvenile hormone-like activity has been demonstrated in other insects[2], in invertebrates[3], in bacteria, yeast, and higher plants[4], in certain organs of mammals[5], and in the fæces of the beetle *Tenebrio molitor*[6]. Recently, the active constituents of the fæces of *T. molitor* were isolated and identified as two related acyclic sesquiterpenes, farnesol (I) and farnesal[7], and the activity of one of them, farnesol, was examined in detail on the assassin bug, *Rhodnius prolixus*[8]. Although the juvenile hormone activity of farnesol was demonstrated, its unit activity was far less than those of partially purified extracts of cecropia moths, and therefore it is not likely that farnesol is the true juvenile hormone of insects[8,9]. Nevertheless, interest is centred on these terpenes as possible precursors of the juvenile hormone.



$$\underset{\displaystyle CH_3}{CH_3{-}\underset{|}{C}}{=}CH{-}CH_2{-}CH_2{-}\underset{\underset{\displaystyle CH_3}{|}}{C}{=}CH{-}CH_2{-}CH_2{-}\underset{\underset{\displaystyle CH_3}{|}}{C}{=}CH{-}CH_2OH$$

(I)

We have also tested, and are able to confirm, the juvenile hormone activity of farnesol and farnesal and another related terpene, nerolidol, on *T. molitor* pupæ; no activity was found in geraniol, citronellol, squalene, and mevalonic acid lactone.

Table 1. JUVENILE HORMONE ACTIVITY OF ISOMERS OF FARNESOL

Concentration	*Trans, cis*	*Trans, trans*	*Cis, trans*	*Cis, cis*
77–129 μg/pupa	0	100	100	0
30–65 μg/pupa	0	100	100	0

The activity at two different concentrations is expressed as the percentage of *Tenebrio* adults showing pupal–adult intermediate genitalia. Ten pupæ were used for each isomer at each concentration.

There are four possible steric isomers of farnesol, the isomerism occurring at the Δ2 and Δ6 positions of the molecule. These four isomers were recently prepared in pure form[10] and we were able to obtain small amounts of them for testing. (We thank R. B. Bates, Department of Chemistry, University of Illinois, for supplying samples of the isomers of farnesol.) The assay used was the quantitative *Tenebrio* test[9], modified to the extent that concentrations injected into the pupæ were in micrograms and the results assessed by recording the presence or absence of pupal–adult intermediate genitalia in the adults. The isomers were dissolved in refined peanut oil and injected into 24–48-h-old pupæ of *T. molitor* at two ranges of concentration. The ranges of concentration were preliminarily determined to give 100 and 70 per cent adult–pupal intermediates, respectively, with commercially obtained farnesol.

In our tests, 100 per cent pupal–adult intermediates at both ranges of concentration were obtained with the *cis*-2, *trans*-6 and *trans*-2, *trans*-6 isomers of farnesol (Table 1). The results of the assay indicate that the biological activity of farnesol and related terpenes is resident in the *trans* configuration at the Δ6 or middle linkage, and further tests with homologues and analogues of farnesol are in progress.

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MICROBIOLOGY

Effect of Various Bile Salts on the Anticandidal Activity of Amphotericin B and Gentian Violet and on the Anti staphylococcal Activity of Neomycin

A MIXTURE of bile salts contained in 'Bacto Bile Salts' (Difco) has been found capable of: (*a*) neutralizing the anti-candidal activity of a number of antimycotic agents including amphotericin B and gentian violet[1]; (*b*) potentiating the anti-staphylococcal activity of neomycin[2]; (*c*) effecting a mucin-like enhancement of intraperitoneal staphylococcal virulence for mice[3]. Considering the last, with the exception of sodium dehydrocholate, each salt contained in the bile salts mixture is capable of exerting this *in vivo* action[4]. Mucin, cholesterol and lecithin, which are present in unspecified amounts, also possess this same capability[5]. The work recorded here is concerned with the effectiveness of various bile salts as well as of mucin, cholesterol and lecithin on the first two *in vitro* activities already noted.

Sodium taurocholate, sodium glycocholate, sodium glycotaurocholate, sodium dehydrocholate, lecithin (animal) and cholesterol were obtained from Nutritional Biochemicals Corporation; sodium cholate from K. and K. Laboratories; sodium deoxycholate from Fisher Scientific Co. and gastric mucin from Wilson Laboratories. All were sterilized by autoclaving at 15 lb. for 15 min. Media were 1 per cent dextrose trypticase soy agar for *Candida albicans* and dilute trypticase soy agar containing 3 parts trypticase soy broth and 5 parts distilled water for *Staphylococcus aureus*. The former was adjusted to *p*H 6·8; the latter to 7·3. After melting and cooling to approximately 60° C, amphotericin B, gentian violet and neomycin were added as indicated to achieve a final concentration of 5·0, 10·0 and 0·1 μg/ml. medium, respectively, as well as varying concentrations of each substance under test. Each tube was well mixed mechanically and poured into a Petri dish. After cooling and solidification, 0·1 ml. of an 18-h culture at 37° C of *Candida albicans* or of *Staphylococcus aureus* in liquid medium, adjusted to an optical density of 0·1 and 0·05 respectively on a Lumetron colorimeter at 650 mμ, was spread evenly over the surface. Inoculated plates were incubated for 48 h at 37° C, and then observed for the presence or absence of distinct microbial growth. Growth control plates containing the various test substances alone without any antimicrobial agents were also prepared and tested for their possible growth inhibitory effect on both micro-organisms being investigated. None inhibited their growth in the maximum concentrations used. Neutralization of the antimycotic agents was evidenced by the production of definite candidal growth when a particular test substance was added to the medium as contrasted to complete lack of growth when it was omitted. 0·5 μg of neomycin/ml. medium was found to be the minimal inhibitory concentration for the standard *Staphylococcus aureus* inoculum and neomycin potentiation was indicated by the achievement of bacterial inhibition with only 0·1 μg/neomycin/ml. medium as a result of the addition of the particular test substance to the medium.

The minimal concentration per ml. of medium of each substance under investigation that neutralized the anticandidal action of 5 μg/ml. of amphotericin B and 10 μg/ml. of gentian violet and which effected a 5 times or greater enhancement of the anti-staphylococcal activity of neomycin is contained in Table 1.

As may be noted from Table 1, 15 mg/ml. or less of each bile salt elicited both effects under consideration, with one exception. 80 mg/ml. sodium dehydrocholate was required to inactivate the gentian violet

Table 1. Minimal concentration in mg/ml. of various bile salts and other test substances required to neutralize the anticandidal action of amphotericin B and gentian violet; and to effect a five times or greater potentiation of the anti-staphylococcal activity of neomycin

Substance	Neutralization 5 μg/ml amphotericin	Neutralization 10 μg/ml. gentian violet	Five times or greater potentiation of neomycin
Bile salts mixture ('Difco')	5·0	5·0	10·0
Sodium taurocholate	12·5	6·0	5·0
Sodium glycocholate	10·0	8·0	10·0
Sodium glycotaurocholate	10·0	10·0	15·0
Sodium cholate	15·0	6·0	1·0
Sodium deoxycholate	1·5	1·6	1·0
Sodium dehydrocholate	> 100·0	80·0	> 100·0
Gastric mucin	> 50·0	20·0	> 50·0
Animal lecithin	> 50·0	6·0	> 50·0
Cholesterol	1·0	50·0	> 50·0

whereas 100 mg/ml. of the same salt failed to neutralize amphotericin B or potentiate neomycin. As for the other substances investigated, 20 mg/ml. of gastric mucin was able to neutralize the anti-candidal activity of gentian violet but the maximum concentration employed with this substance, 50 mg/ml., had no such effect upon amphotericin B. Similarly, while lecithin, 6 mg/ml., neutralized gentian violet, 50 mg/ml. was ineffective against amphotericin B. As little as 1 mg/ml. cholesterol, on the other hand, did neutralize amphotericin B but 50 mg/ml. of the same compound was required to achieve the same effect with gentian violet. No potentiation of the anti-staphylococcal activity of neomycin was observed with the maximum concentration used of mucin, lecithin or cholesterol, 50 mg/ml.

It is noteworthy that every bile salt that proved effective in amphotericin B and gentian violet neutralization, neomycin potentiation and enhancement of intraperitoneal staphylococcal virulence for mice, is able to lower surface tension significantly as determined by the capillary tube method[6], whereas the one bile salt that was ineffective, sodium dehydrocholate, does not possess this property[4]. For this reason the existence of a possible relationship between the possession of this physical property by a substance and its ability to effect both the *in vitro* and *in vivo* effects under consideration is readily suggested. No explanation is forthcoming for the irregular action in this regard of mucin, lecithin and cholesterol, all which possess significant surface tension lowering ability[5].

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CYTOLOGY

Isolation of Nuclei from Animal Cell Suspensions

IN a previous communication, a method for the quantitative isolation of nuclei from HeLa cells was described[1]. The nuclei were isolated by stirring the cells in a dilute solution of 'Tween 80' under controlled conditions. It seems worth reporting that the method has now been successfully applied to strain *L*, Chinese hamster, Ehrlich ascites and Krebs 2 ascites tumour cells. It appears likely that it will prove to be applicable to a wide range of animal cell suspensions.

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GENETICS

Homeostasis and the F_1 Hybrid

IN his letter defining 'social homeostasis' Mackintosh[1] has referred to the uniformity of first crosses of inbred strains as compared with the parental stocks citing the monograph by Lerner[2] and the more recent review by Biggers, McLaren and Michie[3]. These authors are perhaps influenced by their genetic theories. The results of experiments performed by Dobzhansky and Wallace[4] justify their conclusions that "homeostatic adjustments are superior in heterozygotes to those in homozygotes". However, Lerner himself points out that most other experimental work cannot provide unequivocal evidence on the role of heterozygosity in developmental homeostasis as "even when the phenotypic $F1$ variance is lower than the parental average, the reduction may have been due to the genetic and not to the environmental component".

It is unwise to suppose that because results with a particular anæsthetic using two inbred strains and their reciprocal hybrids suggest superior development homeostasis of the heterozygotes all other first crosses will fall into line. In work with pentobarbitone sodium, Brown[5] showed that both F_1 crosses out of $DBA/1$ and $A2G$ strains resembled $DBA/1$ strain more nearly in the precision of their responses measured by λ. One of these hybrid strains $A2DB/1F_1$ gave a response to insulin (Brown[5]) well outside either of its parent strains and significantly less precise than its parent the $DBA/1$ strain. Further, the responses of the F_1 hybrid strains $A2CF_1$ and $CA2F_1$ each more closely resembled its maternal parent. These results may be compared with those noted by Chai[6] and by Grüneberg[7]. From the literature it would appear, therefore, that there is no general rule as to the response of inbred strains and F_1 crosses and for any particular experimental procedure the choice between inbred and F_1 animals can only be made after preliminary testing and observation.

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THE second paragraph in Brown and Dinsley's communication might be construed to refer to my communication on the effects of strain and group size on the response of mice to 'Seconal' anæsthesia. If this is indeed true, I would like to point out that I nowhere suggest that "because results with a particular anæsthetic using two inbred strains and their reciprocal hybrids suggest superior development homeostasis of the heterozygotes all other first crosses will fall into line". In fact, if Table 1 of that communication is referred to it can be seen that particular environmental conditions can result in equal amounts of variation being shown by both the heterozygote and the homozygote, for example, Part 2, two per cage, all strains, and Part 2, eight per cage *CE*/*CE*♂ × *CBA*♀ F_1.

Brown and Dinsley also refer to the work of Brown, who showed that the reciprocal hybrids of *DBA*/1 and *A2G* resemble the *DBA*/1 strain in the precision of their response as measured by λ, defined by her as the ratio, standard deviation : slope of regression.

This may be used as a measure of the accuracy of an assay but it is not, in a quantitative response, a measure of variation. In this connexion Chai is explicitly concerned with precision of assay rather than with variation and many of his data are concerned only with the sensitivity of the response. He also points out that to suggest that equivalent precision between hybrids and pure lines renders them equally suitable for bioassay may be to ignore other advantages which have been reported to accrue with hybrids.

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PSYCHOLOGY

Relationship between Test Difficulty and the Visual Impairment in Monkeys with Ablations of Temporal Cortex

BILATERAL ablation of inferior temporal cortex gives rise to impairment of visual discrimination performance in monkeys. This visual impairment is not associated with a comparable tactile or auditory deficit. Nor is it a simple consequence of damage to the geniculo-striate visual afferent system. Such damage can often be excluded histologically; and direct comparison between the effects of temporal and striate ablations gave distinct patterns of impaired performance[1].

All conventional tasks suitable for testing the monkey's visual performance require the animal to learn to discriminate between two or more test objects. The animals with temporal ablations may be impaired at learning the solutions of visual problems (a defect of learning), or at discriminating by vision between the test objects (a defect of discrimination, perception or differentiation) or in some other way. In many studies the relative importance of learning and discrimination has been varied for a series of tasks, to establish the kind of test giving maximal impairment. No definitive conclusion has been reached as there are probably no pure tests of visual learning (without some discrimination) nor of discrimination (without some learning). A different approach is to analyse the nature of a certain class of tests (retention or relearning tests) and to examine the results relating to test difficulty for such tests.

The effects of inferior temporal ablations in monkeys may be assessed by examining discrimination performance either during post-operative (new) learning or during post-operative retention testing (re-learning): in post-operative learning the animals are trained to discriminate between test objects which have never previously been shown to them; with post-operative retention testing objects that had already been used pre-operatively are again made available, but after surgery. A general finding, applicable both to post-operative learning and retention, is that impairment is greater with increasing test difficulty. Thus "previous studies have shown that monkeys with lesions of both temporal lobes fail on 'difficult' and do better on 'easier' tasks"[2]. Tasks are graded as 'difficult' or 'easy' by reference to the learning scores of unoperated animals.

The relationship between impairment and test difficulty is readily understood for post-operative new learning. The operated animal's learning and/or discrimination capacities may be insufficiently taxed on the easy tests and its defects remain masked; on the difficult tests its capacities are more fully taxed and are shown to be impaired. In the same way a backward child may do as well as a clever child on an easy task but not on a difficult task. However, the greater impairment on difficult tasks during post-operative new learning does not indicate the nature of the defect (learning, discrimination or other).

In the case of post-operative retention testing animals are by convention trained before surgery to an identical standard level of performance (criterion) irrespective of task difficulty; so that, on a naive view, differences in test re-learning difficulty have been eliminated. The greater impairment on the more difficult tests has then not been attributed to a defect of (re-)learning capacity as this is thought, on the naive view, to be equally taxed on both easy and difficult retention tests.

However, training before surgery to a constant criterion involves proportionately more overtraining with easy tasks, proportionately less overtraining with difficult tasks. Overtraining improves retention in unoperated monkeys, and may eliminate impairment otherwise expected in operated animals[3]. The impairment on the difficult tasks during post-operative retention testing may then generally reflect the relative lack of overtraining on these tests. The relatively greater amount of pre-operative overtraining on the easy tests is likely to have eliminated a defect of learning, rather than of discrimination, associated with the temporal ablations.

This interpretation accords with the proposals of others that on easy discrimination tests "long criterional runs may provide sufficient overtraining to produce habits resistant to the operation"[3], and that certain ablations might be associated with "a permanent impairment in the ability to acquire discriminations"[4]. It is also in agreement with the conclusions of Mishkin and his colleagues[1,5] based on evidence unrelated to test difficulty. However, emphasis on factors related to a defect of discrimination, not of learning, has previously been placed by others[2,6].

Irrespective of the process, whether learning or discrimination, influenced by overtraining, it would be useful to examine the effect of temporal ablations on easy and difficult tasks learnt before surgery but with equivalent amounts of overtraining. A first attempt has been made to equate the overtraining for tasks of different difficulty by training unoperated animals to a directly proportional criterion[7]. Six unoperated animals were trained (before dietary treatments) on three tests of different difficulty to a proportional criterion: to a level of 90 per cent correct responses in 10 consecutive trials if the criterion performance began between trials 1–100; to 90 per cent correct in 20 trials if this criterion began to be met between trials 101–200; to 90 per cent correct in 30 trials if between trials 201–300; and so on. All animals were re-trained to a fixed criterion during retention testing (also before dietary treatments began). Differences in test difficulty which existed during learning were found on retention testing to have been reduced by the use of a directly proportional criterion during training. Such differences might have been eliminated by use during training of a criterion bearing a geometrical relationship to test difficulty.

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STATISTICS

k-State Markov Chains

EXAMINATION of random sequences from Markov chains enables us to judge the relative efficiency of various methods which have been developed for testing different statistical hypotheses. A good deal of work, therefore, has been done recently by various authors[1-7] on the distribution of the number of transitions between adjoining observations of sequences from k-state Markov chains. The methods and results obtained in these investigations are rather unwieldy and cumbersome. The object of the present communication is to obtain the results for k-state chain from those of the two-state.

For a two-state chain defined by states a and b with the transition matrix (p_{ij}), i, $j = a$ or b, the probability that the r-th observation is in state a is given by:

$$P_r(a) = P_a + \{P(1 - P_a) - (1 - P)P_a\}\delta_{aa}^{r-1} \quad (1)$$

where P_a is the asymptotic probability of a for the chain, P the initial probability for the chain to commence with a and $\delta_{aa} = (p_{aa} - P_a)/(1 - P_a)$.

Assuming the initial observation to be a, that is, putting $P = 1$ in equation (1) reduces it to:

$$\begin{aligned} P_r(a/1a) &= P_a + (1 - P_a)\delta_{aa}^{r-1} \\ &= P_a + (p_{aa} - P_a)\delta_{aa}^{r-2} \quad (2) \end{aligned}$$

$Pr(a/1a)$ is the conditional probability for the r-th observation to be a when the first one is a. It can easily be seen that the conditional corresponding probability for the k-state chain is given by the same expression as equation (2), the values of P_a and p_{aa} being those for the k-state chain.

The conditional probability $P_r(a/1b)$ has been obtained from first principles for $r = 2,3 \ldots$ for the k-state and reduces to:

$$P_r(a/1b) = P_a + (p_{ba} - P_a)\delta_{aa}^{r-2} \quad (3)$$

Expression (3) is same as (2) with p_{aa}, the transitional probability of aa changed to p_{ba}, the transitional probability of ba.

Following this the conditional probability for the r-th observation to be in state l subject to the condition that the first observation is m is given by:

$$P_r(l/1m) = P_l + (p_{ml} - P_l)\,\delta_{ll}^{r-2} \quad (4)$$

where p_{ml} is the transitional probability for ml and:

$$\delta_{ll} = (p_{ll} - P_l)/(1 - P_l)$$

From the foregoing results using the properties of simple chains we can easily write down the probability of t-th, u-th and v-th observations of the sequence to be in states t, u and v as (asymptotic probability for t) (conditional probability of u when t-th observation is t) (conditional probability of v when u-th observation is u) which is equal to:

$$P_t\{P_u + (p_{tu} - P_u)\delta_{uu}^{u-t-1}\}\{P_v + (p_{uv} - P_v)\delta_{ll}^{v-u-1}\} \quad (5)$$

Using these results the cumulants for the various distributions arising from k-state Markov chain sequences can be obtained in a form much simpler than has been done so far. This is being worked out and the results will be published shortly.

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MISCELLANEOUS

Rocket Engines

IN his review of my book *Rocket Engines*[1], Mr. J. E. P. Dunning takes me to task for stating that the change in total pressure of the burning gases consequent on heat addition in the combustion chamber is not a true loss. He writes, "When heat is added to a gas flowing in a duct it suffers a fundamental loss in total pressure *which cannot be recovered by subsequent cooling*" (my italics).

Mr. Dunning's statement is not strictly true, however, since the equations for heat addition to a flowing gas do not necessarily involve any irreversibility: a decrease in stagnation temperature should increase the stagnation pressure by just the same amount that an increase in temperature reduces it. This point has been clearly demonstrated by Shapiro, who followed the theoretical arguments[2] by actually building a machine in which evaporative cooling was used to increase the stagnation pressure of a flowing stream[3].

It is, of course, indisputable that in any practical rocket combustion system there are other pressure losses, due to friction, turbulence and so on, which are irreversible. But a large part of the stagnation pressure change is that due to heat addition, and my original statement that this is not a loss, since the original pressure could theoretically be restored by cooling, was quite justifiable.

May I add that I was otherwise much heartened by the generous tone of the review.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 3

University of London (in the New Engineering Building Lecture Theatre, University College, Torrington Place, London, W.C.1), at 5 p.m.—Prof. S. A. Schelkunoff (Columbia University): "Generalized Telegraphist's Equations and Their Applications".* (Further lectures on December 5 and 7).

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. H. Woodger: "Lopsidedness, its Cause and Cure".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London W C.2), at 5.30 p.m.—Discussion on "Plugs, Sockets and Interconnexions" opened by Mr. N. E. Hyde,

Royal Institution, Library Circle (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. J. S. Chapman: "English Explorers and Their Books".

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. G. C. Bunn, Mr. D. M Pilcher and Mr. T. W. Ball: "Reconstruction of Sewers for the Metropolitan Borough of Shoreditch".

British Society for International Health Education (at the Draper's Hall, Throgmorton Street, London, E.C.2), at 6 p.m.—Dr. Brock Chisholm: "New Horizons in Health".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 6 p.m.—Mr. Jens Bjerre. "Rendumu" (Colour films with commentary).

Society of Chemical Industry, London Section (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. J. E. Davis: "Recent Developments in the Gas Industry".

Tuesday, December 4

Council for Microphotography and Document Reproduction (at the Connaught Rooms, Great Queen Street, London, W.C.2), at 1 p.m.—Sir Frank Francis: Presidential Address, followed by Mr. K. A. Fairthorne: "Microphotography and Information Retrieval—Implications of the Alliance".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. A. P. Goffe: "Active Immunization Against Measles".* (Fifteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Plastics Institute (at the Wellcome Building, Euston Road, London, N.W.1), at 6.30 p.m.—Mr. W. B. D. Brown: "A Different Way of Looking at Management".

Wednesday, December 5

Royal Society of Arts (at John Adam Street, London, W.C.2), at 2.30 p.m.—Dr. D. A. Sutton· "Modern Natural and Synthetic Glues and Adhesives".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Mr. J. A. Bather: "Control Charts and the Minimization of Costs".

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. I. J. Smith: "The Precision of IP Test Methods".

Institution of Electrical Engineers, Science and General Division P.G.S.7 (at Savoy Place, London, W C.2), at 5 30 p.m.—Discussion on "Character Recognition" opened by Mr. P. M. Hall.

British Institution of Radio Engineers, Radar Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Dr. J. W. R. Griffiths: "Signal/Noise Performance of Radar and Sonar Displays".

Institution of Mechanical Engineers, Steam Plant Group and Nuclear Energy Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. T. P. Webb and Mr. W. R. Wootton: "The Design and Construction of Heat Exchangers for Gas-cooled Nuclear Power Plant".

Society for Analytical Chemistry (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Meeting on "Applications of X-ray Fluorescence".

Wednesday, December 5—Friday, December 7

Royal Photographic Society of Great Britain (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2)—Symposium on "Quality in Photographic Reproduction".

Thursday, December 6

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Prof. G. Pontecorvo, F.R.S.· "Microbial Genetics: Achievements and Prospects" (Leeuwenhoek Lecture).

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London, S.E.1), at 5.30 p m.—Mr. N. A. Tapsfield: "The Development of Two-Temperature Domestic Refrigerators".

Institution of Electrical Engineers (at Savoy Place, London. W.C.2), at 5.30 p.m.—Mr. R. H. Tizard: "Data Processing".

University College, London (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. H. Gaddum, F.R.S. "Chemical Transmission in the Central Nervous System".*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1) at 5.30 p.m.—Dr. H. J. Rogers· "Bacterial Cell Walls and Membranes".* (Sixteenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation)

Radar and Electronics Association (at the Royal Commonwealth Society Hall, 18 Northumberland Avenue, London, W.C.2) at 7 p.m.—Prof. Sir Bernard Lovell, F R.S.: "Investigation of Space by Probes and Radio".

Royal Institute of Chemistry, London Section (at the Northampton College of Advanced Technology, St. John Street, London. E.C 1), at 7 p.m.—Dr. G. H. Williams "Some Aspects of the Chemistry of Free Radicals".

Oil and Colour Chemists' Association, London Section (at the Royal Society of Tropical Medicine and Hygiene, Manson House. 26 Portland Place, London, W.1), at 7 p.m.—Mr. J. S. Marsh "Colour Match Predictions using Organic Pigments".

Women's Engineering Society (at "Hope House", 45 Great Peter Street, London, S.W.1), at 7.30 p.m —Miss L. Chitty. "Some Aspects of Water Supply".

Friday, December 7

Institution of Electrical Engineers, Electronics Division P.G.E5 (joint meeting with the British Institution of Radio Engineers Medical Electronics Group, at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Masers and Lasers and the Possible Applications in Medicine and Biology".

Institution of Mechanical Engineers, Education and Training Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Overseas Training of British Graduates".

Society of Chemical Industry, Fine Chemicals Group (joint meeting with the London Section, at 14 Belgrave Square, London. S.W.1), at 6.30 p.m.—Dr. A. Olow: "The Chemical Industry before Muspratt".

Royal Institute of Chemistry, London Section (joint meeting with the East Anglia Section, at the Mid-Essex Technical College and School of Art, Market Road, Chelmsford, Essex), at 7 p.m.—Dr. I. G. Holden: "Science in the Detection of Crime".

Saturday, December 8

Association of Agriculture and the British Agricultural History Society (at the London School of Economics, Houghton Street, London, W.C.2), at 11 a.m.—Conference—"Agrarian History Some Vistas and Pitfalls (with special reference to Medieval England)".

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3 30 p.m.—Mr. Barrie Reynolds "Magic and Witchcraft Practices in Barotseland".*

Monday, December 10

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Universal Excavators *versus* the Front End Loader".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Sir John Hunt, C.B.E., D.S.O.: "A British-Russian Expedition to the Pamirs".

Monday, December 10—Tuesday, December 11

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (at Church House, Great Smith Street, London, S.W.1), at 11.15 a.m. on Monday and 10 a.m. on Tuesday—Winter Meeting.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Junior Demonstrator (with a special interest in palæomagnetic research) in the Department of Physics—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne (December 7).

Research Officer (with sound postgraduate experience in pig or animal nutrition) in Pig Nutrition in the Department of Agriculture—The Registrar, University College of North Wales, Bangor, North Wales (December 7).

Assistant Lecturer, Grade B (graduate in chemistry, and preferably the ability to teach physics or an applied science), at Northampton College of Technology, to teach physical and organic chemistry to Higher National Certificate standard—Chief Education Officer, Northampton County Borough Education Committee, "Springfield", Cliftonville, Northampton (December 8).

Lecturer in Inorganic and Physical Chemistry; and a Lecturer in Mathematics—The Principal, Nottingham and District Technical College, Burton Street, Nottingham (December 8).

Lecturer in the Department of Physiology—The Secretary, The University, Aberdeen (December 10).

Lecturer in Pure Mathematics; a Lecturer in Applied Mathematics; an Assistant in Pure Mathematics; and an

ASSISTANT IN APPLIED MATHEMATICS at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (December 14).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF SOCIAL ANTHROPOLOGY—The Secretary, The University, Edinburgh (December 14).

LECTURER IN THE DEPARTMENT OF BOTANY at the University of Natal, Pietermaritzburg—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, December 15).

LECTURER or ASSISTANT LECTURER IN THE DEPARTMENT OF CHEMICAL ENGINEERING—The Deputy Registrar, The University, Birmingham 15 (December 15)

SENIOR LECTURER IN GEOGRAPHY; and a LECTURER IN GEOGRAPHY at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W 1 (New Zealand and London, December 18).

READER (with research interests which include non-numerical applications of computers) IN INFORMATION PROCESSING at the Computer Unit—The Academic Registrar, University of London, Senate House, London, W.C.1 (December 19).

LECTURER (with a good honours degree in chemistry or pharmacy or an equivalent qualification) IN PHARMACY—The Registrar, The University, Manchester 13 (December 20)

SENIOR LECTURER or LECTURER IN CHEMISTRY at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 30).

SENIOR LECTURER (with an honours or higher degree in physics, and preferably with previous teaching and research experience) IN THEORETICAL PHYSICS at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (December 31).

READER IN THEORETICAL NUCLEAR PHYSICS—The Clerk to the Governors, Battersea College of Technology, London, S.W.11 (January 15).

LECTURER IN THE DEPARTMENT OF THEORETICAL PHYSICS—The Registrar, The University, Liverpool, quoting Ref. No. CV/208 (February 1).

SENIOR LECTURER (with a degree in pure science and/or in agriculture, and preferably postgraduate experience in genetics) IN PLANT GENETICS—The Registrar, Lincoln College (University of Canterbury), Canterbury, New Zealand (February 1).

LECTURER (graduate in engineering or mathematics) IN ENGINEERING (Applied Mechanics or Electrical Engineering, or Thermodynamics)—The Registrar, The University, Leicester.

SENIOR LECTURER or LECTURER (preferably with an interest in numerical analysis) IN THE DEPARTMENT OF MATHEMATICS, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

SENIOR SCIENTIFIC OFFICER and a SCIENTIFIC OFFICER (with at least a good honours degree in chemistry or equivalent) IN THE SCIENTIFIC BRANCH, for a wide variety of interesting work on research development and scientific control—The Medical Officer of Health (D.1/O/2706/11), L.C.C., County Hall, London, S.E.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Procter and Gamble, Ltd., (formerly Thomas Hedley and Co. Ltd.). Financial Statement for the year ended 30th June, 1962. Pp. 16. (Newcastle upon Tyne · Procter and Gamble, Ltd., 1962.) [910

The National Central Library. 46th Annual Report of the Executive Committee for the year ending 31 March 1962. Pp. 26. (London: The National Central Library, 1962.) [910

Social Policy in a Free Society. By Prof. Francois Lafitte. (An inaugural lecture delivered in the University of Birmingham on 18th May, 1962.) Pp. 17. (Birmingham: The University, 1962.) 2*s*. 6*d*. [910

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University of Bristol. The Annual Report of the Agricultural and Horticultural Research Station, (The National Fruit and Cider Institute), Long Ashton, Bristol, 1961. Pp. 208+13 plates. (Long Ashton, Bristol: Agricultural and Horticultural Research Station, University of Bristol, 1962.) 15*s*. [910

Department of Scientific and Industrial Research: National Engineering Laboratory. Heat Bibliography, 1961. Pp. vi+325. (London: H.M. Stationery Office, 1962.) 25*s*. net. [910

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Building Research Station Digest, (Second Series). No. 27 · Rising Damp in Walls. Pp. 6. (London: H.M. Stationery Office, 1962.) 4*d*. [910

Other Countries

South Africa: Council for Scientific and Industrial Research. National Building Research Institute. C.S.I.R. Special Report No. D4: Thermodynamics of Expansive Soils. Part 1: Volume Changes. Part 2: Energy Changes. By C. M. A. de Bruijn. Pp. viii+19. (Pretoria: South African Council for Scientific and Industrial Research, 1962.) [110

Arkiv för Zoologi, Andra Serien, Band 14, Häfte 1–6: Die Hydracarinen Schwedens, II. Von O. Lundblad. (Utgivet av Kungl. Svenska Vetenskaps-akademien.) Pp. 625+123 plates. (Stockholm: Almqvist and Wiksell, 1962.) Sw. Crs. 75. [110

Comité International des Poids et Mesure. Procès-Verbaux des Séances. 2ᵉ Série—Tome 29: 50ᵉ Session, 1961, (9–12 Octobre). Pp. 85. Comité Consultatif pour la Définition de la Seconde, 2ᵉ Session, 1961, (11–12 Avril). Pp. 123. Comité Consultatif pour les Etalons de Mesure des Radiations Ionosantes, 3ᵉ Session, 1961, (3–4 Octobre). Pp. 32. (Paris · Gauthier-Villars et Cie., 1962.) [110

Institut Royal Météorologique de Belgique. Contributions. No. 70: Profil Magnétique de Mombasa (Kenya, Océan Indien) à Tororo (Uganda) au Travers de la Great Rift Valley (Kenya). Par E. Hoge et K. Whitham. Pp. 6. No. 71: La Météorologie dans les Mémoires de Saint-Simon. Par Dr. L. Dufour. Pp. 10. No. 72. La Mesure de la Distribution Electronique de l'Ionosphere. Par L. Bossy. Pp. 28. No. 73: Pour une Exploitation Rationnelle des Indices Bioclimatiques: Distributions de Frequencies Conjointes et Ellipses de Concentration. Par F. Bultot. Pp. 16. Publications, Série B, No. 37 · Sur l'Empêchement Volumétrique de Nucléation. Par Prof. R. Defay et L. Dufour. Pp. 10. Observations Ionospheriques—Station de Dourbes. Juin, 1962. Pp. 26. Juillet, 1962. Pp 26. (Uccle-Bruxelles: Institut Royal Météorologique de Belgique, 1962.) [110

Occasional Papers of the California Academy of Sciences. No. 34. The Geological History of Eagle Lake, Lassen County, California. By G. C. Gester. Pp. 29+1 plate. (San Francisco. California Academy of Sciences, 1962.) [110

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Bulletin of the Museum of Comparative Zoology at Harvard College Vol. 127, No. 3: Scolopophorous Organs in the Pterothorax and Abdomen of *Gelastocoris oculatus* (Fabricius) (Hemiptera. Heteroptera). By Margaret C. Parsons. Pp. 205–236. Vol. 27, No. 4 · The Mammalian Fauna of the Divisadero Largo Formation, Mendoza, Argentina. By George Gaylord Simpson, José Luis Minoprio, and Bryan Patterson. Pp. 237–294+7 plates Vol. 127, No. 5. On Some Burmese Earthworms of the Moniligastrid Genus *Drawida*. By G. E. Gates. Pp. 295–374. Vol. 127, No. 6: A Study of *Echmatemys callopyge* from the Uinta Eocene of Utah, and Its Redefinition as a Subspecies of *E. septaria*. By David C. Roberts. Pp. 375–400+2 plates. Vol. 127 No. 7: Behaviour of *Daceton armigerum* (Latreille), with a Classification of Self-Grooming Movement in Ants. By Edward O. Wilson. Pp. 401–422. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1962.) [110

Museum of Comparative Zoology at Harvard College. Breviora. No. 161, (July 16, 1962): A Comparative Study of the Respiratory Muscles of Chelonia. By R. V. Shah. Pp. 16. No. 162, (July 25, 1962): Australian Carabid Beetles 10: *Bembidion*. By P. J. Darlington, Jr. Pp. 12. No. 163, (July 26, 1962): New Worm-Lizards (*Ancylocranium* and *Amphisbaena*) from South-eastern Tanganyika Territory. By Arthur Loveridge Pp. 6. No. 164, (August 22, 1962): Notes on the Herpetology of Hispaniola. 7: New Material of Two Poorly Known Anoles: *Anolis monticola* Shreve and *Anolis christophei* Williams. By Ernest E. Williams. Pp. 11. No. 165, (August 22, 1962): An Extinct Solenodontid from Hispaniola. By Bryan Patterson. Pp. 11. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1962.) [110

By Their Fruits: Some Historic Contributions to the Chemistry of Life. Pp. 48. (Rahway, N. J.: Merck Sharp and Dohme Research Laboratories, 1962.) [110

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United States Department of the Interior: Geological Survey. Bulletin 1146-D: Geophysical Abstracts 187, October–December, 1961. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others. Pp. iii+631–708. 40 cents. Bulletin 1146-E: Index to Geophysical Abstracts 184–187, 1961. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others. Pp. ii+709–822. 40 cents. Water-Supply Paper 1535-G: Rainwater as a Chemical Agent of Geologic Processes—a Review. By Dorothy Carroll. Pp. iii+18+1 plate. Water-Supply Paper 1575: Quality of Waters for Irrigation, Western United States, 1958. Prepared under the direction of S. K. Love. Pp. viii+177+1 plate. Professional Paper 236-C: Foraminifera from the Arctic Slope of Alaska. Part 3: Cretaceous Foraminifera. By Helen Tappan. Pp. vi+91–209+plates 29–58. Professional Paper 260-AA: Some Physical Constants for the Marshall Islands Area—Bikini and Nearby Atolls, Marshall Islands. By J. H. Swartz. Pp. iv+953–989. Professional Paper 406: The Eureka Mining District, Nevada. By Thomas B. Nolan. Pp. iv+78+11 plates. (Washington, D.C.: Government Printing Office, 1962.) [110

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THE DRUG INDUSTRY AND PUBLIC SAFETY IN BRITAIN

THE rise of the pharmaceutical industry cannot be dated with the same precision as that of the dyestuffs industry, but its development in the latter part of the nineteenth century was closely related to that of organic chemical industry and the wealth of organic intermediates which the growth of the dyestuffs industry made available. Nevertheless, the outbreak of the First World War found Great Britain almost entirely dependent on Germany for her supplies of synthetic organic pharmaceuticals, and the manufacture of such products in Britain is essentially a development of the inter-war years. At the outbreak of the Second World War in 1939, however, the pharmaceutical industry was entering on a new phase of rapid and continuous development as the outcome of a period of intensive scientific research which brought many new products to light and is well illustrated by the simple fact that 70 per cent of to-day's prescriptions could not have been written in 1935, and 45 per cent even five years ago because the drugs did not exist.

The coincidence of the discovery of the valuable but expensive drugs resulting from this research with the introduction of a free and comprehensive Health Service for all was largely responsible for the rise in the cost of prescriptions which led to the appointment in June 1957 of a Committee of Enquiry with Sir Henry Hinchliffe as chairman, though, as the Committee pointed out in its final Report in 1959, inflation, population changes and the introduction of prescription charges all played their part. In that report, however, the Committee could also add: "The community as a whole derives tremendous benefits from the pharmaceutical service, financially as well as in relief of suffering and saving of life. The use of drugs has made a valuable contribution in the treatment of tuberculosis and other infectious diseases and in mental practice, and has relieved the pressure on hospital accommodation. *The Minister should reconsider ways and means of publicising these facts in a telling manner*".

The Committee was concerned to note the totally inadequate publicity given to the remarkable saving in life, improvement in health, increase in efficiency and saving on extensive institutional treatment stemming from, among other things, the use of new drugs. Nor was it in any doubt as to the vital importance of ensuring the continuance of the fundamental research on which the discovery of new drugs depends. Indeed, it recommended specifically that firms should be encouraged to increase their research effort and that the conditions which may make such research profitable, such as patent rights, the publicizing of proprietary names and the price agreement with the Ministry of Health should be accepted.

In spite, however, of its recommendation that the Minister of Health should consider ways and means of publicizing the facts about the industry's research effort and the widespread benefits derived from the introduction of new drugs, little seems to have been done to improve the publicity position, and the discussion on the control of and testing of drugs at the recent Labour Party Conference is the latest illustration of the need for public understanding of a position for which scientists may themselves have to carry some responsibility. The wilder statements about profits were promptly repudiated in the Press by the Association of the British Pharmaceutical Industry; but it might be recalled when Sir Frank Heath and A. L. Hetherington published their survey of *Industrial Research and Development in the United Kingdom* in 1946, pharmaceutical research was not even mentioned, while the alarm aroused by thalidomide is to a considerable extent due to misunderstanding of the position. Nor is it less important that the true position should be widely appreciated because some firms which are not undertaking the risks of research may be making disproportionate profits. It is difficult, for example, to justify the wide variations in price for what purport to be identical preparations. It should be recognized, however, that a firm which waited until the period of patent protection for some highly successful product was ending, and then put a team of technologists to develop an efficient process, could find the manufacture highly profitable and be able to under-sell heavily the rival firm which had spent millions of pounds on the original research and the biological and chemical testing of the product. If the conditions are such as to deter any firms from undertaking the risks of original research, the introduction of new products will soon cease and with it also the opportunities which the less-venturesome have exploited.

The Hinchliffe Report devoted a separate chapter to the question of research within the British industry in which the position is very fairly set forth, though it can scarcely be said that the Government has shown any real appreciation of the position or its implications during the subsequent three years. While the industry has undertaken to do anything in its power to assist the Ministry of Health in the smooth and equitable operation of the revised Voluntary Price Regulation Scheme, the Association of the British Pharmaceutical Industry did not conceal its fear that export and research incentives built into the original scheme had been dangerously diminished. Although firms in Great Britain may, individually, spend from £100,000 to £1 million per annum on research, the Association of the British Pharmaceutical Industry estimated the total expenditure as no more than £5,257,025 in 1959 compared with a reported £7 million in Switzerland and £43 million in the United States. For 1960 the figures are £7·5 million in the United Kingdom and £77 million in the United States. This expenditure is, moreover, recurrent and a firm which is spending £1 million a year may do so for several years without discovering and developing an important new product. Indeed, the Hinchliffe Committee itself suggested that a firm did well if it made one major advance in ten or twenty years, and although less-notable discoveries are more frequent, even here many hundreds if not thousands of new chemical compounds may have to be synthesized and tested before a new drug is introduced for clinical use.

Even among scientists the position is not always understood clearly, and from the point of view of science itself it seems highly desirable that there should be more understanding of what is involved in the research effort of the industry, both in the discovery of a new product and in the testing which it receives before it is submitted for clinical trial. The discovery of new drugs is, of course, related to developments in other branches of medical science, and this is equally true of their testing, whether for toxicity or in clinical practice. It is our lack of knowledge of the biochemical interaction between drugs and living processes, and of the processes themselves, that is the chief obstacle in a field where progress at the best must be slow and difficult.

As the Hinchliffe Committee points out, fundamental pharmacological research is costly. There are as yet no completely satisfactory theories of the action of drugs to provide a sure basis for predicting the therapeutic effects of new chemical compounds. No organic chemist can write down the formula of a new drug and guarantee that it will be a cure for cancer, high blood pressure, schizophrenia or any other disease. In practice, pharmacological research is essentially a matter of trial and error with only very occasional success.

It is also a collaborative effort in which not only the organic chemist but also the biochemist, the physical chemist, the pharmacologist, the bacteriologist, the pathologist and possibly also the endocrinologist, mycologist, botanist and parasitologist, as well as the pharmacist and medical practitioner may all have their place. It is a matter of team work in which the quality of the scientists is the decisive factor and in which the fullest advantage is derived only after several years of working together. As a corollary, it follows that research expenditure must be planned several years ahead, and besides this recurrent expenditure, considerable capital expenditure on plant and buildings may be required.

Nor is it only a matter of the time and effort put into the unpredictable laboratory investigations. Placing a new drug on the market for general therapeutic use always involves a big risk, quite apart from the fact that the new product may oust another product of the same firm which was previously considered the best for a particular purpose. All the time this may happen as a result of the introduction of new products by another firm and there can be few industries in which it is possible for a market to be lost as quickly as in the pharmaceutical industry. Apart from this the successful products have to carry the costs of the unsuccessful products, including the lengthy investigations that may have ended in clinical trials.

The Hinchliffe Committee made no attempt to estimate the efficiency of the research effort of the pharmaceutical industry, nor, in the absence of a much more detailed inquiry, did it recommend any changes in the organization of the industry. It recognized that competition is inherent in the system of private enterprise and, where it functions freely, should lead to the discovery of more effective drugs, and that with established drugs competition must in fact reduce the price. It made, moreover, no suggestion for co-operative research, at least in respect of the fundamental research for new products, and although at the conference on the pattern of research in British industry organized by the Federation of British Industries, in April, Mr. Aubrey Jones challenged the practice of secrecy in the competitive system as wasteful of effort and as a possible disservice to the economy, it is worth recalling that, in January, Dr. J. Y. Bogue told the Pharmaceutical Society that in 43 years not a single worthwhile drug had been discovered by the State-run industry of the U.S.S.R., and that in Austria, State-controlled medicine had inhibited the growth of the pharmaceutical industry.

Where, however, co-operation should be possible is in the clinical trials, which are time-consuming and expensive in skilled manpower. The Hinchliffe Committee expressed the opinion that clinical trials of new drugs and preparations should be organized by the professional bodies who should also be asked to take responsibility for interpreting the results of the trials. It did not indeed suggest that those firms who arrange for such trials themselves should discontinue to do so; on the contrary, they should be encouraged to continue. The Committee's object in recommending that professional bodies should be asked to consider the whole question of clinical trials was rather to provide some authoritative review of all the clinical data.

It is this aspect that has been forced on public attention during the past year or so, and it is well that the public should be aware of the care and effort which the industry is giving to the evaluation of drug toxicity and the arrangement of clinical trials. Since the symposium on the evaluation of drug toxicity which marked the opening of the new research laboratories of the Pharmaceuticals Division of Imperial Chemical Industries, Ltd., there has been a succession of discussions on this theme and the transfer of drugs from the animal laboratory to man before the Royal Society of Medicine, the Pharmaceutical Society and elsewhere. It has been emphasized, moreover, that there is at the moment no prospect whatever of setting up even a remotely relevant screening test for detecting the sensitizing potential in new drugs.

The limitation derives from the nebulous nature of our knowledge of the factors involved in human hypersensitivity already mentioned, and the designing of satisfactory animal tests must await the accumulation of a sufficient body of knowledge about the human conditions which they are intended to model. This in turn is a matter of inter-disciplinary co-operation, but it has also to be remembered that though such collaboration can reduce it will never entirely eliminate the risk when clinical trials are first undertaken.

Sound judgment as the Committee, set up under Lord Cohen of Birkenhead in August, remarked in an interim report published on November 6, can ensure that such risks are not undertaken unless the potential benefits are worth while. In any event, the total risk has to be set against the immeasurable benefits which mankind has gained from the introduction of new means for controlling and treating disease.

In the report just mentioned, the Cohen Committee rejects the idea that the responsibility for testing drugs should in the first place be transferred to a central authority as neither desirable nor practicable. It recommends that the responsibility for experimental laboratory testing of new drugs before they are used in clinical trials should continue to rest on the individual pharmaceutical manufacturer. It was satisfied that the industry as a whole discharges this responsibility effectively within the limits of contemporary knowledge of methods of testing, and it added that this knowledge was constantly being extended by research and that the Medical Research Council is intending to give advice and guidance on this subject from time to time.

The Minister of Health announced, on November 6, that he accepted these recommendations but that he would await further advice before deciding to adopt the third recommendation, to appoint a new expert body to assess the evidence before new drugs are used in clinical trials. Other steps have also been taken recently to improve co-operation and eliminate waste of effort in this field. The Medical Research Council has appointed a Committee, with Sir Charles Harington as chairman, to advise and assist the Council in promoting research into experimental methods of testing drugs and their possible toxicity. Last September a European Society for the Study of Toxicity of Drugs was formed, membership of which is open to all scientists interested, whether employed in industry, universities, Government or international organizations. In a sense, too, the grant of £250,000 which the Distillers Co., Ltd., recently made available to the University of Edinburgh for research into the incidence and causes of congenital abnormality illustrates the widespread implications of pharmacological research. The 1,500 graduate and 3,000 other workers employed in research and development in the industry itself draw also on the work of many others in biology, chemistry, medicine and other sciences in the universities and elsewhere.

It must not be forgotten that if, as the Hinchliffe Committee considered, the cost of clinical trials should be borne by the pharmaceutical manufacturers, this in turn must be reflected in the price of drugs. At the ninety-ninth British Pharmaceutical Conference last September, Mr. J. Hanbury, in his chairman's address, said that since the effects of thalidomide in unborn children were made known, British manufacturers were submitting many of the drugs they had produced during the past ten years to fresh tests to determine their effects on the fœtus. These tests would add several million pounds to the annual cost of research and increase the demand for scientific manpower, but, while Mr. Hanbury once again emphasized that even so complete safety could not be ensured, it seems necessary to stress not only the need for and extent of scientific collaboration in all such developments but also that all this research and development has to be financed.

At present it is only done out of the rewards which accrue from the relatively few successful discoveries, and here, as was pointed out at a scientific meeting of the Pharmaceutical Society last January, the Minister of Health has chosen to ignore or reject the conclusions of the Hinchliffe Committee. His action in invoking Section 46 of the Patents Act may do immense harm to the British industry at home and overseas. His offer to purchase drugs from foreign countries removes the incentive to research, the only result of which in the long run is an ultimate reduction in the research effort and in the number of new drugs available for the home and overseas markets. Payment of a royalty to the true owner of the patent, as offered by the Minister, is manifestly inadequate in view of the published experience of the National Research Development Corporation and the magnitude of the research expenditure of the industry already indicated.

This unrealistic attitude of the Government is as disturbing as the widespread public ignorance of what is involved in the discovery and testing and manufacture of a new drug, which leads both to ill-informed criticism and needless alarm when some mishap occurs in clinical trials. It is a prime responsibility of that wide range of scientists whose work contributes to the continuous emergence of new drugs to do all in their power to dispel such ignorance, as well as to bring about a more realistic approach to the question by the Government. Nor is it less important that the opportunities which the industry affords for satisfying and creative work to the scientist himself should be duly appreciated by those taking up a scientific career. Indeed, fascinating as is the actual search for new drugs in itself, the work which goes into their trials up to, and including, their first use in clinical practice is no less rewarding and responsible. In the end the decision to recommend the release of a new product for trial on human patients is a matter of personal and professional judgment which demands all that the pathologist, the biologist or the medical practitioner can bring to bear. The industry carries no heavier responsibility than to see that those responsible for such judgments are of the highest possible professional competence; any Government whose policy hinders the maintenance of the highest standards here betrays its duty.

AGRICULTURE, FISHERIES AND FOOD

The Ministry of Agriculture, Fisheries and Food

By Sir John Winnifrith. (The New Whitehall Series No. 11.) Pp. 271. (London: George Allen and Unwin, Ltd.; New York: Oxford University Press, Inc., 1962.) 30*s*.

THIS book is primarily meant for those interested in public administration However, agriculture, fisheries and food attract attention from practically every branch of science, and mostly over the full range from fundamental research to simple applications to practice. Hence, readers of *Nature* will have special interest in the sections describing the scientific and technical activities, and the associated administrative procedures.

The book follows the general plan of the Series. The bulk of the text deals with the work of the Ministry; there are a compressed historical introduction and a concluding section describing the organization of headquarters, regional and local centres.

The story begins in 1793, when Sir John Sinclair and Arthur Young persuaded the Government to form an embryonic Board, which was passing rich on £3,000 a year, until they retired in 1820, when first the grant, and later the Board, lapsed. Then followed fifty years of leisurely Government tinkering with problems like land tenure and cattle diseases, until 1889, when the Board of Agriculture was formed It took over Fisheries from the Board of Trade in 1903, but parted with all its Scottish responsibilities to a newly formed Board there, in 1911. After the First World War the Board became a Ministry, through the curious procedural means of a private member's amendment to a Bill concerned mainly with other matters.

From 1930 onwards the work rapidly expanded: measures to cope with the slump in agricultural prices; import duties and quotas; marketing boards. The Second World War saw the immediate formation of a Ministry of Food, equipped with production plans, based on the experiences of 1914–18 and the inter-war years. The two Ministries achieved a remarkable and vital increase in home food production, both during and after the War The present net output is 60 per cent above pre-war. Methods have been revolutionized: the high degree of mechanization on the land and in farm buildings is now common knowledge, but the fact that two-thirds of the calves born in England and Wales are conceived through artificial insemination may be less widely known.

Ultimately the future of the Ministry of Food had to be decided. The possibilities were abolition, continuation, dispersion into existing Government departments, or complete absorption into the Ministry of Agriculture and Fisheries. The last of these was adopted after 'keen controversy'. Thus, the present-day Ministry, with its new unwieldy title, has grown from the £3,000 Sinclair–Young baby to a £278 million adult, and there is also a separately financed Scottish relative. But, of this large sum, nine-tenths is paid out in price guarantees, production grants and other subsidies.

A very summarized account is given of the sequence from 1910, when the Development Commission first made research grants under advice from the (then) Board of Agriculture, to the effectively independent present-day Agricultural Research Council, which dispenses yearly about £6,500,000 for fundamental research, mainly in the agriculture and veterinary sciences. Fishery research is still under the Ministry, and basic food research is mostly the concern of the Department of Scientific and Industrial Research, and various research associations. The Ministry now confines its attention to applied research, and the consequential control and advisory measures. It runs a number of laboratories, and a comprehensive National Agricultural Advisory Service. covering arable and animal husbandry. On the control side, special mention should be made of foot-and-mouth disease. The brief radio and Press announcements of an outbreak give no conception of the swift and intricate actions to contain it and to trace the precise origin of each fresh one. The account is aptly likened to thriller fiction and a Sherlock Holmes story—and, it happens to be true.

Sir John Winnifrith, in his preface, pays tribute to his colleagues' efforts in assembling the data. He has been well briefed, but the information has obviously been sifted and assembled through one mind. The result is a very readable and informative book, with occasional neat touches of humour to please the attentive reader. B. A. KEEN

PLANT SURVIVAL THROUGH THE RUSSIAN WINTER

Wintering of Plants

By I. M. Vasil'yev. Translated from the Russian by Royer and Roger, Inc. Editor of English translation: Jacob Levitt. Pp. xiv + 300. (Washington, D.C.: American Institute of Biological Sciences, 1961.) 9 dollars.

IN a country the size of the U.S.S.R., where even in the warmest latitudes temperatures may drop below −4° C, great importance is attached to research on wintering of plants. Unfortunately it has always been difficult for Western scientists to obtain more than a fragmentary record of this work since there has been no review of the subject translated into English. The American Institute of Biological Science has now provided such a review, originally published in the U.S.S.R. in 1956, which it has made the first of a series of translations of major Russian works in biology. The book aims to be of practical value, so it should have a wide appeal to those interested in either pure or applied botany.

The injurious effects and the causes of death due to cold, including chilling injury to half-hardy plants, are considered first. Apart from the inherent hardiness of the plants, the importance of good agronomic practice, both in land preparation and in subsequent husbandry, is emphasized. Snow is of major importance as an insulator from extremes of cold. As it is of limited value in protecting trees, various other methods of protection are described, ranging from the ingenious to the drastic.

The second and largest part of the book is devoted to the physiological aspects of wintering, and in it Vasil'yev describes many of his own experiments. The ability of a cell to survive at low temperatures rests on the adaptability of its protoplasm to the increasing cold at the onset of winter. The active changes involved in the protoplasm give it properties similar to those in young cells, and so hardening is considered to be in some ways comparable with rejuvenescence.

The vernalization requirement of winter cereals is discussed only in so far as it is associated with a growth form which has far greater survival in cold weather than the spring form. No mention is made of the bulk vernalization of grain prior to planting which was advocated by Lysenko, but has apparently never been used extensively in the U.S.S.R.

In the final part of the book breeding for winter hardiness is described using the principles evolved by Michurin. The normal methods of hybridization and selection are used, but the Russians also 'shatter' heredity by exposing plants to different environments and afterwards 'fix' heredity by 'training', which includes grafting to acquire permanently the desirable characteristics of the stock.

The book is clearly written, but the reproduction of the text figures is poor and there is no index. The text includes references to some three hundred original papers and the bibliography covers more than a thousand altogether. Two-thirds of these are by Russian authors, but since they have contributed more than anyone else to world knowledge on this subject this is to be expected, and the book remains a review of major importance in this otherwise rather neglected field.

R. G. Hurd

PROTEIN BIOSYNTHESIS

Protein Biosynthesis

A Symposium held at Wassenaar, 29 August–2 September, 1960, under the auspices of Unesco and the Council for International Organizations of Medical Sciences. Edited by R. J. C. Harris. Pp. xii + 409. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1961.) 95*s*.

THIS volume contains a collection of papers which were given at a conference held in The Netherlands in 1960. The discussions which followed the presentation of papers are also included. The twenty-seven original papers are accompanied by an introductory paper and concluding remarks given by Siekevitz and Chantrenne, respectively. Both these participants aimed in their thoughtful contributions at placing the subject of protein biosynthesis in a broad perspective; this redresses the balance of the book as a whole, which is affected by the inevitably specialized nature of many of the papers. The mechanism of protein biosynthesis in animals, plants and micro-organisms is considered. Many of the papers deal with the synthesis of proteins by various subcellular components. Most attention is paid to protein synthesis associated with ribosomes or microsome material. For example, McQuillen gives an account of the comprehensive studies carried out at the Carnegie Institution on the involvement of ribosomes in protein synthesis in *Escherichia coli*. Butler *et al.* describe work on *Bacillus megaterium* and consider the possible involvement in synthesis of amino-acids associated with lipid. The structure of ribosomes is considered in a number of papers as is the significance to be attached to the association of proteins such as ribonuclease or γ-globulin with ribosomes. There are also contributions on protein synthesis associated with other subcellular structures, such as mitochondria (Work *et al.*), myofibrils (Winnick and Winnick) and nuclei (in particular, Allfrey and Mirsky). An appreciable number of papers presents information on the role of 'soluble' or 'transfer' ribonucleic acid in protein synthesis, and some are concerned with its characterization. Several articles deal with anomalous nucleotides. Škoda gives an extensive list of such compounds and their biological properties. Gros and Naono describe the effect of fluorouracil on protein synthesis, and a brief mention is made of the concept of 'messenger' ribonucleic acid, which has received much attention since the conference in question was held. Many of the papers deal with general aspects of protein biosynthesis, but a number include consideration of more specialized aspects, such as the synthesis of serum albumin (Campbell), synthesis of globulins by plasma cell tumours (Askonas) and the effects of carcinogens on the function and fine structure of liver cells (Emmelot and Benedetti).

The general standard of the papers is high. Furthermore, the reader is supplied with a summary of most of the papers, the titles are given of most of the papers cited and there is an author index.

Despite the general excellence of the volume, the question does arise as to what useful purpose it can serve at the present time. There have been considerable advances in knowledge concerning the biosynthesis of proteins during the past two years, in particular, with respect to the parts played by the nucleic acids. The volume reviewed, therefore, cannot give an adequate overall picture of present knowledge. I consider, however, that it still retains some value for those who are actively concerned in this field of study.

J. L. Simkin

ANTIBIOTICS

Antimicrobial Agents Annual, 1960

Proceedings of the Conference on Antimicrobial Agents held in Washington, D.C., October 26–28, 1960. Edited by Peter Gray, Benjamin Tabenkin and S. G. Bradley. Pp. xviii + 670. (Washington, D.C.: Society for Industrial Microbiology, 1961. Distributed by Plenum Press, New York; and Heywood and Co., Ltd., London.) 8.50 dollars; 80*s*.

THIS volume containing some ninety papers submitted at the conference held in Washington at the end of October 1960 also records discussions of the papers and details of panel discussions on modes of action, clinical laboratory testing and on clinical problems. The wide coverage is indicated by the section headings: new products, laboratory studies, modes of action, clinical studies, antitumour agents, agricultural and non-medical uses. Inevitably there is some overlap of interests in these sections and it would be well for anyone interested in a particular section to scrutinize the titles in the others. On the whole, however, the editors have carried out a difficult task well.

Of the panel discussion, that on modes of action is less well documented than the others, is rather difficult to follow and leaves the impression that little advance has been reported. The papers and the discussion on clinical laboratory testing should be of interest to both clinicians and laboratory staff. It is clear from the discussion that a great deal of work remains to be done to bring some measure of standardization at national and international levels, and that too little attention is paid to size of inoculum in relation to the amount of antibiotics in sensitivity disks. There are some critical remarks about using disks as a quantitative measure of activity.

Several new antibiotics are described: 'Porfiromycin' (4 papers), 'Mikomycin' (2 papers) and the semi-synthetic penicillins (3 papers) appear to be the more interesting. Work on 'Porfiromycin' is given in detail but toxicity figures are lacking, an omission remarked on in the discussion; that on 'Mikomycin' gives a wealth of clinical results without much detail. The enzymatic cleavage and resynthesis of penicillins, some laboratory work on 'Methicillin' and on lower alkyl and haloalkyl penicillins will be of interest to workers in this field. Two papers deal with 'Cephalosporin *N*' and two with a new sulphonamide containing the isoxazolyl grouping.

In the clinical section of nineteen papers, five deal with the new penicillins, including the early American work on 'Methicillin', three with certain aspects of the use of 'Demethylchlortetracycline', the others with such matters as the action of antibiotics in the gastrointestinal tract, the clinical use of 'Vancomycin' and the treatment of such infections as Friedlander's pneumonia, pneumococcal and H. influenza type *B* meningitis, and ocular toxoplasmosis. The panel discussion is given over very largely to the treatment of the resistant staphylococcus.

The great interest in the antifungal agents is shown by some twelve papers on this subject and the need for more work is stressed.

Of the non-medical uses of antibiotics, the use of 'Tylosin' in chickens and turkeys to control infections in broilers and a paper summarizing non-medical uses of antibiotics in Great Britain with special reference to animal feeds, food preservatives and plant disease control are of interest.

This book will be necessary for most reference libraries, but its price will probably restrict its purchase by many individuals. JOHN FARQUHARSON

ANALYTICAL CHEMISTRY

Comprehensive Analytical Chemistry

Edited by Prof. Cecil L. Wilson and David W. Wilson. Vol. 1*C*: Classical Analysis: Gravimetric and Titrimetric Determination of the Elements. Pp. xxx + 728. (Amsterdam and London: Elsevier Publishing Company, 1962.) 140*s*.

THE publication of this book, part *C* of the first volume of *Comprehensive Analytical Chemistry*, has been awaited with considerable interest. Parts *A* and *B* have described the general techniques of classical analysis, and in this present book their application to individual chemical elements is further detailed. Previous parts of this series have attained a high standard of excellence, and if this present book is not quite of the quality of its precursors it is mainly because no single volume can possibly cover the wide range of problems associated with the classical analysis of the chemical elements.

The plan of this book is rational; each element is treated separately and a periodic system of classification is adopted which permits easy access to material. No space has been found in this present work for systematic schemes of analysis which form an integral part of classical gravimetric analysis, but it is to be hoped that this omission is to be remedied in a future volume.

The editors' intention of providing a working manual as well as a reference work has been met in many sections by some excellent and authoritative contributions. Their authors describe, giving full practical instructions, all stages of a chemical analysis from the pre-treatment and solution of the starting materials to the separation of the interfering elements, leading to the consequent determination of an element by a preferred method. This treatment is supplemented by ample references to the literature. However, not all contributors have adopted such a useful approach and give us instead a general treatment, in the main confining their attention to the actual chemical determinations, which are often only a small part of an analyst's task.

As befits a book of this type, the binding is stout, and the typography clear; this should be an important addition to an analyst's bookshelf. A. D. WILSON

REVIEW OF INFECTIOUS DISEASES

Natural History of Infectious Diseases

By Sir MacFarlane Burnet. Pp. x + 377. (Cambridge, The University Press, 1962, third edition.) 30*s*.

IT is a pleasure to review this, the third edition, of such a well-known work, which now presents an up-to-date account of the ramifications of an important subject, and as such it is welcome to doctors of medicine as well as to students of applied biology alike.

Throughout it adopts a Darwinian attitude, often overstepping the realms of human pathology into all kinds of unexpected avenues, as the result of wide knowledge and extensive reading. In its ambit it presents a picture of the ravages by bacteria, protozoa and viruses. Much of the information under the last-named heading is culled from the author's own original investigations. The philosophical considerations, as regards the origins of these viruses and their relationship to abstruse biochemistry, and even to the creation of life itself, are unique in their clear and delicious presentation. The processes of defence against disease are somewhat gingerly handled. because what was once considered as a simple explanation has now become an involved, intricate and contentious affair till we find ourselves in a globulin-infested land guided by the light of paper electrophoresis. Under mass prophylaxis, on which subject the author uses wise words, he discredits the practical value of antityphoid inoculation and would rather attribute the favourable results obtained to military sanitation.

As an introduction to the study of the zoonoses the student could do worse than read the condensed account of what is pure tropical medicine. Moreover, the reader will find here important information about such diverse subjects as myxomatosis in rabbits, the common cold, plague, German measles, poliomyelitis, the sweating sickness of the Middle Ages and Q. fever (from personal experience). The latter part of the book deals in detail with some important infectious diseases.

An epilogue on new diseases and the rather bleak outlook for the future finds the author in a gloomy mood in an appraisal of bacteriological warfare: but he is more optimistic about the eradication of tuberculosis, which he believes will be complete in due season.

The only criticism which can be offered is on a certain amount of repetition, which is possibly unavoidable in a book of this nature.

PHILIP MANSON-BAHR

THIRD INTERNATIONAL CONFERENCE ON PRECISION ELECTROMAGNETIC MEASUREMENTS

By W. D. GEORGE

National Bureau of Standards, Boulder, Colorado

THE third International Conference on Precision Electromagnetic Measurements was held during August 14–17 at the Boulder Laboratories of the National Bureau of Standards, Boulder, Colorado. Sponsored by the Radio Standards Laboratory of the National Bureau of Standards, the Professional Group on Instrumentation, Institute of Radio Engineers and the Instrumentation Division, American Institute of Electrical Engineers, the conference was concerned with the advancement of standards and accurate measurement throughout the coherent frequency spectrum. Fifty-five papers were presented in 10 sessions. There were technical discussions during an informal evening session, and the work of the Radio Standards Laboratory was demonstrated.

Dr. Frederick W. Brown, director of the Boulder Laboratories, welcomed some 560 members from Australia, Belgium, Canada, England, France, Japan, New Zealand, Sweden, Switzerland and the United States, and gave background information on the activities of the National Bureau of Standards. Dr. John M. Richardson, chief of the Radio Standards Laboratory and chairman of the Conference, spoke on the Conference's organization and objectives, pointing out that the subject-matter of high-level precision and accuracy throughout the entire coherent frequency spectrum involves the national standards laboratories of the world and other laboratories and organizations in contact with them. Also, a closer relationship between the standards laboratories and those who are making the discoveries which lead to improved accuracy and precision in measurement seems to be required.

The technical programme, under the chairmanship of Dr. George Birnbaum (Atomics International), included papers about, and related to, accurate measurement in determining: (1) low-frequency electrical quantities; (2) high-frequency and microwave quantities; (3) properties of materials; (4) applications of quantum electronics to precision measurement, for example, frequency, time and the velocity of light; (5) use of statistics as an aid to measurement; (6) the precision measurement needs of space technology. About 20 per cent of the papers were from outside the United States.

Dr. Robert D. Huntoon, deputy director, National Bureau of Standards, set the theme of the Conference as co-operation in focusing on precision electromagnetic measurements at all coherent frequencies and in pushing for improvement in the state of the art. In his address, he told of how the Conference work is related to the mission of national standards laboratories, and of the need for very close collaboration between scientists and laboratories in keeping on a common basis as closely as possible in standards, and in keeping on a common basis in precision terminology. It seems that each time a new measurement idea comes about it is duplicated, improved and incorporated in a standard which is returned for calibration. The problem is to keep the standards laboratories ahead of measurement requirements.

Session I: Space Electronics

The sessions commenced with five papers on space electronics, relating to space explorations, and in keeping with the world-wide emphasis now placed on this subject. The chairman, Prof. Samuel Silver, University of California, referred to the objectives previously given, namely, a reporting of the precision measurement problems in space.

W. E. Brown, jun. (Jet Propulsion Laboratory), dealt with "Measurement Problems of Space Vehicle Experiments". He pointed out that the need for conducting remote measurements by use of experimental sensors on space-vehicles, in an environment where there is often a large uncertainty in the parameter to be measured, establishes a new area of endeavour for the research worker. The conditions peculiar to this problem are in many instances vastly different from those normally encountered in laboratory research, and are related to the over-all spacecraft design requirements. These new conditions attach a new significance to calibration philosophy, sampling-rates, conversion accuracies and reliability. Furthermore, the ground data handling problem of extracting information from the large quantities of data is extremely severe and usually requires careful planning by the experimenters several months prior to the actual measurement. The paper surveyed the present and future aspects of acquiring accurate and intelligible information from the spacecraft sensors by the utilization of techniques which tend to be more compatible with the spacecraft design requirements.

D. B. Holdridge (Jet Propulsion Laboratory) described the computations and "Error Sources in the Determination of the Astronomical Unit from Venus Radar Observations". Radar reflexions from Venus when it was near the 1961 inferior conjunction were detected using continuous-wave techniques (2,388 Mc/s) in the Doppler and time-of-flight data systems. The data were compared with theoretical values obtainable by use of the available planetary position–velocity ephemerides and yielded a value of 149,498,845 ± 250 km for the astronomical unit (A.U.), or a solar parallax of 8″.7,940,976 ± 147 based on an Earth radius of 6,378,145 m.

Computations of the A.U. were made separately from the 'Newcomb ephemerides' and the 'Duncombe-corrected ephemerides'. Reduction of the Doppler data disclosed a small residual error in the difference between the heliocentric longitudes of the Earth and Venus in Duncombe's corrections to Newcomb's theory.

D. D. McKibbin (Lockheed Missiles and Space Co.) reported results from an ion chamber carried on about 100 orbits through the $F2$ region of the ionosphere. The measurements were made from 73° N. to 73° S. over altitudes of about 210–510 km. Kinetic ion temperatures were found to be 1,500° K and higher. Ion concentrations varied from 5×10^3 to 10^6 particles/cm^3. Fluctuations in the ion chamber values, especially in the southern auroral zone, indicated the presence of large ionization irregularities.

T. J. Crowther (Lockheed Missiles and Space Co.) gave a paper on "Automatic Range Switching of Instrumentation in Space Vehicles for Increased Accuracy". He described a device for making accurate measurements of electronic signals from a space-vehicle using automatic range switching. The device controls an instrument which has many operating ranges or scales and automatically selects the proper scale. The readings are then telemetered back to Earth on two channels, one to indicate which scale is in operation and the second to give the actual reading within the scale. Results from an actual application were given where accuracies of 5–15 per cent were obtained over a dynamic range of 10^4.

E. M. Bollin (Jet Propulsion Laboratory) discussed "Lunar Surface and Sub-surface Magnetic Susceptibility Instrumentation". His paper is a description of the development and the changes necessary in the instrumentation and transducer configuration to adapt a standard field method of magnetic susceptibility measurement to the special restrictions of space-craft electronics, transmission systems, measurement environment, and the restrictions imposed by sample geometry and size.

One of the major considerations is to get accurate information to the scientific community concerning the specific need for these rather specialized measurements. Accurate instrumentation was not previously developed as there was no specific need and no easy way of overcoming difficulties inherent in the measurement.

Session II: Direct Current and Low-Frequency Measurements

Five papers in this basic area of precision electromagnetic measurements were presented under the chairmanship of Chester Peterson (National Bureau of Standards).

G. A. Candela and R. E. Mundy (both at the National Bureau of Standards) described the measurement of "Absolute Magnetic Susceptibilities by the Gouy and the Thorpe–Senftle Methods". Dr. Candela pointed out that most magnetic susceptibility measurements in the past were made by relative methods. These depend on knowing the susceptibility of a reference substance; however, there are very few substances the susceptibility of which is known to better than about 1 per cent. To-day the measurements can be made easily and on an absolute basis on small samples. This results largely from the developments described and from measuring magnetic fields with a proton resonance magnetometer. It was suggested that laboratories equipped with the Gouy and Faraday apparatus should use the absolute measurement methods.

J. J. Hill (National Physical Laboratory, Great Britain) spoke on "The Design and Performance of High Precision Audio Frequency Current Transformers". The principal factor governing the performance of current transformers at high audio-frequencies is the magnitude of the product value of total leakage inductance and total interwinding capacitance. When the equations governing these quantities are examined it is found that there is an optimum thickness for the insulation space between the primary and secondary windings. Further improvement is obtained if the winding resistances, leakage inductances, and capacitances are all symmetrically and uniformly distributed. It is suggested that these conditions can best and most easily be realized by the use of single layer primary and secondary windings on a toroidal core.

The National Physical Laboratory has constructed transformers on this design to measure currents up to 400 amperes in the frequency band 400 c/s to 20 kc/s. The maximum ratio error at any load throughout this frequency-range is 17 parts in 10^6 and the maximum phase error is 50 μradians.

The discussion brought out the need for international agreement on how transformers are tested and the manner in which errors are to be specified.

P. L. Richman (Rotek Instrument Corporation) described "A New Peak to d.-c. Comparator for Audio Frequencies", an adjustment-free transfer technique for making precision comparisons between audio-frequency a.c. voltages and a reference d.c. Limitations and errors were estimated at 50 parts in 10^6 for the worst case in the over-all a.-c.–d.-c. transfer. Experimental verification for many of the error estimates was described, and experimental over-all comparison against thermal calibration and against a modification of the Smith–Clothier calibration technique was given. Agreement in the latter case was of the order of 30 parts in 10^6, indicating good correspondence with calculated error predictions. The comparator was usefully employed at frequencies up to 100 kc/s.

Dr. William A. Geyger (U.S. Naval Ordnance Laboratory) discussed "Low-Power-Operated d.-c. Instrument Transformers". Recent research work has been conducted toward the development of low-power-operated direct-current types of instrument transformers. The term, "d.-c. instrument transformer", has gained acceptance as a general name for those types of saturable-core devices which permit the measurement of direct currents and low-frequency currents, with a high degree of accuracy, without the use of power-consuming shunt resistors.

Dr. Geyger's paper describes a miniaturized d.-c. instrument transformer which is 60 c/s-operated from a correspondingly miniaturized switching-transistor magnetic-core multivibrator. In a special application to the measurement of currents derived from solar batteries, this low-power-operated d.-c. instrument transformer (the power drain on the 6-V battery is about 5 mW) eliminates the considerable power dissipation (up to 5 W) in a 5-ohm shunt resistor carrying the direct current to be measured. The device is also quite valuable for other applications, where small size, light weight, and an extremely low-power drain are of prime importance.

R. P. McKnight (Sandia Corporation) discussed the development and performance of "A Direct Reading Voltage Divider with Standard Cell Reference", in which the output voltage is equal to that of a standard cell when the input resistance has been adjusted so that 1 m.amp of current is flowing through the divider. The range of measurement with direct reference to a standard cell is 2–1,100 V. The range may be lowered to 0·02 V with decreased accuracy by the use of a Lindeck potentiometer in place of the standard cell. Results indicate that accuracies of approximately 0·005 per cent may be obtained from 2 V to 1,100 V without applying corrections.

Session III : Data Reduction in Precision Measurements

This session of four papers was mainly devoted to professional advice on the statistical analysis of data.

The first paper, "Uncertainties in Calibration", by W. J. Youden (National Bureau of Standards), also chairman of the Session, presented striking examples of research values which were improperly given in the literature. He spoke of incorrect simplifying assumptions, for example, treating systematic errors as though they were random. He then described efficient methods of clearly revealing systematic errors. General rules were given: we must improve the meaningfulness of limits set on measurement results, we need to know more about our random and systematic errors, we need to recognize that in many cases the available knowledge to form an estimate of errors is not reliable information, we must not jump the elementary steps, and we must be sure of what the precision errors are.

A. G. McNish (National Bureau of Standards), with a paper entitled "The Speed of Light", complemented the foregoing remarks. He summarized the present state of our knowledge regarding this important physical constant, and in doing so explained methods and techniques of analysis applicable to practically all well-controlled experiments. The concluding value after analysis of the numerous independent or semi-independent measurements was given as 299,792.50 ± 0·10 km/s.

The discussion brought out that there is no evidence either theoretical or experimental that the speed of light changes with frequency or with epoch.

The third paper, "Automatic d.-c. Data Logging System", was given by William Arnett (COHU Electronics, Inc.). He described a system which digitizes d.-c. voltages and records the values on punched cards for subsequent machine data reduction. The system has high precision, a few parts in 10^6, which is new in this field. Statistical analysis of the calibration data and information on stable voltage sources show the probable error to be about one part in 10^6. The speed of measurement, about 100 points in 6 min, and automatic self-calibration are also unusual with this type of system.

The last paper of this session, "Variance of Radio Frequency Caused by Atmospheric Turbulence in Line-of-Sight Transmissions", by K. A. Norton, E. C. Barrows, M. C. Thompson, jun., and H. B. Janes (all at the National Bureau of Standards), was given by Mr. Janes. This paper deals with the errors contributed by the turbulent lower atmosphere to standard radio frequencies transmitted over line-of-sight paths. Some measurements of these errors are discussed in terms of their effect on frequency and time-interval estimation. They are also compared quantitatively with the error contributed by transmitting oscillator instability.

The paper establishes the fact that, at the present state of the art, transmission of standard frequencies over a line-of-sight propagation path through the troposphere will not appreciably further deteriorate their accuracy relative to that available before this transmission.

Session IV : Properties of Materials

Recent developments in solid-state electronics and the increasing need for compact, light electronic devices with minimum power consumption for service in space-craft have focused scientific interest on the electromagnetic properties of materials. The examination of such properties requires the development of precise and convenient methods of measurement such as those explored by this conference. Prof. A. A. Oliner (Polytechnic Institute of Brooklyn) served as chairman and introduced the speakers.

Prof. L. M. Silber (Polytechnic Institute of Brooklyn) began the session with a paper prepared in co-operation with Prof. H. J. Juretschke (also of that Institute): "DC Effects Accompanying Ferromagnetic Resonance in Thin Films". The technique described permits the simultaneous measurement of several properties of a thin film: electrical conductivity, extraordinary Hall coefficient, magneto resistance anisotropy, permeability tensor and gyromagnetic ratio. Since all these properties can now be measured for a single film, their relationships can now be studied without the uncertainties of earlier methods which required the preparation of several identical films.

The second paper, "Microwave Faraday Rotation Using a Bimodal Cavity", by Dr. D. P. Snowden (General Atomic, San Diego, California), described the use of a TE_{111} bimodal cavity which is 1,000 times as sensitive as the direct method for measuring microwave Faraday rotation. Dr. Snowden pointed out that the same cavity can be used to measure the conductivity tensor or the susceptibility tensor of a material. During the discussion, Mr. H. E. Bussey (National Bureau of Standards) indicated that a bimodal TM_{110} cavity can be used in a similar way to measure the susceptibility tensor of a material with the added advantage that an exact mathematical analysis of the TM_{110} measurement is available.

Mr. A. D. Krall (U.S. Naval Ordnance Laboratory, Silver Spring, Maryland) spoke on "The Microwave Measurement of Narrow Line Width Ferromagnets by Radiated Fields". Mr. Krall showed that by placing a pick-up loop in the field radiated from a resonant sample the ferromagnetic line-width can be measured quickly, conveniently and with simple equipment. For narrow line-widths this method gives an accuracy of ± 0·02 œrsted. The first order anisotropy coefficient of the material can also be measured by this technique.

The final paper of session four was, "Measurement and Standardization of Dielectric Samples", by Mr. H. E. Bussey and Mr. J. E. Gray (both at the National Bureau of Standards, Boulder Laboratories). The introduction indicated that the art of measuring dielectric properties at microwave frequencies is maturing as is evidenced by the close agreement of different methods of measurement. Consequently, it now seems appropriate for the National Bureau of Standards to issue standard dielectric samples with specified dielectric constant and loss tangent. Mr. Bussey explained that accurate microwave dielectric constant measurements are made by correcting for the effects of all deviations from the ideal or perfect resonator or line. When this is done accuracies of 0·3 per cent for dielectric constant measurements and 5 per cent for loss tangent measurements become possible.

On August 13, the day preceding the Conference, an important meeting was held on high-temperature dielectric measurements. Attendance included representatives of about ten laboratories active in the field. Present-day methods were examined. A sample standardized to about 1,500° C is desired. To partially satisfy this need a round-robin comparison among interested parties will be conducted immediately.

Session V: Quantum Electronics in Precision Measurements

Seven advanced papers, under the chairmanship of Dr. R. C. Mockler (National Bureau of Standards), treated present and future possibilities of quantum electronics in precision measurements.

N. Bloembergen (Harvard University) discussed the new phenomena of non-linear optics which has become accessible to experiment by the advent of optical masers. The coherent nature of these new sources of light makes possible extremely high electro-magnetic fields within crystals which in turn reveal the non-linear behaviour of the medium. Maxwell's equations have been applied to this situation to obtain new laws of reflexion and refraction. The result is a theoretical basis for optical mixing, harmonic generation, and modulation with high intensity light beams.

A. Javan (Massachusetts Institute of Technology) presented evidence that the continuous-wave gas maser may achieve high reproducibility with the improvement of the mechanical resonator. He cited 3–4 sec stability of 1 part in 10^{13} and a reproducibility of 1 part in 10^{9} at present with a helium neon laser.

A. L. Bloom (Spectra Physics, Inc.) explained the use of optically pumped rubidium vapour as a precision magnetometer. This device is better known as a precision frequency source for atomic clocks. The sensitivity of the rubidium transition to magnetic field allowed a magnetometer to be constructed with a sensitivity of 10^{-8} gauss and a dynamic range of 10^{5}. Fast response and absolute accuracy were also claimed for the instrument.

J. P. Gordon (Bell Telephone Laboratories, Inc.) outlined the limitations of information-rate in communication channels due to quantum noise effects. The optimum signal should have the same statistical form as the noise. The differences between an amplitude sensitive receiver and a power sensitive receiver were explained. Each had advantages in some situations.

A. Ganssen (National Co., Inc.) discussed the advantages of a proton resonance oscillator in which the working substance could be water. This has advantages of room temperature operation and low-frequencies from -2 kc/s to 100 Mc/s. The disadvantage is the external magnetic field of high stability required. High spectral purity has been obtained along with stability of a few parts in 10^{10}.

Y. Beers and G. Strine (National Bureau of Standards) explained the use of the microwave Stark effect to measure high voltages to great precision. The instrument which was constructed consisted of a parallel plate microwave resonator of the Fabry–Perot type in which the Stark splitting of a molecular transition could be accurately measured. If the dipole moment of the molecule is known, the voltage across the parallel plates can be calculated with great accuracy. The instrument can also serve to determine the dipole moment if the voltage is known.

R. C. Mockler (National Bureau of Standards) outlined a proposed experiment to measure the velocity of light (γ-rays) using the Mossbauer effect. The source and detector are vibrated at high frequency in synchronism and the distance between them is varied to observe maxima in γ-ray absorption. This is a very difficult experiment but a measurement to a few parts in 10^{8} is expected to be possible.

Session VI: Atomic Frequency and Time (Hydrogen, Hydrogen Cyanide, Other Molecules)

This session with four papers and Dr. John T. Henderson (National Research Council) as chairman commenced the third day of the conference, a full day allotted to frequency and time.

A report on theoretical and physical properties of "The Atomic Hydrogen Maser", N. F. Ramsey and H. E. Peterson (Harvard University), was presented by Prof. Ramsey. He discussed effects of cavity pulling, wall coating of the hydrogen container within the cavity, magnetic fields, etc., which leads to shifts in the maser output frequency. From measurements made within the past week, the frequency difference between two independently constructed and independently adjusted machines was about 1 part in 10^{12}. A better agreement can be expected. In answer to inquiries he reported that instability in the electronic measuring apparatus (phase comparator) appears to be well under control, and is no longer a significant factor in evaluating the overall instability. The technique of looking at several nearby field-dependent transitions was described as the means of measuring the magnetic field actually seen by the atom in the maser itself.

The hyperfine frequency of atomic hydrogen, previously published as 1, 420, 405, 762. 415 ± 4·000 c/s (with reference to an atomichron), appears now to be known to about ± 0·030 c/s. Prof. Ramsey anticipates a hard push in obtaining a value known to 1 part in 10^{13}; however, he is confident that such can be obtained.

R. F. C. Vessot and H. E. Peters (Bomac Laboratories, Inc.) summarized their work on "Design and Performance of an Atomic Hydrogen Maser". Two objectives were mentioned: (1) design of a compact unit; (2) fundamental investigations on maser theory and performance.

Dr. Vessot reported details on one completely sealed off hydrogen maser, using 'vac ion' pumps, which has operated more than two months and is expected to operate two or more years. It may be likened to a tube with a large capacity getter and could be designed for 20 years performance. With no special precautions, beyond an air-conditioned laboratory, the instability relative to a second hydrogen maser was about six parts in 10^{13} in 1 min and five parts in 10^{12} in 10 h. It was pointed out that as the work progresses a fundamental importance will be given to measurement of the ratios of frequency of masers and other devices operating with different atoms such as cæsium, hydrogen and rubidium.

Dr. D. Marcus (Bell Telephone Laboratories, Inc.) described his "Hydrogen Cyanide Molecular Beam-Type Maser". It is a close relative to the ammonia maser, the first maser ever operated successfully. However, it differs from the ammonia maser in two important points. (1) It uses a different transition. While the ammonia maser uses the inversion of the nitrogen atom perpendicular to the plane defined by the three hydrogen atoms, the hydrogen cyanide maser uses the rotational motion of the molecule as a whole. This is the first successfully operated maser using a rotational transition. (2) The frequency of operation of the hydrogen cyanide maser is more than three times as high as that of the ammonia maser. This maser oscillates at 88·63 Gc/s corresponding to a wave-length in vacuum of 3·4 mm.

The design of this maser is interesting in that it used a great number of molecular beams which emerge from a circular source and converge on the resonant cavity which is located at the centre of the circle. A system of electrically charged rods arranged in two planes below and above the molecular beams removes the lower state molecules from the beams, thus achieving population inversion.

The resonant cavity is of the confocal interferometer type which is at present used in many optical masers.

This maser can be used as a precision frequency standard in much the same way as the ammonia maser.

A study of the possibilities of an electric resonance molecular beam frequency standard, "Choice of a Molecule for a Beam Frequency Standard", was presented by Dr. F. S. Barnes (University of Colorado). The devices described are analogous to the cæsium beam machines except that they operate with electric fields and electric dipole moments instead of magnetic fields and magnetic dipole moments.

Such devices might give nearly exact reproducibility except for: (1) uncertainty in field measurements in the *C*-field region; (2) uncertainties due to residual magnetic fields and a small magnetic dipole moment of many of the molecules which may be chosen; (3) measurement errors inherent in all beam type devices resulting from the finite '*Q*' and electrical noise; (4) shifts in the resonance from nearby molecular transitions; (5) unequal phase in the two cavities required.

Three kinds of detectors were investigated: hot wire, electron bombardment devices, and the Pirani gauge using semiconductive films.

The various sources of instability are hard to evaluate and a device should be built and operated to be sure. A stability of one part in 10^{13} can be expected.

Session VII : Atomic Frequency and Time (Ammonia)

Dr. Henderson continued as chairman and introduced a series of three papers which showed that precision and accuracy are still being improved for ammonia masers. In a paper "Ammonia Masers", K. Shimoda (University of Tokyo) discussed the effects of cavity pulling, longitudinal and transverse Doppler effects, the pressure shift, the dispersion of nearby lines, the effect of unresolved hyperfine structure, and the effects of magnetic field.

Also from Tokyo, Y. Saburi presented a paper "Characteristics of the 3–2 Line Maser of $^{14}NH_3$ and the Precision of Frequency Comparison", by Y. Saburi, M. Kobayashi, and Y. Yasuda (Ministry of Posts and Telecommunication). They considered the effects of changes in experimental parameters such as ammonia pressure, focuser voltage and magnetic field, as well as possible fluctuations from the associated quartz oscillator and frequency multipliers.

The third paper, "$^{15}NH_3$ Double Beam Masers as a Primary Frequency Standard", was given by J. De Prins (University of Brussels). He discussed experiments where the symmetry of double beam masers was made use of to reduce the frequency sensitivity to experimental parameters. From these papers, numbers of the order of 10^{-9} were suggested for the accuracy and 10^{-11} for the resettability of single beam masers and an order of magnitude better for double beam masers.

In 1957, Prof. P. Kusch proposed that a thallium hyperfine structure line may yield a more precise standard of frequency than the use of the cæsium line. The first reported operation of a thallium standard was given at this conference by Dr. Jacques Bonanomi (Neuchâtel Observatory). In his paper, "Design and Performance of a Thallium Frequency Standard", the construction differs from cæsium standards in the following main points: a high (1,000° K) oven temperature, high (14,000 gauss) *A* and *B* fields, and an oxidized tungsten filament detector. In his preliminary attempt, an accuracy was attained which is already comparable to the best accuracy of other kinds of atomic frequency standards.

Dr. Bonanomi presented possibly the most interesting results of the Conference. He showed that the construction problems for a thallium beam machine are not insurmountable, and that there are good reasons to believe that the advantages of a higher frequency and smaller magnetic field sensitivity as compared with cæsium can be realized. Measurements were reported where the whole correction for the magnetic field *C*-field was about 2 parts in 10^{12}. With reference to the unit of time in which the cæsium frequency is 9, 192, 631, 770 c/s, the thallium line frequency was reported as 21, 310, 833, 945·1 c/s ± 1·0 c/s.

Session VIII : Atomic Frequency and Time (Rubidium, Cæsium and Other Matters)

This afternoon session under the chairmanship of Dr. L. Essen (National Physical Laboratory, Great Britain) continued the day's discussion on frequency and time.

A paper by M. E. Packard (Varian Associates), "The Optically Pumped Rubidium Vapour Frequency Standard", reported recent work on gas cell frequency standards and the physics of the quantum mechanical system. Carefully engineered systems have been demonstrated to have high stability with high reliability and small weight and volume.

Frequency stability, which is the important quality of a standard, has been studied over a wide range of averaging and measurement times. A plot of standard deviation of frequency stability shows a broad minimum of $2{\cdot}5 \times 10^{-12}$ for a run of 10 h (24 min averaging time for each measurement); 2×10^{-11} for a run of 10 min (1/4 sec averaging time); and $4{\cdot}5 \times 10^{-11}$ for a run of 160 days.

P. Kartaschoff (Swiss Laboratory for Horological Research) reported results on "Operations and Improvement of a Cæsium Beam Standard Having 4 Meters Interaction Length". By extending the length of an atomic beam frequency standard, the line width is reduced, allowing greater precision of measurement if the signal to noise ratio remains sufficiently high and if other length dependent errors such as *C*-field uncertainty and phase shift are not limiting. He found it possible by using demagnetizable Armco iron shields to reduce errors due to *C*-field uncertainty to $1{\cdot}2 \times 10^{-12}$. Cavity phase shift effects were below $1{\cdot}5 \times 10^{-11}$ and his accuracy was stated to be $\pm 3 \times 10^{-11}$. Two years of comparisons by VLF Standard Frequency Broadcasts showed agreement to about $\pm 1 \times 10^{-10}$ with the United States Frequency Standard.

Manual measurements of cæsium beam resonators are more straightforward and involve less equipment than servo methods, but are also more time-consuming and more dependent on the technique of the individual operator. Servo measurements, however, are potentially affected by many more parameters.

R. E. Beehler, W. R. Atkinson, L. E. Heim, and C. S. Snider (all at the National Bureau of Standards) presented "A Comparison of Direct and Servo Methods for Utilizing Cæsium Beam Resonators as Frequency Standards". This investigation showed that any inaccuracies added to the measurements due to the effects of their servo system parameters are less than several parts in 10^{12}. The precision of measurements has significantly increased to 2×10^{-12} for a 1/2 h average. The servo method is now used for measuring the U.S. Working Standards of Frequency on a regular basis.

William Markowitz (U.S. Naval Observatory), in a paper "The Atomic Time Scale", discussed the system of atomic time, *A*1, derived from the operation of cæsium-beam resonators located in nine laboratories about the world. A re-determination of the cæsium quantum transition frequency, found to be 9,192,631,770 c/s of ephemeris time in 1958, was made, using observations of the Moon through July 1962. This resulted in the same frequency but with the error of observation reduced from ± 20 c/s to ±10 c/s.

The Bell Telephone Laboratories have a programme for improving quartz crystal oscillators. A report on their 2·5 Mc/s and 5 Mc/s oscillators used for standard frequency and time control, "Performance of Precision Quartz Crystal Controlled Frequency Generators", by R. A. Sykes, W. L. Smith and W. J. Spencer was given by Mr. Sykes. Recent improvements in oscillator, oven and crystal unit design have produced 5-Mc/s oscillators with drift rate less than 1×10^{-10} a day, while the 2·5-Mc/s oscillator drift-rate is less than 1×10^{-11} a day.

The short-time stability (10 msec) of both types of units when measured in the Gc/s range appears to be of the order of a few parts in 10^{10}.

Addition of a getter in the quartz crystal unit appeared to have a marked effect in reducing frequency shifts caused by oven and oscillator shut-off.

H. F. Hastings (U.S. Naval Research Laboratory) and P. B. King (Aircraft Radio Corporation), in a paper "Technique for Precise Measurement of Short- and Long-term Stability of Oscillators", discussed improvements in the error multiplication system of frequency measurement. In this system the unknown frequency and standard frequency, nominally the same, are multiplied by different factors, in this case 9 and 10. The resulting beat frequency is again multiplied, the resulting process of error multiplications being repeated until sufficient precision is attained.

In the last paper of this session it was shown that there are certain advantages in using relatively short cæsium beams for frequency standards, among them less difficulty in obtaining a uniform *C*-field over a short region and higher beam intensity, although the cæsium line width is made larger by the short interaction time. The performance of such a beam tube was reported by James George (National Company, Inc.) in his paper "Recent Advances in Cæsium Beam Technology and Characteristics of Rabi and Ramsey Cæsium Beam Tubes 17 Inches in Length". The tube uses a new hot wire ionizer relatively free of potassium with a response of 0–500 c/s. The line width is 1,000 c/s with only 1·5 μW necessary to excite the transition using a single Rabi type cavity. The standard deviation from the controlled output frequency of an 'Atomichron' using the 17-in. beam tubes (Rabi and Ramsey types) was a few parts in 10^{11} and in 10^{12} respectively. This development now permits the realization of 'Atomichrons' small in size, weight and power consumption for use in highly portable systems.

Unit of Time and Velocity of Light

Prof. N. F. Ramsey served as chairman of the informal evening session at which two subjects were discussed: "An Atomic Definition for the Unit of Time" and "Determination of the Velocity of Light".

William Atkinson (National Bureau of Standards) presented results which were of importance to the problem of keeping atomic time scales and to time and frequency comparisons by very-low-frequency broadcasts. He discussed how clocks driven by quartz oscillators would be subject to fluctuations which depend on the spectra of the phase fluctuations of the oscillators, and gave results of measurements on typical oscillators which show an increasing amplitude of fluctuation as the fluctuation frequency decreases. He presented results of comparisons by means of *WWV* of the National Bureau atomic time-scale and the Naval Observatory *A*1 atomic time-scale. He also pointed out that caution must be used in applying statistical treatment to data from very-low-frequency comparisons since his analysis showed that the data did not generally exhibit a gaussian distribution.

There was a considerable amount of discussion concerning what transition would be used for an atomic definition of the second as well as some of the problems in relating atomic time to astronomical time. The unit of time-interval, now identical with the second of Ephemeris Time, may be re-defined in 1966 in terms of an atomic transition by the General Conference of Weights and Measures. This would recognize formally the situation that now exists, that atomic clocks are used to provide the unit of time-interval where very high precision is required. However, the requirements for providing Universal Time along with the interval of atomic time will continue (Universal Time is a time-scale based on the rotation of the Earth). Prof. Ramsey, by way of summary, stated that in order that the Consultative Committee for the Definition of the Second may make a firm recommendation by 1966 to the International Bureau of Weights and Measures, the particular transition should be fairly well settled on in the next two years. Cæsium now appears to be a good possibility with a long head start, but there are still others in the running. The atomic transitions, thallium, cæsium and hydrogen, look a little more favourable than molecular transitions such as ammonia. In the meantime, astronomers are working hard to supply the best value for close agreement between the Ephemeris and Atomic Second.

When the Atomic Second is adopted it will very closely agree with the Ephemeris Second, but will be independent of the latter.

The second part of the evening discussion was devoted to experiments in progress, or proposed, for determining the velocity of light. The points were raised that such experiments should have as their aims an increase in the present accuracy of the value of *c* and a verification of the hypothesis that *c* is independent of frequency.

An experiment in progress at the National Bureau of Standards Boulder Laboratories and reported by Mr. R. C. Baird utilizes a micro-wave interferometer of the Michelson type to measure phase velocity of 50-Gc/s electromagnetic waves. The primary difference between this interferometer and earlier ones is the region of operation. The Bureau model operates in the Fresnel or near field region. This necessitates the use of a diffraction correction and this is calculated on the basis of a rigorous solution of Maxwell's equations. The accuracy of this determination will be about 3 or 4 parts per 10 million, which is equal to the best determination to date. One of the most useful results of this work is the analysis of the diffraction correction; another feature of value is that the experiment represents an independent determination at a different frequency and uses a different method from previous experiments.

Dr. Boyne of the National Bureau of Standards, Washington, analysed a novel method utilizing an optical maser as a source. The method would necessitate the measurements of the difference frequency of two gas laser lines of the helium–neon system. This frequency is about 1,600 Gc/s, and Dr. Boyne explained the use of a modified cathode ray-travelling wave tube using a photo cathode to measure this accurately.

Dr. Essen (National Physical Laboratory, Great Britain) commented on his experiments aimed at determining lengths in terms of frequencies, utilizing the velocity of light. He is of the opinion that their determination of c will be to a few parts in 10^8.

During this part of the discussion Mr. McNish made the point that present measurements of light speed are all less accurate than length and time-interval measurements. Hence attempts to define lengths in terms of a frequency standard and an assumed light speed would make length determinations more inaccurate than they ought to be in comparison with present techniques based on an independent length standard (wave-length of the krypton line). Dr. Ramsey stated also that since we have an *MLT* system, the value of the speed of light, although a theoretically invariant constant, must still be determined experimentally.

There was some discussion of the practical necessity for knowing the speed of light to a high accuracy, and Dr. Richardson expressed an interest in finding out just what are the present theoretical limitations, in concept, on the constancy of the light speed c. Dr. Shimoda noted that estimates of the limit of the deviation of the Coulomb law from the inverse square seem to yield a figure of 1 part in 10^{16} as an interesting region for investigation. Such a deviation would imply a non-zero photon rest mass and consequently a dispersion in the speed of light. Besides atomic effects of this kind, Richardson also had in mind a general relativistic limitation on our definition of standards.

Any co-ordinate systems, as a result of the curvature of space time predicted by general relativity theory, must also partake of this distortion. Hence, the co-ordinate speed of light will differ from point to point due to gravitating masses. However, the speed measured over short enough distances in a freely falling, that is, an inertial reference frame will always be the same invariant, c. A co-ordinate speed could differ, near the Earth by 1 or 2 parts in 10^9, from the co-ordinate value at a large distance from the Earth, or from the value measured in an inertial system. However, a suitable choice of the space and time co-ordinates can, of course, be made so that in any small enough region the co-ordinate speed and the proper light speed are equal. But this cannot be done everywhere simultaneously using only one co-ordinate system. Thus, methods of dissemination of time signals must take this limitation into account.

Such relativistic effects as this furnish us with additional reasons why an atomic standard for time, as well as one for length, should be adopted. Such standard units are definable over restricted regions of space and time in such a manner as to satisfy the conceptual requirements of relativity theory.

Session IX : Micro-wave Power and Attenuation

This session, presided over by K. Tomiyasu (General Electric Co.), had seven papers on microwave power and two on microwave attenuation. Each was concerned with precision measuring techniques and standards.

The session opened with an invited paper by Prof. H. M. Barlow (University College, London), who surveyed the instruments and techniques recently developed in Great Britain for measuring power. Some of these new measuring techniques are attractive in that they do not require absorption of the incident energy, as do ordinary bolometric techniques. The first device is an oscillating dipole, placed in a resonant cavity, and arranged with a fine suspension fibre and optical deflexion measuring system. Peak deflexion of the dipole in response to the torque that results when pulsed power is applied to the cavity can be related to the power-level.

Several of the difficulties inherent in present Hall-effect devices were discussed, and a new method which offers hope of eliminating some of these troubles was proposed. Finally, a new type of electrostatic torque wattmeter, developed by the speaker, was described. It offers the advantage of calibration in terms of mass, length and time, and is thought to be capable of correct power measurements regardless of the nature of the load.

S. Okamura (University of Tokyo) discussed the work being done in Japan on a device similar to the oscillating dipole type designed for use at 35 Gc/s. The basic unit has been refined by the addition of a special photoelectric deflexion-measuring system. Both transmission and cavity types of microwave wattmeters based on the magneto-resistance effect were also described.

Investigations into the use of Hall-effect devices to measure microwave power densities up to 1 W/cm^2 were reported in a paper by R. A. Leavenworth, J. Maczuk, and R. F. Schwartz (University of Pennsylvania). Their studies were primarily concerned with the use of such devices as broadband collectors and transducers of the microwave energy, operating without tuning over the range 0·1–10 Gc/s. Meaurements made covered the range from 0·3 to 3·4 Gc/s and sensitivities of about 1·28 μV/100 mW/cm^2 were obtained.

A paper by K. Sakurai and T. Maruyama (Electrotechnical Laboratory, Tokyo) described a mm-wave microcalorimeter of interesting design. The instrument uses a single load, as opposed to the thermally balanced type developed at the National Bureau of Standards, and uses an electronic servo system to maintain the temperature difference between the load (bolometer mount) and the reference (the shell of the calorimeter) less than 10^{-5} °C. This is accomplished by means of a Peltier thermoelectric cooler controlled by a differential thermopile between the two calorimetric bodies. The current of the cooler furnishes the data in the system. The system measures the effective efficiency of a bolometer mount with an absolute accuracy of 0·6 per cent, making possible accurate power measurement in the 35-Gc/s region.

The next two papers demonstrated the importance of microwave pulse power measurements. The first, by C. A. Denny, C. L. Mavis and C. J. Still (Sandia Corporation), described two systems in which a sample of the unknown pulse is compared with an identical sample of a known generator, using a coherent detector for level comparison. The two methods differ from each other in that one samples the unknown signal directly with a crystal switch while the other uses a heterodyne receiver with a pulsed intermediate frequency amplifier. The pulsed receiver system can be used down to -60 decibels referred to 1 mW with an accuracy of 5 per cent. Somewhat better accuracy is obtained at higher levels. The crystal switch is usable with pulse widths greater than 200 nsec, and the pulsed receiver may be used with pulse widths greater than 350 nsec.

The second paper dealing specifically with peak pulse power measurement was prepared by P. A. Hudson, W. L. Ecklund and R. A. Ondreika (National Bureau of Standards, Boulder). Their system is very similar to that described by C. A. Denny, but features a narrower sampling pulse, typically 100 nsec wide. This, together with the pulse delay and trigger circuitry of the system, permits resolution of the unknown pulse shape as well as measurement of the peak value for pulses longer than about 1 μsec. A special switch design affords greater than 70 db. isolation when open and 1·5 db. loss when closed. The high isolation is necessary to the accuracy of the system, which is given as plus or minus 2·5 per cent. The system is intended for application at levels up to 10 kW peak.

A highly refined and carefully analysed rotary vane attenuator was described by A. V. James (Sperry Gyroscope Co.), who proposed its use in primary calibration laboratories. The design discussed uses a tape wound around the circumference of the rotary drum in place of the usual gear drive. This eliminates the gear backlash and converts the rotary motion to translational motion, where it is measured by means of a dual micrometer mechanism. The translation amounts to 4·5 in. for a 90° rotation and is measured to within 250 μin. A check against an audio-substitution attenuation measuring system showed agreement to within 0·04 db over a 40-db range, in the X band. Calculated limits of error were plus or minus 0·095 db in the range 0–40 db.

A paper by R. F. Clark and B. J. Dean (National Research Council, Ottawa) discussed a precision X-band system capable of an accuracy of $\pm(0{\cdot}010 \text{ db} + 0{\cdot}005 \text{ db}/10 \text{ db})$, with a 40 db dynamic range. The X-band attenuator is compared with a calibrated 30-Mc/s reference attenuator by means of a linear superheterodyne detector. Both frequency and amplitude stabilization are used in the system to obtain for the standard a drift of less than ± 0·005 db per 5 min.

A new and unusual method of pulse power measurement was disclosed in a paper entitled "An Elastic Wave Method for the Measurement of Pulse Power Density", delivered by R. M. White (General Electric Co.). The method is based on pulse heating of the surface of a body which is mechanically coupled to a sensor such as piezoelectric crystal. The absorbed energy pulse produces a rise of temperature in the surface of the body which creates an elastic stress wave that propagates through the body to the sensor. Experiments have shown that the effect is due to heating and not to radiation pressure; indeed, the system is much more efficient than one designed to respond only to the radiation pressure. In an experimental X-band detector, an output of 6 mV was obtained from the sensor for a peak pulse power density of 12 kW/cm^2. Experiments were also made with different energy sources, including light pulses from a laser or stroboscopic lamp, and a pulsed beam of electrons in an evacuated chamber. It may be possible to obtain ultimate sensitivities of a few hundred microwatts per square centimetre by using resonant techniques.

Session X : Micro-wave Techniques

Session X on "Microwave Techniques", with Harold M. Barlow (University College, London) as chairman, consisted of six papers.

S. B. Cohn (Rantec Corporation, Calabasas, California) presented the first paper, entitled "Swept Phase-Measurement Techniques with CW and Pulsed Signals", in which he described recent advances in instrumentation for measuring phase shift versus frequency for linear two port devices and phase shift versus control voltage or drive power for non-linear devices. The instrumentation, for either continuous-wave or pulse-modulated signals, had a scale expansion such as to allow errors of only a few tenths degree around 90° for single frequency measurement.

The second paper, entitled "A Phase Measuring System for Short RF Pulses", presented by R. A. Sparks (Emertron, Inc., Silver Spring, Maryland), described a novel technique of measuring the relative phase characteristics of pulsed microwave amplifiers. The method utilizes a two-channel system and the principles of homodyne detection. The capability of handling very narrow radiofrequency pulses with high resolution is obtained through generation of an unusually high offset frequency and the use of a wide band intermediate frequency amplifier. A 100 Mc/s oscilloscope is utilized to display the undetected bipolar signal that contains the translated phase information of the pulsed carrier.

Paper three, "A Technique for Precise Measurement of the Radio Frequency Structure of Pulsed Signals", was given by W. D. Griffin (HRB-Singer, Incorporated, State College, Pennsylvania). The technique described here is capable of detecting a zero-beat to one one-hundredth of a cycle during a pulse. It is based on measurement of the instantaneous relative phase of the pulsed signal with respect to a stable local oscillator (stalo) at approximately the same frequency. This is accomplished by dividing the pulsed signal into two equal parts and heterodyning these in balanced crystal mixers with two equally divided continuous-wave signals from a stalo. Equal path-length is maintained throughout except for a 90° phase shift inserted in the path of one of the two oscillator signals. One mixer output is used as the vertical deflexion voltage on a cathode-modulated cathode ray oscilloscope, while the other is used as the horizontal deflexion voltage. This then produces a unique display of the relative phase of the pulsed signal with respect to the oscillator as a function of time.

The next paper, "Report on Standardization of High-Precision Coaxial Connectors", was presented by D. E. Fossum (Sandia Corporation, Albuquerque, New Mexico). In the present state of the art of coaxial measurement, errors due to connexion uncertainties limit the accuracy of nearly all quantities. The report briefly described the co-operative effort of the National Bureau of Standards and instrument manufacturers with university and foreign laboratories to recommend practices for precision coaxial connectors. Such connectors would be used on standard and high-quality instruments to ensure minimum loss of accuracy in transferring information with a small number of compatible connectors. Such connectors would be made to accuracies now used for precision wave-guide flanges giving voltage standing wave ratios ranging from about 1·002 at 1 Gc/s to 1·01 at 13 Gc/s.

D. Woods (Ministry of Aviation, London) presented the fifth paper, "Improvements in Precision Coaxial Resistor Design". Discussed were: (1) the successful development of cylindrical metal film resistors of uniform surface resistivity with an adjustment tolerance of 0·1 per cent; secular stability, 0·03 per cent; temperature coefficient less than 0·002 per cent/°C, suitable for use with a tractorial jacket in a coaxial mount up to 4 Gc/s; (2) the proposal to apply the technique to the manufacture of conical resistors for use with a cylindrical jacket. The advantages of this type of mount are pointed out, namely, the simplicity of the mechanical construction and the reduction of residual discontinuities to a bare minimum, even to the elimination of the dielectric support in a matched termination, provided a suitable coaxial connector is used, similar to one developed by the author; (3) a simple explanation of the principle of operation of the cylindrical resistor with a tractorial jacket and the conical resistor with a cylindrical jacket in terms of electromagnetic wave propagation over a resistor film surface; (4) the effects of field penetration through the resistive film into the resistor substrate and means for compensating the effect in a precision coaxial resistor.

The last paper, "Microwave Measurements at 150 Gc/s", was presented by J. B. Thaxter (Lincoln Laboratories, Lexington, Massachusetts). Mr. Thaxter discussed methods presently used at his laboratory for the measurement of frequency, power, and impedance in the two millimetre wave-length region of the spectrum. Frequency is measured by heterodyning the unknown with a signal obtained by multiplication from a highly stable low-frequency source. Power at 100–200 Gc/s in the fractional mW range is measured by a water-flow calorimeter utilizing a d.-c. comparison method. Impedance is measured by the usual bridge technique utilizing a hybrid *T* along with a calibrated attenuator and sliding short. Lack of standards in this frequency-range and the component sizes dealt with severely limit the accuracy of measurements in this new field.

Numerous persons, including many of the authors already mentioned, contributed to and reviewed this summary. Their efforts are greatly appreciated.

INTERPRETATION OF MICRO-STRUCTURES IN CARBONACEOUS METEORITES

By PROF. G. MUELLER

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THE considerable number and variety of microscopical objects within the Orgueil and several other carbonaceous stones were interpreted by some authors[1,2] as of likely organic origin. These conclusions have been contested[3] and critically discussed[4,5].

The aim of the present article is to attempt to cast more light on this controversy through additional evidence furnished from observations under the polarizing microscope of fifteen previously recognized and five new stones with a carbonaceous phase. It was found that the majority of the structures claimed as of organic origin could be readily and quite unequivocally identified as mineral grains.

From the point of view of types and abundance of micro-structures present, the meteorites so far investigated could be divided into three broad classes as follows:

Microstructure type 1: these include the two stones so far examined from compositional group I of Wiik[6]. They are characterized by the presence of troilite plates and abundant glass globules (Table 1). This group includes the Orgueil meteorite.

Microstructure type 2: includes mostly the compositional type 2 of Wiik. In these there are no troilite plates, and the glass globules are less abundant, whereas freely grown, cored olivines are the

Table 1. MICROSTRUCTURES IN CARBONACEOUS METEORITES

Type	Meteorite	Troilite plates	Limonite after troilite	Glass globules	Pele's hair	Opaque (Fe_3O_4, FeS NiFe) globules	Olivine globules	Cored olivine crystals	Cored felspar, enstatite, etc., crystals	Sintered and sheathed olivines
(1)	Orgueil	+	+	+ + +	+	+ + +	+ +	+ +	+	+ +
,,	Alais	+	+	+ + +	+ +	+ + +	+	+ +	+	+ +
(2)	Cold Bokkeveld	–	–	+	–	+	+ +	+ +	+	–
,,	Kaba	–	–	+ +	+	+ +	+ +	+ +	+	+ + +
,,	Karoonda*	–	–	+	–	+	+ +	+ + +	+ +	+ +
,,	Mighei	–	–	+ +	–	+ +	+ + +	+ + +	+	+
,,	Mokoia	–	–	+ +	+	+ + +	+ +	+ +	+ +	+ +
,,	Murray	–	–	+	–	+	+ +	+ + +	+	+ +
,,	Nagoya	–	–	+	+	+ +	+ +	+ +	+	+
,,	St. Caprais*	–	–	+ +	+ +	+ +	+	+ + +	+	+ +
,,	Staroye Boriskino	–	–	+	–	+	+ +	+ +	+	+
(3)	Bali*	–	–	+ +	+	+	+	+	+ +	+ + +
,,	Goalpara	–	–	–	–	+	+	+	+	+ + +
,,	Indarch	–	–	+	–	+	+	+ + +	+	+ +
,,	Lancé	–	–	–	–	+	+	+ +	+	+ + +
,,	Ornans	–	–	–	–	+ +	+	+	+	+ + +
,,	Renazzo*	–	–	+	+	+	+	+	+	+ + +
,,	St. Seveur*	–	–	–	–	+ +	+	+	+	+ + +
,,	Warrenton	–	–	–	–	+	+	+	+	+ + +
–	Erakote	–	–	–	–	–	–	–	–	–

* Carbonaceous stone recognized for the first time.

Approximate abundance of microstructures: + + +, above 10 per mg; + +, 1–10 per mg; +, below 1 per mg; –, not observed, less than 0·2 per mg.

most common. This group contains the Mighei meteorite.

Microstructure type 3: consists mostly of the 'low carbon' group of Wiik; it contains no, or very little, glassy structures, and the cored olivines are of the coated or sintered type.

The only meteorite so far examined which does not fall into any of the foregoing groups is Erakote, which consists of radiating, acicular aggregates of a slightly translucent, dark mineral of straight extinction, which has not been identified so far.

It is interesting to note that both the diameters of the high-temperature mineral grains present, and their ratio to the ground-mass of hydrated silicates and carbonaceous phase, do increase with decreasing volatile, carbon, water and sulphur percentage of the stones.

The markedly rounded or globular habits of both the glass and the olivine, etc., microstructures seem to indicate that these solidified when freely suspended in gas. This is reminiscent of conditions prevailing in cases of terrestrial ash eruptions and nuée ardentes, where often the high-temperature pumice grains tend to intermix with low-temperature fragmented matter, as is the case in the structure of the fresh olivine, etc., grains spread throughout the hydrated silicate and carbonaceous phase containing matrix of the carbonaceous chondrites. The markedly more freely formed habits of the grains in meteorites, when compared with those of terrestrial tuffs or welded tuffs, can be explained possibly through these staying suspended for a longer period of time within the gases, due to the lower gravitational field which prevailed on the parent body. The presence of cores in most of the crystals indicates a process of unmixing of impurities during cooling. It is significant that, in the case of the olivine grains, the ratio diameter of crystal/diameter of core remains approximately constant at 5 (compare Figs. 7–12), indicating the unmixing of a reasonably constant proportion of impurities from a spray of essentially 'olivinic' composition.

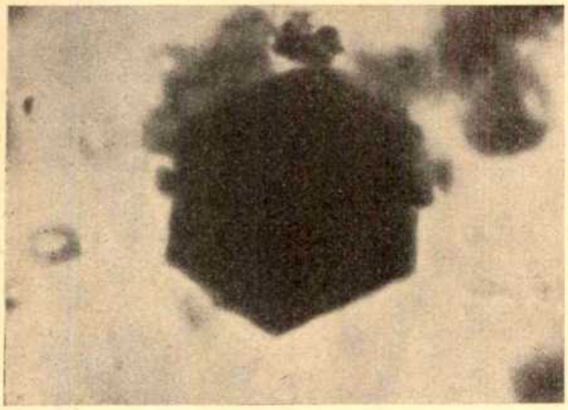

Fig. 1. Troilite plate with two magnetite knobs in diagonal position from Orgueil. (×1,000)

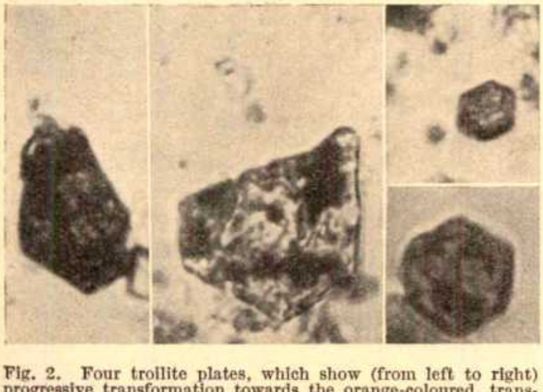

Fig. 2. Four troilite plates, which show (from left to right) progressive transformation towards the orange-coloured, translucent limonite pseudomorph; the upper right-hand pseudomorph shows no more vestiges of the opaque troilite. (×1,000)

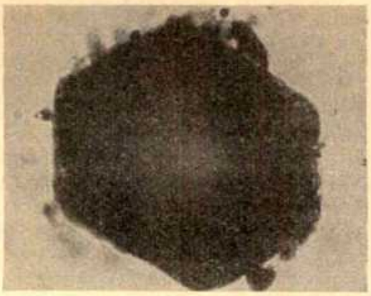

Fig. 3. Troilite plate with incipient oxidation to limonite and three magnetite concretions on alternate faces. (×1,000)

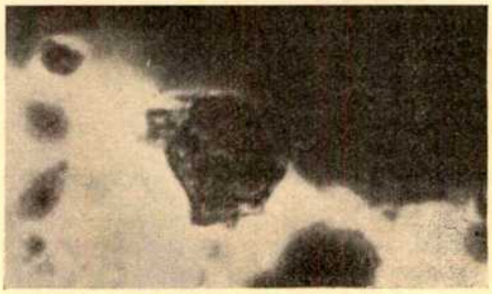

Fig. 4. Limonite pseudomorph with some traces of troilite, and three appendages of lighter and more greyish shade on alternate faces, from Orgueil. (×1,000)

The only microstructures which cannot be readily explained as of mineral origin are the jointed double walled filaments observed in Orgueil[7], which are very rare, and, judging from their fresh appearance, may be contaminations.

The grains found in the meteorites examined divide into three rather broad mineralogical-genetical groups as described briefly here.

(1) *Hexagonal plates of troilite with occasional magnetite overgrowth and their limonite pseudomorphs.* The object of without doubt the greatest theoretical interest, illustrated by Claus and Nagy[1], is the orange-brown, hexagonal, rounded plate with the three roughly cylindrical-conical protrusions of greyish-brown colour, situated at alternate sides. Its morphology is too dissimilar from that of any known terrestrial microfossil to be conceived as a possible contamination; on the other hand, a mineralogical interpretation does not seem to present itself at first sight. The previously noted[3] similarity between the foregoing structure and the hexagonal. magnetic opaque troilite plates present does not account for the colour and the rather symmetrically arranged protrusions.

It was found in the course of the present investigation that a perfectly gradual transition exists between the relatively larger, 30–50μ diameter, troilite crystals, which show invariably brown surface tarnishing, and the smaller, 10–20-μ plates, which are completely or almost completely converted to the limonite pseudomorphs (compare Figs. 1 and 2), which, in turn, is identical in all respects with the central plate of the structure of Claus and Nagy. The detection of a very rare troilite plate, with three rather cylindrical overgrowths of magnetite aggregates on alternate faces (Fig. 3), appears to give decisive evidence for the complete mineralogical derivation of the object in question. In oblique reflected light, the central plate appears concave and stained brown, whereas the three magnetite knobs are lustrous, black—which fact might account for the differences in colour between the pseudomorphosed plate and its protrusions, as shown in Fig. 4.

The control of nucleation of the magnetite is not a crystallographic one, as the troilite does not contain a trigonal element in its symmetry, but is due to the fact that a once nucleated magnetite aggregate on a given face would absorb so much substance from the setting that nucleation on the two neighbouring faces would be unlikely. As a result of this, troilite with magnetite on one face only, on the two opposite faces (Fig. 1), or on two or all the alternate faces would be theoretical possibilities, and, indeed, all these types of overgrowth have been observed.

The genesis of these freely grown troilite plates may be explained through a late-stage penetration of the loose matrix of the meteorite with hydrogen sulphide containing gases, the latter reacting with the abundant Fe^{++}, producing ferrous sulphide. This process is further indicated through the presence of numerous calcite, gypsum, sulphate of magnesium, etc., veinlets which render the aspect of the stone very similar indeed to that of a specimen of terrestrial ash traversed by a fumarola vent. As a rule the latter contains innumerable pyrites cubes, which in turn may oxidate, producing the familiar limonite pseudomorphs, and, indeed, a few of such oxidated pyrites cubes were encountered in the Orgueil meteorite (Fig. 5). The fact that both the crust and the central portions of the Orgueil meteorite proved to contain limonite pseudomorphs after troilite seems to indicate that at least part of the oxidation occurred in outer space.

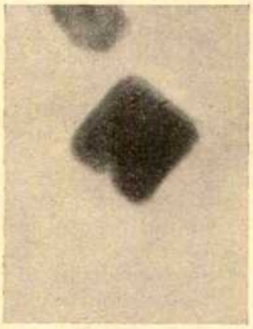

Fig. 5. Limonite pseudomorph of pyrite cube from Orgueil. (× 1,500)

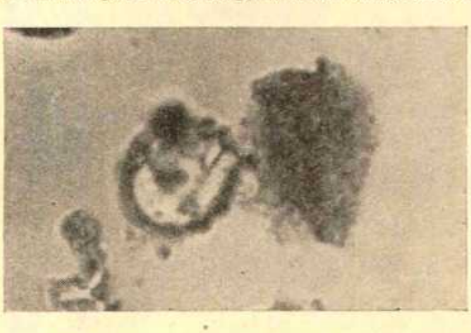

Fig. 6. Glass globule from Orgeuil. × 1,000)

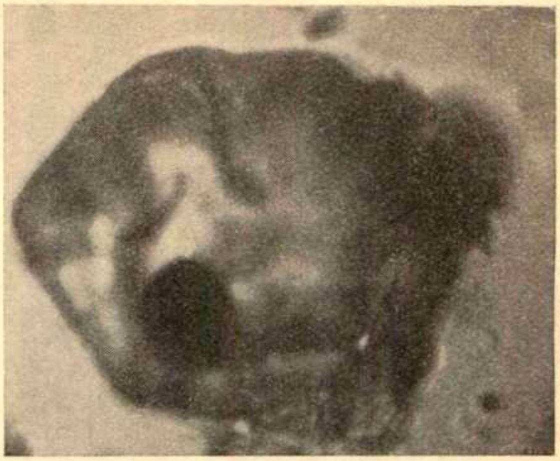

Fig. 7. Cored olivine crystal from Mighei. (× 1,000)

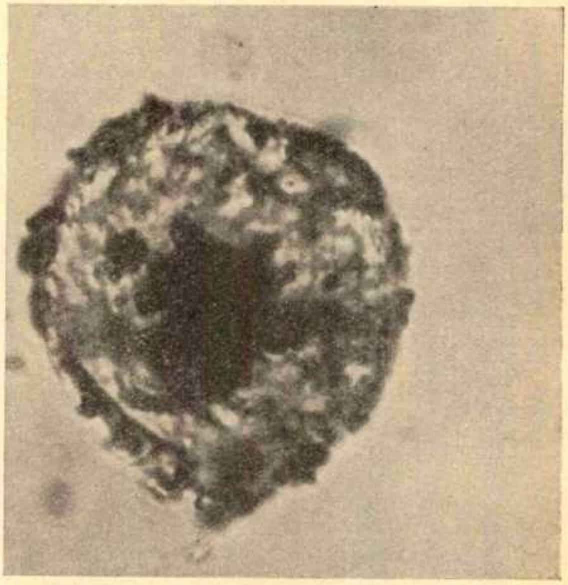

Fig. 8. Cored and sheathed globule of olivine from Alais. (× 1,000)

(2) *Globules, etc., of glass.* Most of the stones proved to contain rather crinkled globules (Fig. 6) or expanded threads—'Pele's hairs'—of glass, with N: 1·5–1·52, only occasional slight stain-birefringence and colours ranging from pale yellow to greyish-brown; all these properties are well within the range of glasses described previously from meteorites. A few of the globules were heated to about 700° C, with a filament directly under the microscope, without any change being observed. It seems that most of the globular objects illustrated by Claus and Nagy[1] may belong to this group, although, as the absence or presence of birefringence was not mentioned in the paper, some of them may belong to group 3, which follows.

(3) *Single crystals (mainly olivine) of rounded or globular habit.* Crystals or occasionally also crystalline aggregates of the opaque minerals (mostly magnetite, troilite and nickel-iron) are always of markedly globular or ellipsoidal habit. The very common olivines (identified through their optical properties,

habits, cleavage angles, etc.) show in all the meteorites an approximately constant radius of rounding of some 5μ; as a result of this the crystals of less than 18μ diameter are markedly globular.

An interesting feature of most of these olivine crystals—and occasionally also of the rarer felspar, enstatite and glass globules—is the presence of one or more globular cores (Figs. 7–12). The most common core is opaque, of low reflectance and granular appearance; it seems to consist mainly of fine-grained magnetite with other impurities. Cores of bronze colour (troilite ?) and of high metallic reflectance (nickel-iron ?) were also noted, usually within the multi-cored crystals, in which the common, black core is rarely if ever absent. Sometimes light-coloured glassy cores and gas bubbles appear as well. The study of thin sections of Murray revealed that sometimes the core of impure magnetite contains a small core of silvery lustre (nickel-iron ?), producing a globular crystal which resembles closely the models of internal structure of the Earth. This similarity is even more accentuated in the case of the 'coated globules' (Figs. 8, 9, 10), which apparently contacted the matrix of hydrated silicates and carbonaceous matter in a still tacky state, their outer sheaths being rather analogous to our sediments. Olivine globules, expanded into 'Pele's hairs' (Fig. 10), have been observed as well.

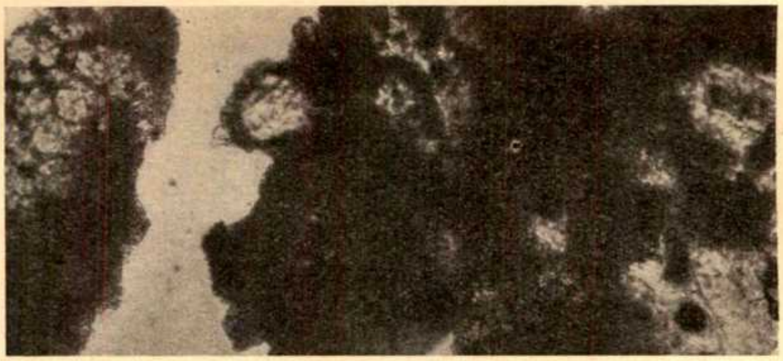
Fig. 11. Several olivine globule-chondri in Mokoia, showing partial sintering of the individual cored crystals

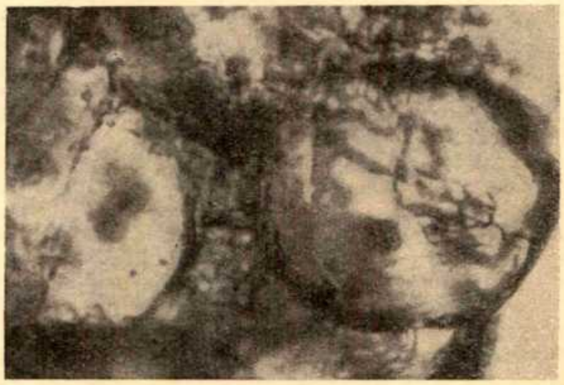
Fig. 12. Cored olivine globules in enstatite, from Indarch. (×1,000)

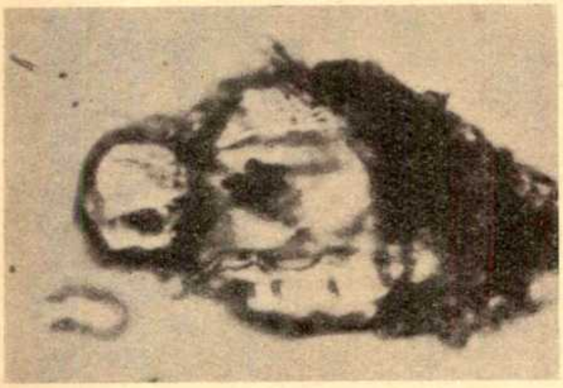
Fig. 9. Two cored and sheathed olivine globules from St. Caprais; note smaller size of core in smaller globule. (×1,000)

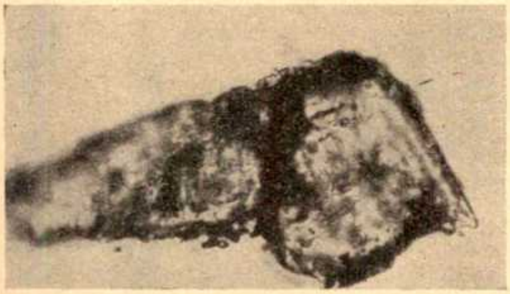
Fig. 10. Expanded olivine globules from Renazzo, with cores (out of focus) and partial sheaths. (×500)

With the increasing mechanical strength and decreasing carbon content of the meteorites, the individual cored crystals tend to sinter into larger globule-chondri. The sectioned grains of Mokoia, illustrated in Fig. 11, show several of these with diverse grades of sintering of the cored olivines and, also, occasional opaque globules, including the formation of single olivine crystals in which part of the original cores, however, apparently still remained separated. Sometimes the globules tend to decrease in size when proceeding from the centre towards the surface of the chondrule. Globular olivine crystals embedded in enstatite were also observed (Fig. 12).

The extensive range of stones examined and the consistence and graded character of the mineral grains observed in them would seem to remove the necessity to postulate an organic origin for any of them. The problem of how they actually originate and their relations to carbonaceous matter in the meteorites will evidently require more detailed and systematic study before any general theory of their genesis can be safely postulated.

The work described here was carried out with funds made available by the Nuffield Foundation. I thank Prof. J. D. Bernal for most inspiring and fruitful conversations on the subject and, also, Prof. J. Orcel, of the Natural History Museum, Paris, for his kind help in placing at my disposal samples of carbonaceous chondrites from his Museum.

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SCIENTIFIC METHOD AND THE NATURE OF TECHNOLOGY

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TECHNOLOGY has been defined as "the scientific study of the practical or industrial arts" and science as "truth arrived at by observation, experiment, induction" (*Oxford English Dictionary*). A scientist is one who applies scientific method in the fundamental natural sciences while a technologist is one who engages in the scientific study of the industrial arts. He is a kind of practical scientist. A technologist has studied the fundamental principles of his chosen technology and should be able to use his knowledge and experience to initiate practical developments[1].

It is evident from these definitions that a technologist must be a scientist though a scientist is not necessarily a technologist. In considering the nature of technology one must therefore look into the difference between the two. What must a scientist do to become a technologist ? It is clear from the definitions that he must study the practical and industrial arts; but, although his study of these is supposed to be scientific, he must learn to understand something of the crafts and craftsmen associated with his chosen technology.

In studying crafts one must learn to appreciate rule of thumb. Sir David Pye, in his address to the Institution of Mechanical Engineers (October 1952), said: "The art of engineering includes a very great deal that lies outside formulated theory. The rule of thumb plays and will always play an important part; and rules of thumb are accumulated by experience". When scientists choose cloth from a pattern book for a new suit they are not likely to apply any scientific tests but will almost certainly feel the cloth between the fingers and thumb after inspecting its colour. What can one find in this way ? If the ordinary buyer can obtain useful information in this way, the buyer skilled in the arts can learn much more. Enormous quantities of manufactured goods and articles are bought and sold after simple subjective tests of this kind. A good technologist must, therefore, try to acquire as much rule of thumb as he can, and as quickly as he can.

He must also learn that in the real industrial world there is seldom a unique solution. Unlike the idealized systems of the pure scientist where chemicals are pure and variables limited he must cope with mixtures of impure materials and with systems containing numerous variables. There are, nevertheless, several unifying principles running through technology. Scientific method should be the same in its essentials wherever it is applied and and it is very important to understand the way in which it is applied in industry.

As used in a straightforward experiment in a school or university laboratory scientific method may be represented schematically by Fig. 1. An experiment is designed, possibly after a survey of the literature, in order to test a particular hypothesis. The experiment is executed, and any conclusions drawn from the observations made are considered along with the hypothesis. In the terminology of modern automatic control theory, the conclusions may be said to be fed back into the hypothesis. If they are considered along with existing theory this will be derived from a survey of the literature and also fed back into the hypothesis. After this a new or revised hypothesis may be drawn up and the process repeated. The process is essentially cyclical, and each part of the cycle is equally essential. Extra information on side-issues may be obtained from the observations. According to the popular Press, discoveries result from chance observations but a chance observation implies the presence of an observer. A scientific experiment carried out in the presence of a skilled observer may easily lead to useful chance observations but the observer must be there.

Many industrial experiments and indeed much industrial planning can be analysed according to the scheme shown in Fig. 2. Someone in authority, shown as M.D. (managing director), puts forward a problem or idea for investigation. This is passed to the appropriate experimental officer or technologist through the accepted channels of communication. A survey of the literature will, of course, include existing company reports. Some industrial experiments may be criticized on the grounds that too little effort is made to record data in such a way that they can be published for the general benefit. Nevertheless, an industrial experiment follows just the same cyclical course as any other scientific experiment, and the conclusions must again be fed back into the original idea. After several cycles each involving reconsideration of the problem in the light of the observations

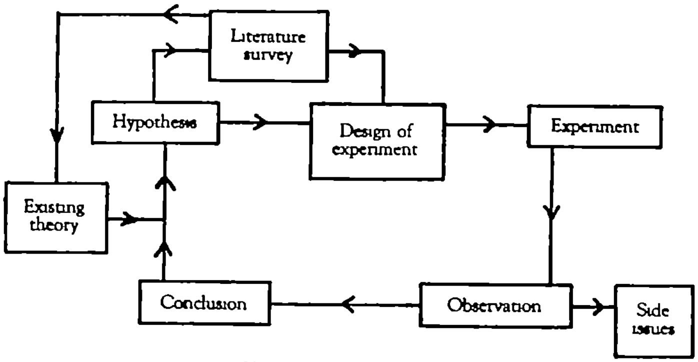

Fig. 1. Scientific method

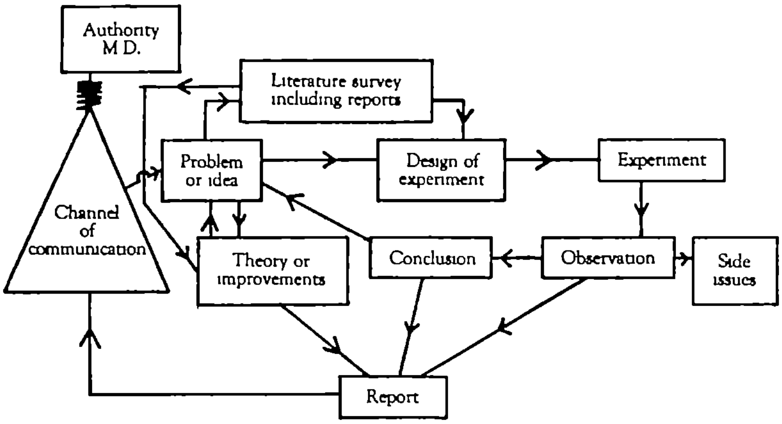

Fig. 2. Industrial research diagram

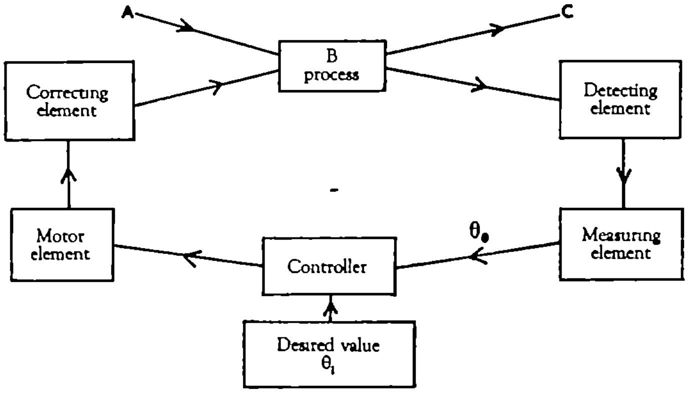

Fig. 3. Automatic control

made and the existing literature it may be possible to suggest some improvements. The industrial equivalent of the pure scientist's 'new theory' is often just 'improvements', and "improvements in and relating to . . . " has become standard jargon in the titles of patent specifications.

At this stage in the technological process a report must be written. When written the report must be fed back through the official channel of communication to the particular authority who originally called for the investigation.

If no report is written at this stage, the work is quite useless industrially. It is essential that the authority—M.D. in Fig. 2—who initiated the work should receive a report on which he can act. Only the initiating authority will have sufficient power in the manufacturing or sales organization to take any action, and unless the report reaches him, in a form which he can assimilate and use, the work might just as well not have been done.

It is at this stage that many young scientists in industry fail. They have been taught by pure scientists whose aim in research is publication. The comparison of the conclusions with the original hypothesis in order to develop new theory completes the pure science cycle of Fig. 1. After that, it is up to others to read the publication and take action if they think fit. There is no obligation on the scientist to put his conclusions in simple English to encourage them to read it—the reverse is indeed often the case.

In industry this method will just not do. The authority is often a layman and invariably busy so that the report must be well written, well summarized and emphasize the action required rather than the prolonged processes by which the conclusions have been reached. Owing to the difficulties to be overcome at this particular stage of the feed-back required in the industrial cycle, the 'channel of communication' has been shown as complicated in Fig. 2 and as containing a resistance. It is most important that the report should be so written that it overcomes this resistance and that the young scientist should understand that his responsibilities do not end until the report has either been discarded or translated into action. If it does result in action he may himself be appointed to develop the processes resulting from it.

Stimulated by authors such as Parkinson[2] there is a tendency to assume that anyone who is 'chair-borne' is not doing useful work. This attitude is particularly prevalent in the less-enlightened industrial laboratories. Actually as more apparatus is invented and particularly more recording apparatus, the time spent in experimentation is bound to decrease and the time spent on interpretation to increase.

In the simplest of industrial scientific experiments, for example, routine control by physical chemical testing, the information may be 'reported' merely by a written entry in a book or a spoken message over the telephone, but the essential principle still remains. It must be 'fed-back' as in Fig. 2 to someone with the authority to take a decision on the process being controlled.

Those responsible for directing this kind of work should always consider whether it could be done better by an automatic controller (Fig. 3). In a process controller the detecting element working in the plant (for example, a thermometer) passes information to the measuring element (for example, a pressure gauge connected directly or through relays with the thermometer). In the controller the measured value θ_0 is compared with the desired value θ_i, and the difference between the two (error) used to actuate a motor element (such as a rubber diaphragm balanced against a spring by air pressure) which will move the correcting element (a valve supplying steam to the process).

This is a very elementary example of the partial application of scientific method. The negative feed-back is there, the observation is compared with the desired (or theoretical) value and action is taken to correct the process, so as to make it produce more closely the desired result. The element of induction, argument from the particular to the general and the development thereby of new theory or knowledge, is absent and the application of scientific method is only partial.

Action by an automatic controller is rapid; routine control by laboratory analysis is slower, and for this reason more wasteful in both time and material; but both emphasize the great importance of negative feed-back. Anyone directing simple routine control should consider the theory of automatic controllers, whether he is able to use controllers or not. Speed of feed-back is so important that it may be worth sacrificing accuracy in order to attain it. Methods should always be under review to see if they can be simplified and speeded up.

Enough has been said to show that scientific method is fundamental to any technology. What, if any, are the other fundamentals ? Here it is desirable to set up the concept of the 'ideal technologist', unattainable in practice just like other ideals. The ideal technologist would be well versed in mathematics and the fundamental natural sciences, physics and chemistry. He would also be an engineer and a biologist. He will be working in industry and be familiar with administration in all its branches, particularly sales, economics, accounting, sociology, personnel management. As Revans[3] has said, in order "to make a steady hundred tons a day" of sulphuric acid "it is probably much better to know how to get one's maintenance engineers to work hard at the week-end than it is understand the surface properties of vanadium pentoxide; it is quite surprising—especially to young graduates—how much easier it is to be informed about catalysts than to understand the behaviour of riggers or pipe fitters". The ideal technologist must clearly be a craftsman too.

In practice, of course, the ideal technologist does not exist, and industry is served by experts in the various crafts, arts and sciences on which it depends. It is often said that some of these experts, particularly those engaged in research, tend to learn more and more about less and less until they know everything about nothing. Others may, having started as experts in one subject, gain information, by accretion or by deliberate learning, in some or all of the other subjects important to their particular industry. It is again said that those who get far enough up the industrial administrative ladder have to learn less and less about more and more until they know nothing about everything. This is not an unfair picture of the end-state of a successful executive. If he is a technologist as well as an executive he must have started with, or acquired a knowledge of, one of the sciences sufficient to equip him with a knowledge of scientific method.

We are now back at the original question: Which of the sciences ? Are there really any fundamentals other than scientific method itself ? Many writers on this subject have assumed that there are, but it is usually found that the chosen fundamental science is the one with which the particular writer started himself. This, too, is usually chemistry or engineering according to the nature of the industry. Either of these sciences may be learned up to an appropriate standard at a university or technical college; each of them is served by an adequate number of professional institutions. Having started in either of them a graduate is well placed to pick up the 'know-how' of the industry he serves. Over the past thirty years physicists and mathematicians have entered industry in much smaller numbers than chemists and engineers, but are now in great demand. It is not surprising, then, that one frequently hears that physics is fundamental to all technology. If, however, fundamentals vary according to the person defining them can they still remain fundamental ? One is left with the view that there is only one real fundamental in technology, and that is scientific method itself.

This is not to deny the importance of the experts in the sciences already listed; but it is most important to remember that each science as taught in a three- or four-year university course is only a tiny selection from the almost illimitable field of knowledge. It becomes conventionalized, rationalized and reduced to almost standard proportions simply because the amount a student can be expected to assimilate usefully in three or four years is more or less the same all over the world. It is three- or four-year brain capacity that produces the arbitrary limits between the fundamental sciences—not anything inherent in the sciences themselves.

If this view is once accepted, the number of subjects which can be properly taught under the heading 'technology' ceases to be limited. One must first teach the real fundamental—scientific method. One must then teach as much as one can of the conventional (not fundamental) sciences most appropriate to the industry which requires the technologist. One must put in the bare minimum of industrial 'know-how' to enable the students to take advantage of vacation periods spent in industry. In content a course of this kind may be broader than a normal science course but will otherwise not be very different. In intent it will, however, be different. It is designed primarily to train men for industry and not for academic research.

The well-trained technologist must be prepared to take methods from various branches of science, and, if, as is likely, he is not expert in them all himself, to employ, or in any event to take advice from, men who are. He should therefore strive to appreciate the methods used in more than one branch of science.

The conventional knowledge of to-day is liable to become the classical knowledge of to-morrow. It was a certain ecclesiastic (a prebendary of Durham—c. 1870—quoted by B. V. Bowden in *Proposals for the Development of Technology*, published by the College of Science and Technology, Manchester, in 1956) who said that "the advantages of a classical education are two-fold; it enables us to look with contempt on those who have not shared its advantages, and it fits us for places of emolument, both in this world and the next".

When this was written it was true that a knowledge of Latin and Greek was essential for places of emolument. Latin was fundamental to all knowledge, and educated men had to learn it to understand each other. In industry to-day, however, the classically educated literate but 'innumerate' leaders of yesterday are being replaced by the 'numerate' (and one hopes not illiterate) leaders of to-day. ('Numerate' and innumerate' are defined in the *Crowther Report*, 15. A report of the Central Advisory Council for Education (England). Ministry of Education, 1 (H.M.S.O., 1959).) Physical science, so far as we understand it, is therefore conventional. It is important and necessary in technology to-day. This, however, does not really make it fundamental, and it is safest to assume that the only fundamental is scientific method itself.

I thank Mr. W. P. Scott. who inspired this article. It is based on some lectures given, under titles chosen by him, to a group of graduate trainees in the Wiggins Teape group in 1957 and has been discussed with him several times since then.

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[1] White Paper (H.M.S.O., Cmd. 9703).

[2] Parkinson, C. N., *Parkinson's Law, or the Pursuit of Progress* (J. Murray, 1958).

[3] Revans, R. W., *Brit. Chem. Eng.*, 5, 260 (1960).

NEWS and VIEWS

The Royal Society: Officers for 1963

At the Anniversary Meeting held on November 30, Sir Howard Florey, professor of pathology in the University of Oxford, was re-elected president.

The other officers re-elected for the ensuing year were: *Treasurer*, Lord Fleck, formerly chairman of Imperial Chemical Industries, Ltd.; *Biological Secretary*, Sir Lindor Brown, Waynflete professor of physiology in the University of Oxford; *Physical Secretary*, Sir William Hodge, Lowndean professor of astronomy and geometry in the University of Cambridge; *Foreign Secretary*, Sir Patrick Linstead, rector of the Imperial College of Science and Technology, London.

Other members of Council elected (or re-elected) were: Mr. F. C. Bawden, director of Rothamsted Experimental Station; Mr. G. B. R. Feilden, group technical director, Davy-Ashmore, Ltd.; Prof. E. F. Gale, professor of chemical microbiology in the University of Cambridge; Sir William Glanville, director of road research, Department of Scientific and Industrial Research; Prof. W. K. Hayman, professor of pure mathematics in the Imperial College of Science and Technology (University of London); Prof. E. L. Hirst, Forbes professor of organic chemistry in the University of Edinburgh; Prof. D. Lewis, Quain professor of botany in University College (University of London); Dr. P. B. Medawar, director of the National Institute for Medical Research; Prof. A. A. Miles, director of the Lister Institute and professor of experimental pathology in the University of London; Prof. W. T. J. Morgan, deputy director of the Lister Institute and professor of biochemistry in the University of London; Prof. C. F. Powell, Melville Wills professor of physics in the University of Bristol; Prof. F. W. Shotton, professor of geology in the University of Birmingham; Prof. J. E. Smith, professor of zoology at Queen Mary College (University of London); Sir Gordon Sutherland, director of the National Physical Laboratory; Sir Graham Sutton, director-general of the Meteorological Office; Dr. H. W. Thompson, university reader in infra-red spectroscopy in the University of Oxford.

(Publication of the presidential address and award of medals at the anniversary meeting of the Royal Society has been deferred until the issue of December 15.)

Chemistry, Physics and Metallurgy at the Royal Aircraft Establishment: Dr. B. P. Mullins

As from November 19, the Chemistry Department and the Metallurgy and Physics Department at the Royal Aircraft Establishment, Farnborough, became amalgamated into a Chemistry, Physics and Metallurgy Department. Dr. B. P. Mullins, at present head of the Chemistry Department, has been promoted to deputy chief scientific officer and head of the new Department. The present head of the Metallurgy and Physics Department, Mr. L. G. Carpenter, who was recently promoted on individual merit to deputy chief scientific officer, is being partially relieved of administrative responsibility, in accordance with the aims of the individual merit promotion scheme, by appointment as head of the Physics and High Temperature Materials Division in the new Department. Dr. Mullins was born in 1920 and educated at Shooters' Hill School, London, which he left when sixteen to join the Engine Branch of the Air Ministry. At nineteen, after two years' attendance at the Woolwich Polytechnic Evening Institute and a year's private study (spent as a Ministry of Aircraft Production evacuee in Harrogate) he obtained a London B.Sc. degree in physics, chemistry and mathematics. He immediately commenced research in the Engine Department of the Royal Aircraft Establishment, Farnborough, where at twenty-two he became head of the Fuels and Lubricants Laboratory. After a year's research on high-vacuum gas analysis under Prof. R. G. W. Norrish at the University of Cambridge on secondment, he returned to Farnborough, where between 1944–60 he undertook physical and physical–chemical researches in the field of combustion science at the National Gas Turbine Establishment (formerly Power Jets, Ltd.). At thirty-one, as a principal scientific officer and having just been awarded an external Ph.D. degree in fuel technology by the University of London, he began to widen his interests, and at thirty-three was appointed the first head of the Chemical Physics Department at the National Gas Turbine Establishment, where he remained until July 1960, when he was appointed head of the Chemistry Department at the Royal Aircraft Establishment. During the mid-1950's Dr. Mullins was U.K. member and chairman of the Combustion and Propulsion Panel of AGARD–NATO.

Physical Chemistry at Leeds: Prof. P. Gray

Dr. P. Gray has been appointed to a personal chair in physical chemistry in the University of Leeds. Dr. Gray was educated at Newport High School and Gonville and Caius College, Cambridge, where he obtained first-class honours in Parts I and II of the Natural Science Tripos in 1946. Following graduation, he was engaged in research in the Department of Physical Chemistry at the University of Cambridge from 1946 until 1951. He was elected to the Dunlop studentship in 1946, and awarded a Ph.D. in 1949. In the same year the Ramsay Memorial fellowship was awarded to him and he was elected a Fellow of Gonville and Caius College. In 1949 he was appointed University demonstrator in chemical engineering. Dr. Gray joined the staff of the University of Leeds in 1955 as lecturer in physical chemistry and received in the same year the Meldola Medal of the Royal Institute of Chemistry. The title of reader was conferred on him in October 1959. He was awarded a Commonwealth Bursary in 1958 and went as visiting professor to the University of British Columbia in Vancouver. The Marlow Medal of the Faraday Society was conferred on him in June 1959. Dr. Gray's research work at Leeds has been in several fields. These include the study of flames and explosions, work on thermochemistry, thermodynamics and heat transport, and the study of free radicals.

Directorship of Twyford Laboratories, Ltd.

The director of Twyford Laboratories, Ltd., Dr. H. D. Kay, has announced his retirement as from the end of the year, when he will have completed his pioneering three-year term on the board of the company. As from January 1, Dr. F. A. Robinson, chairman of Crookes Laboratories, Ltd., will be the managing director of Twyford Laboratories, Ltd., but will also continue as chairman of Crookes Laboratories. Twyford Laboratories, Ltd., a wholly owned subsidiary of Arthur Guinness Son and Co., Ltd., was established to conduct fundamental research into animal and plant biochemistry and microbiology, and was recently expanded to enable research into chemotherapy and virology to be carried out.

U.K. Nuclear Reactor Research and Development

In a written answer in the House of Commons on November 5, the Parliamentary Secretary for Science, Mr. D. Freeth, stated that the Capenhurst Plant was not being closed down. Its output would be reduced gradually by the end of 1963 to the minimum level to keep it in production and available to meet any future increase in demand. Difficulties in the operation of the Dounreay breeder reactor experiment had been overcome and the reactor resumed operation at 30 MW (Heat) on July 26 and since October 14 had been supplying 3 MW of electricity to the national grid. It was intended as the next phase to run the reactor for some months at about 30 MW (Heat) to obtain information on fuel element behaviour. The Authority now has in hand the design and development of a prototype fast power reactor drawing on the experience at Dounreay.

Expenditure on Buildings and Plant for Scientific Research

In the House of Commons on November 5 the Chancellor of the Exchequer, Mr. R. Maudling, announced in the debate on the Address to the Throne that he proposed to raise the investment allowance on new plant and machinery and other assets from 20 to 30 per cent and on industrial buildings and other assets from 10 to 15 per cent. The cost of this change was estimated at some £8 million next year and about £50 million the following year. He also proposed that for new heavy capital plant and machinery the lowest actual annual rate of depreciation should be 15 per cent corresponding to a 12 per cent basic rate. The cost of this change was estimated at £15 million in the second year, rising to as much as £35 million a year. He also proposed that capital expenditure on buildings and plant for scientific research should in future be written off 100 per cent in the first year, as well as qualifying for the increased investment allowance of 30 per cent. The cost of this change was estimated at £4 million in the first full year.

The Building Research Station and the Ministry of Public Buildings and Works

In reply to a question in the House of Lords on November 7 regarding the functions of the newly appointed Director-General of Research and Development of the Ministry of Public Buildings and Works, Sir Donald Gibson, and whether this appointment would involve the removal of the Building Research Station from the responsibility of the Minister for Science, Lord Hailsham said first that he hoped that two principles would remain inviolate. First, research under Government auspices should be undertaken under the general supervision of scientists: this was the purpose of the Department of Scientific and Industrial Research and of the Research Council system. Secondly, the organization of research should be co-ordinated centrally, usually through one of the Research Councils. In spite of the importance he attached to these two principles, Lord Hailsham said that he did not deprecate research by executive departments provided these principles were accepted. The functions of the Director-General of Research and Development would be to co-ordinate and extend the activities of the various research and development groups throughout the Government service; to assume direct responsibility for the work of the development groups in the Ministry of Public Building and Works; to encourage and develop generally the use of new and rapid methods of construction; to standardize the use and production of building components to the greatest possible extent, and to secure the widespread dissemination of the best modern practices. The appointment did not involve the removal of the Building Research Station from the responsibility of the Minister for Science or of the Department of Scientific and Industrial Research. It was a necessary step in the assumption of responsibility by the Minister of Public Building and Works for co-ordinating the extensive work already proceeding within Government departments in the development and use of new, cheaper and speedier methods of construction. Almost half the total output of the construction industries was for public authorities. Sir Donald Gibson's functions would lie far more in the operational field than in that of pure research, although undoubtedly he would be greatly interested in research and would wish to stimulate and direct research along the most fruitful lines.

The National Research Council of Canada

Founded in 1916, the National Research Council of Canada now has an operating budget of about 42 million dollars. Some 14 million dollars are required for 'foundation' work—scholarships and research grants, in science, engineering and medicine—and the remainder is used to operate the laboratories. High standards are required of the 2,600 personnel, of whom 730 are scientists and engineers. About 690 of these are engaged in research, 360 having a Ph.D. degree and 330 having a Bachelor's or Master's degree. Approximately 250 are engineers. A detailed report of all the Divisions, including the Atlantic Regional Laboratory and the Prairie Regional Laboratory, gives considerable information about the contribution the Council is making in building up knowledge and the economy of Canada (*Canada. Review of the National Research Council, 1962*. Pp. 383. Ottawa: National Research Council, 1962).

Grants to Students in the United Kingdom

The important changes in grants to students operating from September 1, 1962, are detailed in Part 1 of a pamphlet issued as a short guide for students resident in England and Wales who are taking first degree or comparable courses (*Grants to Students—First Degree and Comparable Courses*. Pp. 8. London: Ministry of Education, 1962). Part 2 explains the contribution to be paid by students and parents. An appendix, which lists full-time or sandwich courses designated by the Ministry as comparable to first degree courses, also indicates where further information can be obtained.

Carnegie Grants for Field Work in Natural History

The Carnegie Trust has approved grants to five societies for field work projects. Assistance is being given to the Suffolk Naturalists' Society for population studies on noctule bats and to the Castleford and District Naturalists' Society for a study of a large hirundine roost. The Selborne and the Hampstead Scientific Societies are receiving grants for ecological surveys, and a grant has been approved to enable the Wharfedale Naturalists' Society to have a set of aerial photographs made in connexion with their survey of Ilkley Moor (*News for Naturalists*, 4, No. 1; July, 1962.)

University News :

Birmingham

The following appointments have been announced: *Lectureships*, Dr. J. F. Barrett (mechanical engineering); Dr. A. P. Brown (biochemistry); F. W. Darwin (zoology and comparative physiology); C. S. Dunn (highway and traffic engineering in the Department of Civil Engineering); Dr. B. P. Hughes (civil engineering); Dr. H. G. Klemperer (biochemistry); Dr. A. E. Pedler (chemistry); P. J. Reilly (civil engineering); Dr. A. E. Wright (geology). *Research fellowships*, K. I. Bartlett (engineering production); S. Brojdo (Associated Television Fellow in the Department of Electrical Engineering); R. M. Burstall (Kenward Memorial Fellow in the Department of Engineering Production); E. Carel (mathematical physics); B. T. Davies (engineering production); P. W. Graves (physical metallurgy); Dr. R. Hancock (biochemistry); A. A. Knight (engineering production); Dr. S. K. Mitra (physical metallurgy); J. C. Morris (electrical engineering); M. Richer (engineering production); L. F. Turner (Associated Television Fellow in the Department of Electrical Engineering); Dr. R. L. Cochrane (anatomy); Dr. B. Tallini (staff Fellow in nuclear physics); P. J. Waterton (staff Fellow in nuclear physics); M. Cordey-Hayes (nuclear physics); D. Smith (electron physics); H. F. Ross (neurocommunications); R. M. Norris (medicine).

The title of reader in chemical engineering has been conferred on Dr. R. Long, senior lecturer in the Department of Chemical Engineering. Titles of University Research Fellow have been conferred on O. C. Schauer (geology) and A. H. Barth (electrical engineering).

Cardiff

Dr. K. S. Dodgson, senior professor in the Department of Biochemistry in the University College of South Wales and Monmouthshire, has been appointed to the chair of biochemistry at the College in succession to Prof. J. Pryde, as from October 1, 1963.

London

Dr. J. T. Eayrs, professor of neuro-endocrinology in the University of Birmingham, has been appointed to the Fitzmary chair of physiology tenable at the Institute of Psychiatry. Dr. John Gloster, formerly a member of the scientific staff of the Medical Research Council, Ophthalmological Research Unit, has been appointed to the readership in experimental ophthalmology tenable at the Institute of Ophthalmology. Dr. G. S. Nelson, parasitologist in the Kenya Medical Department, has been appointed to the readership in medical parasitology tenable at the London School of Hygiene and Tropical Medicine.

Sheffield

R. Barer, at present University demonstrator in human anatomy in the University of Oxford, has been appointed to the Arthur Jackson chair of human biology and anatomy. Dr. G. E. Bacon, deputy chief scientific officer, Atomic Energy Research Establishment, Harwell, has been appointed to the chair of physics and headship of the Department of Physics as from October 1963. (It has been decided to experiment with a system in which the headship of the Department of Physics will be held in turn for a period of three years by each of the two professors concerned.) Dr. M. G. H. Wallbridge has been appointed as a lecturer in chemistry.

Announcements

Sir Hans Krebs, Whitley professor of biochemistry in the University of Oxford, has been elected an honorary member of the Belgian Royal Academy of Medicine.

The third Joint Health Physics symposium organized on behalf of the Joint Health Physics Committee of the British Institute of Radiology will be held at St. Bartholomew's Medical College, Charterhouse Square, London, E.C.1, on January 25. The subject will be "Metabolism of Biologically Important Radionuclides". Further information can be obtained from the General Secretary, the British Institute of Radiology, 32 Welbeck Street, London, W.1.

"Viruses, Nucleic Acids and Cancer" is the title of the seventeenth annual symposium on Fundamental Cancer Research which will be held at the M. D. Anderson Hospital, University of Texas, during February 20–22. The symposium is being organized by the Hospital in conjunction with the U.S. National Cancer Institute and the Texas Division of the American Cancer Society. Further information can be obtained from the chairman of the symposium, Prof. L. Dmochowski, Department of Biology, the University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston 25, Texas.

A symposium on "Recent Developments in the Chemistry of Genetic Processes" will be held at the Bradford Institute of Technology on February 9. The symposium is being organized by the Bradford Chemical Society and the Department of Chemical Technology of the Bradford Institute of Technology in conjunction with the Yorkshire Council for Further Education. The programme will include the following papers: "Recent Developments in the Chemistry of Nucleic Acids", by Dr. R. Markham (Agricultural Research Council); "Protein Synthesis in Mammalian Tissues", by Dr. H. N. Munro (University of Glasgow); "Some Aspects of the Action of Chemical Mutagens", by Dr. D. M. Brown (University of Cambridge); "Biochemical Aspects of Genetics", by Dr. J. R. S. Fincham (John Innes Institute). Further information can be obtained from Dr. R. P. Sheldon, Department of Chemical Technology, Bradford Institute of Technology, Great Horton Road, Bradford 7.

Erratum. The sentence beginning on line 19 of the second paragraph of the article entitled "Coordination Compounds as Resolving Agents", by the late F. P. Dwyer and Dr. B. Halpern, which appeared on p. 270 of the October 20 issue of *Nature*, should read: "The specific rotation $[\alpha]_D$ was $-35°$ (0·125 g in 1 N sodium hydroxide, 2 dm. tube)".

CLINICAL TESTING OF NEW DRUGS IN THE UNITED STATES

REGULATIONS strengthening control over the testing of new drugs in clinical trials have been approved by the U.S. Department of Health, Education and Welfare. The new regulations would require: that the Food and Drug Administration be given the full details about the distribution of drugs for investigation ; that clinical investigations be based on adequate pre-clinical studies to assure safety; and that the clinical investigations themselves be properly planned, executed by qualified investigators, and that the Food and Drug Administration authority be kept fully informed during the progress of the investigations.

The present regulations require that drugs used in clinical trials be labelled for "investigational use" only and that manufacturers keep records of distribution. The regulations also require that investigators have adequate facilities, and that names of investigators be made available to the Food and Drug Administration on request.

Existing regulations, however, do not require either an initial notice to the Food and Drug Administration of a clinical trial of a new drug or subsequent reports on such use. Now, once a clinical investigation has been undertaken, if a substantial doubt developed as to the safety of the drug, the Food and Drug Administration and all investigators using the drug would be notified immediately. If necessary, the trial would be halted by the Administration. Similarly, if a plan proved to be inadequate to assure safety, or if the plan as presented was not being followed, the Administration could require corrective action and would, if necessary, terminate the work.

In notifying the Food and Drug Administration of his intention to begin a clinical investigation with a new drug, a manufacturer would be required to provide information concerning all the pre-clinical investigations, including experimental trials which had been undertaken with animals, to show that it is reasonably safe to test the drug on human patients. Where the clinical investigation involves use on infants or pregnant women, special assurances of safety for such use would be required; copies of the complete information about the pre-clinical investigation and experience with the drug which will be supplied to each investigator, including all that is known about the relevant side effects and precautions suggested by the pre-clinical data; and the names and a summary of the training and experience of each person who will participate in the investigations and an outline of what is planned in the clinical trials, how long they will take, how many patients will be involved, and when progress reports will be made.

Once an investigation was undertaken, the sponsors would be required to maintain complete records covering the distribution of the drug being investigated, and monitor the progress of the investigation.

Each investigator in the clinical trial would be required to supply the sponsor with a full statement of his education and experience, a description of the hospital, institutional and laboratory facilities available to him, and an outline of the plan that he intends to follow. He must fully inform himself about all the pre-clinical investigations before giving the drug to any patient, maintain complete records of his disposition of the drug and case-histories of the patients to whom it is administered, and furnish adequate reports to the sponsor promptly after the completion of the clinical trials. These records must be open to inspection by the Food and Drug Administration on request.

The proposed regulations were published with an invitation for all interested persons to comment within 60 days. Copies of the full regulations are available from the Commissioner of Food and Drugs, U.S. Department of Health, Education and Welfare, Washington 25, D.C.

LIBRARIES AND PUBLIC SERVICES

THE annual conference of the Library Association at Llandudno, September 25–28, was the first annual conference to be held since the Association was transformed into a purely professional body, but although the president, Mr. W. B. Paton, considered the implications of this change in his presidential address, "The Profession of Librarianship", the proceedings otherwise presented fewer features of general scientific interest than usual.

Mr. Paton boldly challenged the profession to tackle its objectives with unity and courage, particularly the problems involved in relating public libraries to the library service of the country as a whole, and attributed the failure of the Roberts Committee to face that problem to the negligence of the Library Association itself. His exposition of the professional ideal contains much that is to the point for any professional man or woman, whether in a particular section of the profession of librarianship or in any other profession. So, too, Mr. A. H. Bill's discussion on September 26 of the problems of the library in the community indicates ways in which readers themselves might co-operate in the development of a service which meets far more adequately and effectively the needs of to-day. However, in the first instance, he urges librarians themselves to discard the erroneous ideas that the public library is a collection of books and provides a service for various types of readers and to seek instead to develop the layout and arrangement of our libraries in accordance with the three-fold conception of their function and purpose. First, there is the provision of a community information service; secondly, that they act as a collection of information in various forms which has to be gathered, organized and fully exploited to be made fully and speedily available to every user; and thirdly, that the service given is to individuals, not to groups or types of readers. He believes that the public library could be a most potent means of achieving Dr. J. R. Oppenheimer's idea: "to keep our minds open and to keep them deep, to keep our sense of beauty and our ability to make it".

Mr. A. Duggan's stimulating annual lecture, also on September 26, "Personal Experience and the

Interpretation of History", aptly illustrates the possibilities in this direction and also has some implications for the writing of industrial and scientific history that the scientist or technologist might ponder. Mr. D. D. Haslam, when discussing on September 27 the problems of library staffing, covered university and Government libraries as well as those of commerce and industry, the technical colleges and the public libraries, and presented a balanced picture of trends and difficulties and their implications which is of wide interest. The planning of central libraries was considered by Mr. F. M. Gardner on September 25 in a paper of interest to industry and commerce as well as to public libraries, while on September 26 Mr. P. H. Sewell examined generally present-day thought on library co-operation though without considering the technical implications of the Roberts report. Miss C. Jones, on September 27, under the title "From Book-Box to Telex: Mechanization for County Libraries", discussed some of the developments and opportunities which mechanization had brought to the county library service. Other papers presented at the conference were concerned with libraries and youth, children's reading and aspects of library service in hospitals.

FORCES IN COLLOIDAL SYSTEMS

An informal discussion of the Faraday Society on the nature of forces in colloidal systems was held in the Chemistry Department of the University of Nottingham during September 20–21, by kind permission of the Vice-Chancellor. The meeting was attended by 108 people, 69 of whom came from industrial organizations and 39 from educational institutions.

Colloidal systems are very diverse in character, and the early research on colloids was essentially based on phenomenology. It has therefore become increasingly important, in order to understand systematically the behaviour of such a wide range of systems, to obtain a deeper understanding of the fundamental forces which act between particles, or molecules, of colloidal dimensions. The forces have the same origin as those acting between simple ions and molecules but in colloidal systems the situation becomes more complicated since the 'ion' may consist of a particle with a number of charges on the surface, for example, a micro-crystal of silver iodide. or an assembly of soap ions as in a micelle. Both repulsive and attractive forces have to be averaged over a surface, and in recent years theoretical work has been devoted to obtaining equations which enable the variation of the force to be predicted over distances of the order of those separating colloid particles. The equations require experimental verification of their validity, and in recent years work on the stability of dispersions, on the structure of soap micelles, and on the stability and structure of thin soap films has yielded some measure of the relative contribution of electrical, attractive and solvation forces. This trend was clearly illustrated during the course of the discussion.

On September 20, the afternoon session was entitled "Stability of Dispersions in Aqueous and Non-aqueous Media". After an address of welcome by Prof. D. D. Eley, the chair was taken by Sir Eric Rideal, who, in his introductory remarks, stressed the importance of colloids as a field of study and the need for deeper understanding of the fundamentals of the subject. A great deal of work was still required to elucidate the part played by the solvent both in aqueous and non-aqueous media.

The first paper, by Dr. J. A. Kitchener and Mr. F. Z. Saleeb (Imperial College of Science and Technology), reported work on the adsorption of 'Aerosol *OT*' by a graphitized carbon black in aqueous media. The adsorption isotherms showed saturation adsorption corresponding to a close-packed, vertically oriented, monolayer of 'Aerosol *OT*' ions having their sulphonate head groups exposed to the liquid. Complementary measurements of zeta potential, surface conductance and the negative adsorption of co-ions were also made. Results suggested that the majority of the counter-ions were located in the Stern plane and that the plane of shear in the electrokinetic experiments was close to the plane of the head groups of the adsorbed 'Aerosol *OT*' ions.

Mr. P. Dawson and Dr. D. A. Haydon (University of Cambridge) presented work on the adsorption of a series of *n*-alkyl carboxylic acids by titanium dioxide suspensions in benzene. The dispersions used were of small particle size (radius *c.* 25 Å) and it was found that the stability of the dispersion increased as the length of the alkyl chain was increased. It was suggested, therefore, that stabilization did not occur until the chains were sufficiently long for steric hindrance to occur between the adsorbed layers. An exception was found in the case of acetic acid, which also appeared to stabilize the system; it seemed improbable, however, that in this case stabilization could arise from steric hindrance.

An account of adsorption on to 'Graphon', from binary liquid mixtures of monoalkyl benzenes and *n*-heptane, was given by Dr. G. D. Parfitt and Mr. E. Willis (University of Nottingham). The apparent isotherms changed in nature as the length of the alkyl side-chain increased, indicating an increasing preferential adsorption of the alkyl benzene. The adsorption isotherms calculated for the individual components suggested a saturation monomolecular layer of alkyl benzene with the molecules lying flat on the surface. Complementary measurements of stability were made on dilute dispersions of 'Graphon', and it was found that the stability increased, with the proportion of alkyl benzene in the mixture and the length of the alkyl chain. The van der Waals attraction constant calculated for this system, with due allowance for the thickness of the adsorbed layer. appeared to be too large to account for the stability observed experimentally.

Drs. K. G. Mathai and R. H. Ottewill (University of Cambridge) presented the results of work on the stabilization of aqueous inorganic dispersions by non-ionic detergents. The rate of flocculation of sols by bivalent and trivalent ions was measured in the presence of various concentrations of non-ionic detergents. The latter were polyoxyethylene glycol monoalkyl ethers, the alkyl chain length of which was varied from eight carbon atoms to sixteen; the length of the polyoxyethylene glycol chain was maintained constant at six units. As the concentration of non-ionic detergent was increased the concentration of inorganic salt required to produce flocculation also increased. Above the critical micelle concentration of the detergent, however, a large increase in stability of the sol occurred. Below the critical micelle concentration the increase in stability was attributed to the increase in hydration of the sol particles caused by adsorption of a thin layer of detergent molecules. The large increase in stability observed above the critical micelle concentration was considered to be due to the fact that the adsorbed molecules prevented the particles from approaching sufficiently closely to enter the primary minimum.

On September 21, the session was entitled "Micellization", and the chair was taken by Prof. D. D. Eley. The first paper in this session, by Dr. A. S. C. Lawrence and Messrs. R. Aveyard and J. T. Pearson (University of Sheffield), presented the results of studies on micellization using calorimetry. Results were presented for micellization and co-micellization (the case of a polar micelle incorporating amphiphilic molecules) and also for the effects of salt concentration on these phenomena. The heats of solution of a series of *n*-alcohols in water and in soap-water mixtures were discussed in terms of the heats of hydration of the hydrocarbon chain and the polar or ionic head group. The contribution to the energy terms due to increased unbinding of counter-ions in the co-micellization process was discussed, and the calorimetric measurements were supported by measurements of conductance, *p*H and sodium ion activity in these systems; the latter measurements involved the use of a sodium responsive glass electrode.

Mr. J. Clifford and Dr. B. A. Pethica (Unilever, Port Sunlight) reported the results of measurements on the self-diffusion of micelles of sodium dodecyl sulphate (dye labelled) and on the self-diffusion of sodium ions (radio-tracer labelled) in the presence of these micelles. The mechanism of binding counter-ions to the micelle by means of electrostatic forces was discussed. The results of some preliminary studies on micelles using nuclear magnetic resonance were also presented. A change in spin lattice relaxation time of the water protons was found in the presence of the micelles, suggesting that water had been immobilized near the micelle surface. The spin lattice relaxation time of the protons in the CH$_2$ groups in the interior of the micelles was approximately ten times shorter than the relaxation time of CH$_2$ groups in liquid hydrocarbons. This suggested that the alkyl chains in the interior of the micelle were under compression.

The structure and stability of black foam films were discussed by Dr. J. F. Goodman (Procter and Gamble, Ltd., Newcastle upon Tyne). Experiments were described in which simultaneous measurements were made of the total film thickness, using an optical method, and of the water content using infra-red spectroscopy. The detergent content of the film was determined by radio-tracer experiments. It was shown that the black film (50–100 Å thick) has a simple lamellar structure in which two monolayers of surface-active molecules are spread on a thin aqueous core. In mixed cationic–anionic surface-active systems it was found that the ratio of anionic to cationic material in the film was always 1 : 1. irrespective of the ratio in the bulk liquid. Under these conditions it appeared unlikely that the film could be stabilized by electrostatic repulsion between opposing monolayers of soap ions. The factors influencing the stability of the thin films were discussed in terms of the evaporative equilibrium of the volatile film component. It was suggested that the stability of a black film could be maintained by an excess of solute, relative to the bulk solution, in the aqueous film core.

Prof. B. Stuke (University of Munich, Germany) discussed the thermodynamics of micellar systems. In the first part of the paper a discussion was given of the respective merits of using the law of mass action to describe micellization and of considering the micelle as a separate phase. It was concluded that thermodynamically the law of mass action gives the sound approach, the phase approach being only an approximation. Starting from a model for the process of micelle formation at interfaces the second part of the paper was concerned with the influence of micelles on the stabilization of thin films.

R. H. Ottewill
G. D. Parfitt

GEOLOGY AND GEOPHYSICS IN THE U.S.S.R.

THE following are abstracts of geological topics that appeared in the Soviet journal *Priroda* during 1962; for convenience they are arranged under general headings (in italics).

Tunguska 'meteorite'. The so-called Tunguska 'meteorite', which fell in Siberia (60° 55′ N., 101° 57′ W.) on June 30, 1908, is once more in the news as the results of the 1961 expedition are made known. This expedition remapped the directions of the fallen trees over an area of 2,000 km^2, and located and investigated the nature of the so-called 'impact' craters and the composition of spherical granules extracted from the ground. According to the conclusions of V. G. Fesenkov (*Priroda*, 8, 24; 1962) and K. P. Florensky and I. T. Zotkin (*Priroda*, 8, 31; 1962), the impact was not that of a meteorite but of a comet which struck the Earth at a glancing angle and exploded. However, according to N. I. Piavchenko (*Priroda*, 8, 39; 1962) the observed cavities in the peat bog are not due to impact but to ordinary processes occurring in regions of permafrost.

Meteorite crater in Sayan Range. According to M. V. Voroshilov (*Priroda*, 3, 107; 1962) an elliptical hollow, rimmed by a 'bulge' and filled by a lake, some 150–400 m in diameter, situated on the eastern foothills of the Western Sayan Range, represents a meteorite crater. The author suggests that the meteorite responsible for this hollow fell about 150 years ago.

Biogeochemistry. Recent research in biogeochemistry carried out in the U.S.S.R., including the part played by micro-elements in animal nutrition, rational compensatory feeding and biogeochemical provinces and zones, is discussed by V. V. Kovalsky in the June issue of *Priroda* (6, 3; 1962).

Arctic regions. A brief review of recent expeditions to the Soviet Arctic has been made by B. A. Kremer (*Priroda*, 6, 61; 1962), while an up-to-date general account of the geology of the Arctic region is provided by Y. M. Pushcharovsky (*Priroda*, 4, 35; 1962). The latter article contains a reduced version of a tectonic map of this region compiled by the members of the Institute of Geology of the Arctic. In the descriptive part, the author provides a brief geological–tectonic account based on recent publications in the U.S.S.R., Finland, Norway, Greenland and Canada.

Elbrus. According to J. Y. Panteleev and Y. P. Masurenkov (*Priroda*, 5, 61; 1962), Elbrus (5,630 m) —the highest mountain in Europe—is not an extinct volcano, but only dormant. This is testified by its extensive fumarolic field giving out numerous steam and gas emanations under its ice cap. The principal gases emitted are carbon dioxide, chlorine, fluorine and boron compounds. Some emanations are 200°–300° C at their source.

Geothermy. The first All-Union Conference on the problems of geothermy was held in Moscow during March 1–6, 1956, and the transactions, edited by V. I. Vlodavets and others, were published in the form of two volumes entitled "Problems of Geothermy and Practical Utilization of the Heat of the Earth" (*Pub. Acad. Sci. U.S.S.R.*, 1; 1959; 2; 1961). The first volume contains 22 articles, including contributions by I. D. Dergunov, E. A. Lyubimova (both dealing with the thermal regime and history of the Earth), B. N. Dostovalov (the thermodynamic study of the Earth), V. I. Vlodavets (the hydrothermal regions of Italy and New Zealand) and A. M. Zhirmunsky (problems of geothermo-energetics). These volumes also contain sections dealing with problems of regional geothermy and methods of geothermal studies. The second volume contains 26 articles dealing with problems of hydrogeothermy and detailed regional investigations of hydrothermal regions of the Soviet Union. One of the articles, by S. A. Dzhamalov, deals with the thermal waters in Daghestan. In a more popular article (*Priroda*, 6, 74; 1961) the same author describes the progress made in the utilization of thermal waters in Makhach-Kala, the capital of Daghestan. This town has already six active boreholes supplying 4,000 m³ of water at 55°–68° C per 24 h. This water is used for heating and supply of hot water for domestic and industrial needs. A new borehole is projected to supply 2,000 m³ of water up to 160° C per 24 h to a geothermal power station.

Geocryology. Geocryology, or the science of permafrost, as studied in a special Institute of the Academy of Science, U.S.S.R., is of great importance due to its wide application in the Soviet Union, especially in connexion with buildings, canals and roads. A brief account of permafrost in the Union, illustrated by a new geocryological map, has been provided by I. A. Nekrasov (*Priroda*, 2, 19; 1962).

Slow movements of the Earth's crust. In 1960, at the seventh general assembly of the International Union of Geodesy and Geophysics held in Helsinki, a special commission for the study of micropulsation and slow movements of the Earth's crust was established, under the chairmanship of Y. A. Meshcheryakov. Various aspects of this work are presented by L. E. Setunskaya (*Priroda*, 3, 27; 1962) and V. F. Bonchkovsky (*Priroda*, 7, 48; 1962), while V. V. Lamakin (*Priroda*, 7, 53; 1962) discusses the slow movements of the Earth's crust in the region of Lake Baikal.

Geochronology. Much work is being carried out in the Soviet Union on geochronology. Recent research in this field is summed up by I. E. Starik and E. V. Sobotovich (*Priroda*, 5, 75; 1962), who provide a summary date of absolute age for the geological periods. They also conclude that the ancient parent matter of meteorites is 4,500 million years old, while the minimum age of the Earth is of the order of 3,500 million years. In another article, V. V. Cherdyntzev (*Priroda*, 6, 42; 1962) gives an account of the origin and development of the potassium-argon method of geochronology. This method was originally demonstrated by V. G. Khlopin and E. K. Gerling and rapidly developed by numerous research workers, among whom was prominent the group led by A. A. Polkanov in the Laboratory for the Study of the Precambrian at Leningrad. At the present time, more than twenty laboratories carrying out 'potassium-argon' measurements for dating are functioning in the U.S.S.R.

S. I. TOMKEIEFF

THE ATOMIC ENERGY COMMISSION OF JAPAN

THE fifth annual report of the Atomic Energy Commission of Japan, which was established in 1956, is the first to be published in English*. It describes in some detail the aims and activities of the Commission and its subsidiary organizations, the Japan Atomic Energy Research Institute (JAERI), the Atomic Fuel Corporation (AFC) and the National Institute of Radiological Sciences (NIRS). The total number of employees of these organizations now exceeds 1,900. About 60 per cent of the Governmental budget (about 8,000 million yen) for atomic energy is allocated to JAERI. The Research Institute has three reactors: *JRR*-1, which has continued normal operation during the year under review; *JRR*-2 which reached criticality during October 1960 and which is used by private companies and universities; and *JRR*-3, which was due to be completed during September 1961 and to become critical by March 1962. The Japan power demonstration reactor (*JPDR*) is being constructed by the General Electric Co., United States, and the construction of *JRR*-4 (shielding research reactor) by JAERI was to be completed by the end of the fiscal year 1961. Reactors are under construction also at Rikkyo University, the Musashi Institute of Technology and Kinki University. Among the private concerns, both Hitachi, Ltd., and Tokyo Shibaura Co., Ltd., have decided to build training reactors and to gain atomic energy experience under Government subsidy. During August 1960, the JAERI concluded a contract for the purchase of a *BWR*-type power demonstration reactor.

On February 8, 1961, the Commission announced a new long-range programme of nuclear power generation. On the assumption that nuclear power will become competitive with thermal power in cost in about 1970, it is estimated that new power-generating capacity of 36,000 MW will be required between 1961 and 1970, and 48,000 MW between 1971 and 1980, and that of this 1,000 MW and 6,000–8,000 MW will be provided by nuclear power plants during the respective decades. Private companies are expected to install the nuclear power stations, which will be of the light-water-cooled and gas-cooled types, and possibly in later years the organic cooled type.

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* Japan, Atomic Energy Commission. Fifth Annual Report, 1960–61. Pp. iv+167. (Tokyo: Atomic Energy Bureau, Science and Technology Agency, 1962.)

A centre for nuclear research and development is being completed at Tokai-mura, and an institute for plasma physics is being set up on the Higashi-yama campus of Nagoya University. The institute will consist of seven research divisions covering theoretical studies and high-temperature generation experiments. An investigation of the required high-voltage supply and of the pinch effect was initiated in 1957 at Osaka University and since then plasma physics research has been carried out at Tohoku University, Tokyo University of Education, Tokyo University, Nagoya University and Kyoto University. At present there are approximately sixteen different forms of apparatus planned or under construction by twelve plasma physics research groups in Japan.

In the chapter of the annual report dealing with uses of radiation it is stated that most of the radioisotopes used in Japan are imported. During 1960 a total of 55,000 curies were imported, consisting mainly of cobalt-60, phosphorus-32 and iodine-131. By the end of March 1961, 867 establishments, representing medical organizations, private firms and universities and research institutes, in approximately equal numbers, were using radioisotopes. JAERI is preparing for full-scale production of radioisotopes in order to fulfil the future domestic requirements. The first six isotopes to be produced will be sodium-24, phosphorus-32, sulphur-35, potassium-42 and gold-198.

Other chapters of the report deal with nuclear equipment and materials; radioactivity surveys; treatment of radioactive waste; regulations and radiation protection; and training of scientists and engineers. It is considered that 11,000–13,000 scientists and engineers in nuclear energy will be required in the next ten years and it will be necessary to train and educate some 8,000 in those ten years. At present, the possible number to be trained each year is 150–200 at universities, about 200 at JAERI and NIRS, and 90 at institutions outside Japan.

S. Weintroub

GASEOUS OPTICAL MASERS

Bell Telephone Laboratories Inc. have issued a brochure describing recent work at Murray Hill, New Jersey, on the gaseous optical maser. The Bell Laboratories are the most active single organization in this field, and have recently extended the range of wave-lengths available from the maser. The first type of gas discharge optical maser, initially operated by Javan, Bennett and Herriott in February 1961, used a helium–neon mixture and produced an output at 1·15μ. Since then, population inversion sufficient to support maser oscillation has been obtained in gas discharges in pure inert gases (helium, neon, argon, krypton and xenon) providing a total of fourteen different wave-lengths in the range 1·618–2·189μ. Inversion has also been achieved between two levels of the oxygen atom by dissociating molecular oxygen in collisions with an inert gas atom, either argon or neon, in a metastable state, using a discharge in a mixture of oxygen and the inert gas. The maser output in this case is at 8446 Å. The shortest wave-length, and consequently the most useful for applications involving high-efficiency photoelectric detection, lies in the visible at 6328 Å, and has been obtained from a helium–neon maser with mirrors having a high reflectivity at this wave-length.

Power outputs of these new masers are in the range from a fraction of 1 mW to 10 mW. The experimental work has been done with masers using external confocal mirrors and a discharge tube having end windows at the Brewster angle to the emergent beam, thus eliminating, for the appropriate plane of polarization, reflexion and consequent energy loss at the windows.

The information in the Bell Telephone Laboratories brochure is an indication of the great effort which is being put into the development of the gaseous maser, which is already being applied to a number of research problems and may, in the future, find uses in the communications field.

J. H. Sanders

EFFECT OF MOISTURE MOVEMENT OF NATURAL AGGREGATE ON CONCRETE

Some years ago investigations of concrete failure, thought to be due to the chemical reactivity of certain rock-types involved with the cement during hydration, were carried out in the United States and later in the United Kingdom. Arising from this, certain rock-types, for example, some dolerites, especially those containing the chlorite mineral chlorophaeite, became suspect. Experimental work was based largely on chemical and petrological studies to determine potential reactivity with alkalis released during hydration of Portland cement in concrete involving aggregates dominated by supposedly unstable components. Methods of determination of such components were tentatively laid down by the American Society for Testing Materials (*Proc.*, 48, 1948; and *Standards*, 1952), and in the United Kingdom F. E. Jones has discussed the subject in some detail (*National Building Research Studies*, Nos. 14, 15, 16; 1952).

The evidence from this experimental work cannot be considered as conclusive; in fact, some of it is so conflicting as to raise grave doubts on the alleged chemical vulnerability of certain dolerite aggregates, including natural gravels containing these rock-pebbles, or others loaded with acid volcanic types, also suspect. There is at present what appears to be a more convincing explanation of this type of concrete failure. In a paper by L. C. Snowdon and A. G. Edwards, entitled "The Moisture Movement of Natural Aggregate and its Effect on Concrete" (*Concrete Research*, 14, 109; 1962), is described the work of the Scottish Laboratory of the Building Research Station on the failure of pre-cast concrete products in which certain crushed dolerite aggregates had been used. It is pointed out that in Scotland dolerite aggregate is extensively used in concrete.

The interest of the Department concerned ". . . became focused on its performance in 1952 when very severe cracking developed in concrete specimens after a single winter's exposure on the exposure site at Thorntonhall, near Glasgow. It was later found that the dolerite rock itself exhibited large dimensional changes in water content". Volume changes due to chemical reaction were investigated but not substantiated. Since dimensional changes on wetting and drying of the rock itself occurred, a study was made of the expansion and contraction caused by moisture in various Scottish dolerites and concretes made from them. The experiments were extended to other rock-types as well as natural sands and gravels used as aggregates for concrete. The arguments in this paper are convincing and establish that many natural crushed rock and gravel aggregates undergo changes of volume as they take up water, thus influencing both the shrinkage and the durability of the concrete in which they are incorporated. H. B. MILNER

DEMOGRAPHY OF MAURITIUS

THREE hundred and fifty years ago the island of Mauritius was uninhabited by man. The only creatures there were some indigenous reptiles and birds, and a few domestic animals left by passing sailors. To-day, the dodo is extinct, but within the island's narrow confines lives one of the most rapidly growing human populations in the world.

Mauritius is a hurricane-battered island in the Indian Ocean. It is about 500 miles east of the island of Madagascar, more than twice that distance from the south-eastern coast of Africa, and 200 miles north of the Tropic of Capricorn. In a land area of 720 sq. miles, there are 990 Mauritians for every square mile, an immense burden for the island's meagre natural resources and limited facilities. Until 1946, the rate of natural increase was low. Immigration accounted for almost half the total growth. In 1946 there were 428,000 people on the island. Recently, a dramatic shift in the survival pattern upset the two-centuries-old balance between births and deaths which had held the population in check. In just five years, the death-rate dropped from 36 to 14 and the birth-rate rose from 38 to 50 per thousand of the population per year.

It is estimated that, in 1961, there were 656,000 people on the island, an increase of more than 50 per cent in only 15 years. During the period, natural increase averaged 2·9 per cent a year, one of the highest rates in the world. Immigration had not been an important feature in population growth. By 1961 the Mauritian death-rate was 10, lower than that of the highly urban-industrial United Kingdom. The Mauritial birth-rate had receded to 40, a rate typical of those found in less-developed countries. Should the phenomenally rapid multiplication of people continue, Mauritius would have a population of more than two million in the year 2,000—a three-fold increase in only 39 years.

One of the most serious consequences of this over-rapid growth is a population with a bottom-heavy age structure which adds to Mauritius's economic difficulties. To-day, 44 per cent of the population is under 15 and another 3 per cent is 65 years or older. If the rate of population growth is maintained, the increase in the economically dependent age-groups could strangle the economy. In less than 25 years, the number of children under 15 alone would be larger than the entire population in 1960.

This island is one of the few less-developed countries with a tradition of reliable vital statistics, introduced by the British. Thus, Mauritius serves as an almost perfect small-scale example of how too-rapid population growth can depress living-levels when the resource base is meagre and limited.

There seems to be but one ray of hope in Mauritius. Although the birth-rate is still alarmingly high, it has declined from its record peak of 50 in 1950 to 40 in 1961. The way in which the rising population of Mauritius may be controlled has been described by Robert C. Cook, editor of *Population Bulletin**.

* Population Reference Bureau. *Population Bulletin*, 18 No 5, August 1962: *The Story of Mauritius—From the Dodo to the Stork*. Pp 93–116 (Washington, D.C: Population Reference Bureau Inc, 1962.) 50 cents.

LACK OF ACTIVATION OF PHOSPHORYLASE BY ADRENALINE DURING ITS PHYSIOLOGICAL EFFECT ON INTESTINAL SMOOTH MUSCLE

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AN activation of phosphorylase by adrenaline has been observed in liver, skeletal muscle and heart muscle *in vitro* and *in vivo*[1]. When conventional experimental procedures were used, similar results were obtained from isolated intestinal smooth muscle preparations from the taenia coli of the guinea pig[2]. An improved technique was used for the experiments to be described in this communication, and the results show that an activation of phosphorylase does not occur in the tissue while the physiological effect takes place, and that earlier results[2,3] must be ascribed to a conversion of phosphorylase *b* to phosphorylase *a* during the period which elapsed between the removal of the tissue from the adrenaline-containing medium and the assay of enzymatic activity. As these observations are limited to a single type of smooth muscle, they do not rule out the possibility that activation of phosphorylase has a relationship to the physiological actions of adrenaline in other tissues. However, recent observations of Mayer, Cotten and Moran[4] have also indicated a lack of correlation between phosphorylase activation and the positive inotropic effect of adrenaline on the myocardium.

Phosphorylase activity was measured by the determination of glucose-1-phosphate produced from glycogen in the presence of inorganic phosphate. Conversions of phosphorylase *a* to *b* and of *b* to *a* were inhibited by the addition of sodium fluoride and of ethylenediamine tetraacetic acid, respectively. The possibility of these interconversions was minimized further by carrying out the assays at a 1,200-fold dilution of the tissue constituents, a procedure which reduced the final concentration of endogenous AMP to less than 2×10^{-7} molar. Activation of taenia coli phosphorylase *b* under these conditions was not observable below an AMP concentration of 1×10^{-5} M.

The tissue was homogenized at 0° with 100 vol. of a solution containing 0·02 M citrate buffer (*p*H 6·7) 0·001 M EDTA, 0·01 M sodium fluoride and 0·3 per cent serum albumin. Immediately thereafter 0·05 ml. of the homogenate were incubated in a centrifuge tube for 30 min at 37° C in a total volume of 0·6 ml. of a solution which had the following constitution: 0·5 per cent glycogen, 0·05 molar potassium phosphate buffer (*p*H 7·5), 0·01 M sodium fluoride, 0·001 M EDTA and 0·3 per cent serum albumin. Another 0·05 ml. of the homogenate was incubated in the same reaction mixture containing, in addition, 5-adenylic acid (AMP) in a final concentration of 1×10^{-3} M. At the end of the incubation period, 0·09 ml. of N hydrochloric acid were added. The tube was placed in an ice bath and 0·09 ml. of N potassium hydroxide and 0·23 ml. of a solution containing 0·2 M imidazole buffer (*p*H 7·5) (ref. 5) and 0·005 M magnesium chloride were added. The mixture was centrifuged at 8,000*g* and 0° C for 30 min. Glucose-1-phosphate was determined spectrophotometrically in an aliquot of the supernatant solution by the use of crystalline phosphoglucomutase[6] as described elsewhere[7]. The amount of glucose-1-phosphate formed in the absence of AMP was a measure of phosphorylase *a* activity, while total phosphorylase (*a* + *b*) activity was equivalent to the formation of glucose-1-phosphate in the presence of AMP.

Weighed pieces of taenia coli were suspended either isometrically (ratio $\frac{\text{wet weight (mg)}}{\text{length (mm)}}$: 0·65–0·8) or isotonically (initial tension, 1–3 gm) in 120 ml. of a modified Krebs solution[8] at 37° ± 0·5° C and gassed with oxygen containing 3 per cent carbon dioxide. After a period of 1–2 h, control samples were removed for assay, and adrenaline was added to the bath medium to yield a final concentration of 5×10^{-7} M. Tension recordings with the aid of spring balances[8] revealed that, without exception, addition of the hormone produced a decrease in tension within 5–10 sec. The tissue samples were removed for assay 5 sec–2 min after addition of the hormone. The results are shown in Table 1.

Table 1. PHOSPHORYLASE *a* ACTIVITY (IN PER CENT OF TOTAL PHOSPHORYLASE ACTIVITY) OF TAENIA COLI BEFORE AND AFTER EXPOSURE TO ADRENALINE (5×10^{-7} MOLAR)

Group	(*a*) Assayed at once			(*b*) Assayed after drawing or rubbing tissue on dry glass surface		
	No. of samples	Limits	Average	No. of samples	Limits	Average
Control	21	0–3·8	1·6	17	1·4–4·6	3·1
Adrenaline	46	0–4·1	1·6	21	5·1–11·4	8·3

In series (*a*), 27 out of the 46 samples were taken 5–15 sec after addition of adrenaline, that is, as soon as maximal relaxation of the muscle had occurred. In the control samples, phosphorylase *a* activity of taenia coli was minimal and was not increased by adrenaline (Table 1, series *a*). These results were obtained consistently if the tissue was placed immediately in the homogenizing medium and homogenized at once after removal from the adrenaline-containing bath medium.

In series (*b*), on the other hand, the phosphorylase *a* activity of the controls was higher and it was significantly increased by adrenaline. In this series the muscle samples were first dried by drawing them repeatedly over a glass plate for 5–8 sec, or they were placed on the upper dry part of the inner wall of the homogenizer and then pushed down along the wall into the homogenizing fluid. In a previous study[3], when the tissues were dried in this manner for the purpose of weighing, an increase in phosphorylase *a* occurred with adrenaline 5×10^{-7} M, but not at a 20-times lower concentration. When the tissue was cut in an ice bath prior to the assay, phosphorylase activation was observed also with 2×10^{-8} M adrenaline[2]. When the muscle was handled in this way, the increase in phosphorylase *a* activity by adrenaline was the more pronounced the higher the concentration of adrenaline.

It is possible that manipulation of the muscle after removal from the bath results in phosphorylase activation by cyclic 3′,5′-AMP. Since adrenaline stimulates the production of 3′,5′-AMP (ref. 9) more 3′,5′-AMP may have been available after treatment with adrenaline. Thus the formation of 3′,5′-AMP would take place in the intact tissue, while the activation of phosphorylase would take place after the removal of the tissue from the bath. In our work, during relatively short periods of manipulation, only some (less than 10 per cent) activation of phosphorylase was observed in control samples. But it has been reported[10] that, after prolonged grinding with sand, more than 60 per cent of the total phosphorylase activity in tracheal and arterial smooth muscle may be present as phosphorylase *a*. It would appear that phosphorylase *a* activity, determined after this treatment and delay, bears no relationship to the enzyme activity in the intact tissue, and the very high initial value may explain the failure to observe in these conditions a further increase after exposure to adrenaline.

Total phosphorylase activity of taenia coli varied between 2·06 and 3·88 μM of glucose-1-phosphate formed per min and per g of tissue in the absence of sodium fluoride. Fluoride in a concentration of 0·01 M inhibited total phosphorylase activity to approximately 20 per cent. Nevertheless, this concentration of sodium fluoride was used in the series of experiments summarized in Table 1 in order to ensure inhibition of the ('*PR*') enzyme converting phosphorylase *a* to phosphorylase *b*. Another series of experiments carried out in the absence of sodium fluoride, but otherwise under identical conditions, yielded the same results, that is, phosphorylase *a* activity was only increased following exposure to 5×10^{-7} M adrenaline, if homogenization was carried out after some manipulation.

It is concluded that adrenaline causes no activation of phosphorylase in the intact tissue at the time when it produces relaxation of intestinal smooth muscle by causing cessation of spike activity and hyperpolarization. Phosphorylase *a* activity may be increased afterwards by subjecting the tissue to certain manipulations, and this activation is greater after previous treatment with adrenaline. No activation is demonstrable with an improved technique. Since the changes in the electrophysiological and mechanical state of taenia coli produced by adrenaline require metabolic energy[12], it appears that biochemical mechanisms other than activation of phosphorylase or of phosphofructokinase[3] must be associated with these effects.

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OVARIAN TUMOURS INDUCED BY A STERILIZING STEROID

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PROTRACTED sterility was induced in mice by subcutaneous pellets of 19-nor-progesterone; when the pellets were removed after 108 or 176 days (comparable with 5 or 10 years in the reproductive life-span in women) the animals recovered fertility[1]. Reproduction has been examined in these females for as long as 8 months after removal of the sterilizing pellets; reproductive behaviour remained quantitatively the same as in normal females, and the decline of fertility set in, as in the latter, at the age of about 13–14 months[2].

It was thought that experiments with protracted sterility may offer the opportunity to approach experimentally fundamental gerontological problems, that is, the mechanism underlying the decline and cessation of reproduction, as conceived already in the classic work of Hunter[3] and more recently studied by Jones and Krohn[4]. We shall not enter here into major details referring to these gerontological problems or to the results obtained in new experiments of ours in which the steroid-induced sterility was maintained in mice (*BALB*) with subcutaneous pellets of 19-nor-progesterone for as long as 13 months or more[5]. We shall deal here with only one observation.

Quite unexpectedly ovarian tumours were discovered in these animals. A detailed microscopical study of the ovaries of 33 experimental animals was made; the results were compared with those in 14 normal animals. The latter were of the same strain and of the same age (Table 1), mostly even of the same litters as the experimental animals; each normal and corresponding experimental animal was maintained during the whole life-span together in the same cage, with the male also.

Table 1

Group	Age range (average) days	Duration of treatment* days	No. of animals Total	With gran.-cell tumour	With abundant lut.-cells 'luteoma' (?)
Normal	451–705 (597)	0	14	0	0
With 19-nor-progesterone	478–718 (600)	395†–559	33	8	17‡

* Implantation of pellet every 6–7 months.
† 2 animals 375 days only.
‡ Omitting animals with granulosa-cell tumour in the contralateral ovary.

There were granulosa-cell tumours in no less than 8 animals treated for 13 months or more; the granulosa-cell tumour was unilateral in 7 cases and

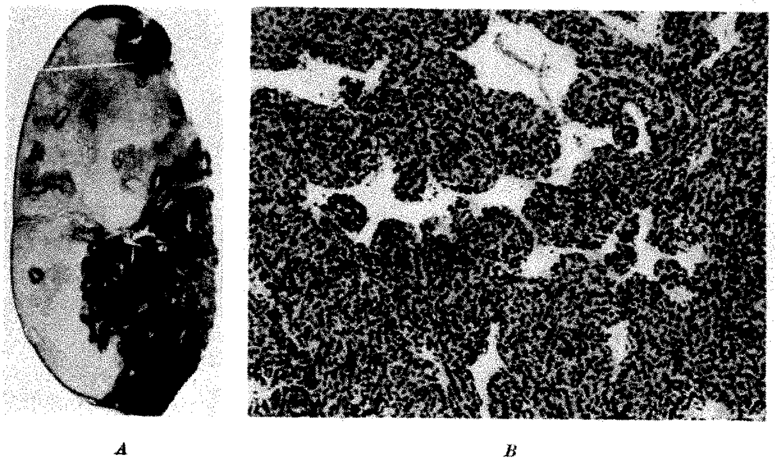

Fig. 1. 402 days with subcutaneous pellet of 19-nor-progesterone (40 per cent). Killed at an age of 693 days. Weight of right ovary, 1,250 mg. *A*, right ovary (× 5). Granulosa-cell tumour with extended degeneration of the tumour (evolutional phase 17, according to our scheme[7,8]). *B*, Same ovary (× 100). Typical granulosa-cell tumour. (Weight of the left ovary: 3 mg; great abundance of 'lutein' cells; follicles were present but no corpora lutea: non-exclusive luteoma)

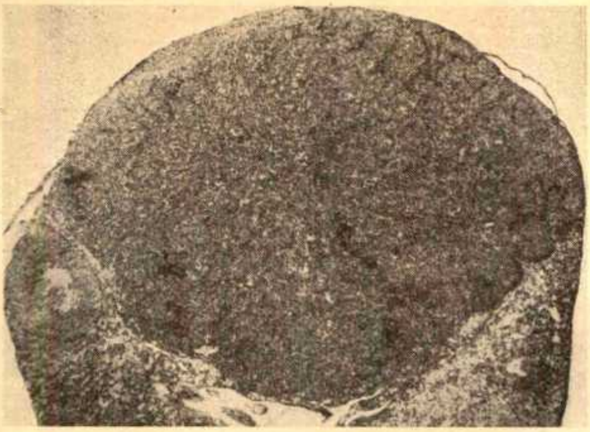

Fig. 2. 510 days with subcutaneous pellet of 19-nor-progesterone (40 per cent). Killed at an age of 591 days. Weight of the ovaries: 4 and 4 mg (× 35). Granulosa-cell tumour immediately beneath the ovarian surface, occupying great part of the ovary. Graafian follicles present; no corpus luteum. (Out of the 9 ovaries with granulosa-cell tumours 5 belonged to the type of Fig. 2, but they were smaller than the latter)

bilateral in 1 case. Examples of these tumours are given in Figs. 1 and 2.

The ovaries of most of the treated animals contained a great abundance of nodules of large cells containing vacuolated protoplasm ('lutein' cell). Similar cells are present also in the ovary of mice of normal age of different strains[6] and so also in our present strain[7]. But the difference between the ovaries of treated and normal animals with regard to size of these nodules was considerable. The nodules of large cells are very similar to those of the so-called luteoma in mice with sub-total castration and intrasplenic ovarian transplantation[8]. However, there was no 'exclusive' luteoma as in the last-mentioned experiments. Follicles, including large Graafian follicles, were absent in only 3 or 4 of the ovaries with granulosa-cell tumours; but they were present in all ovaries with the whole stroma transformed into nodules of 'lutein' cells.

It is not for us to discuss here whether our findings are of importance concerning the control of reproduction in women by the use of 19-nor-steroids. We may only mention that a steroid-induced sterility of 13 months in mice would correspond to one of 25 or more years in women; and that the use of anti-ovulatory steroids in women is never a continuous one as in our experiments in mice, but always a discontinuous one, thus allowing for regularly intercalated 'menstruations'[9]. Indeed, one may wonder whether a steroid-induced sterility of shorter duration than in our present work, or even a steroid-induced discontinuous anti-ovulatory condition as in women, may in the long run also be harmful for the ovary similarly to what happened in our experiments with protracted sterility of 13 months.

On the other hand, our results are of considerable interest from the point of view both of ovarian physiology and pathology. By the removal of CH_3 in C–19 as effected in the laboratory, transforming progesterone into 19-nor-progesterone, the progestational action of progesterone is increased about tenfold. One may then ask why the normal body is working with progesterone and not with 19-nor-progesterone; the question is quite justified in view of the fact that the body is able to remove CH_3 in C–19 as in œstrogens. Is it "The Wisdom of the Body" (these are Cannon's words) to prefer progesterone to 19-nor-progesterone, the latter being toxic since it is too active, and thus rendering rather difficult the adjustment of the progesterone homeostasis in the body? Indeed, like progesterone, 19-nor-progesterone is inactivated in the liver[10]; its metabolic fate in the body[11] also is seemingly coincident with that of progesterone. The problem of the comparative toxicity of the two compounds can be dealt with in experiments with protracted steroid-induced sterility; such work is in progress in this laboratory.

Of a more definite kind are our new findings for the understanding of ovarian pathology. From former work with sub-total castration in mice[7] and with intrasplenic ovarian grafts[12] it was concluded that there is, in these experimental conditions, an ovarian-hypophyseal imbalance primarily due to a non-adjusted steroid homeostasis. Adjustment of the steroid homeostasis was thought to be one of the bodily mechanisms of 'antitumoural autodefence'[13]. The implication of steroid homeostasis in ovarian tumourigenesis was made especially evident in experiments with combined intrasplenic and intrarenal ovarian grafts[14]. The present work with the production of ovarian tumours by the protracted administration of a steroid gives definite experimental evidence that a failure in the adjustment of the homeostasis of progestational steroids may be at the root of ovarian tumourigenesis. [*Note added in proof*. Recently granulosa-cell tumours have been elicited in female dogs by the administration of diethylstilbœstrol[22].]

Are the dynamics of ovarian tumourigenesis, as due to the administration of steroids, coincident with those in experiments with sub-total castration and intrasplenic grafts? Are the tumours induced by the administration of steroids also due to a pituitary imbalance? The same question arises in experiments in which ovarian tumours were elicited with carcinogens[15,16]; certain findings speak decidedly in favour of a pituitary imbalance being implicated in these latter experiments[15]. Since progestational steroids act on the ovary most probably via the hypophysis[17] it would seem reasonable to suppose that the same dynamics are involved also in the tumourigenic action of 19-nor-progesterone on the ovary. The very pronounced antiluteinizing action of 19-nor-progesterone has been evidenced already in former work[18]. How can then the exorbitant development of 'lutein' cells in the ovarian stroma in the present work be explained? There are two possibilities: first, that the 'lutein' cells as present in these ovaries are not to be identified with corpora lutea cells; or secondly, that the gonadotrophic functional pattern of the hypophysis which emerges from the protracted action of the progestational steroid is fundamentally different from any one hitherto known.

When contemplating tumourigenic results as obtained with partial castration or intrasplenic ovarian grafts, and now with the administration of an artificial steroid as 19-nor-progesterone, one may argue that all this is no more than experimental ovarian pathology far removed from reality. However, what comes out of this experimental pathology is but a replica of those spontaneous ovarian tumours which have been described in 11 different strains of mice[19] and in rats[20].

At the time of finishing this preliminary review of our experimental results we were impressed by reading an article just published in the *Lancet*[21] which ends as follows: "The possibility that orally active gestagens exert an unfavourable influence on the hormonal environment controlling fertilization and nidation [in women] needs to be investigated" (from Sir Solly Zuckerman's Department, University of Birmingham).

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FLUORESCENT COMPOUNDS AND NON-ENZYMATIC BROWNING

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THE Maillard reaction, in which sugars and amino-compounds react to form brown insoluble melanoidins, has been examined by many workers[1-4]. On a number of occasions fluorescent substances have been reported both in model browning systems and in foodstuffs, but they have apparently proved intractable and their role has not been disclosed. There have been speculations that they may be by-products, and some fluorescent compounds are known to be formed from amino-acid reactions in the absence of sugar. Simonson and Tarassuk[5] observed that in milk products the development of fluorescence, browning and evolution of carbon dioxide all appeared to progress together, and speculated whether the level of fluorescence could be used as a guide to the browning potentialities of foodstuffs.

In a separate publication[6] we have mentioned the isolation of yellow fluorescent substances from scald liquor and that these substances gave brown polymers when incubated by themselves, and with glycine[7,8]. A number of crude fractions from our model browning systems have also shown a more rapid development of browning in the presence of a nitrogen function that in the presence of aldoses[9,10].

Initial investigations on sugar/amino systems (with P. J. Moody) revealed that fluorescent substances appeared in all sugar/amino-acid incubates, and that the degree of fluorescence and number of fluorescent substances increased as the incubation proceeded; the R_F values of the fluorescent zones varied with the amino-acid used.

Further investigation showed that the sugars, incubated with an amino-function, developed fluorescence much more rapidly and to a greater extent than in its absence. This occurs even at alkaline pH's which would not favour the production of furfurals. We have found that the rate of development of fluorescence of various sugars with amino-compounds in solution follows the same order as does the early browning, the less-stable sugars[11] giving a more rapid development of fluorescence, for example, aldopentoses > aldohexoses, galactose > glucose. We have already reported[12,13] an examination of sulphited glucose/glycine systems which showed that after the initial period (during which α-amino-NH_2 dropped and colourless intermediates were formed) an increase took place in the conjugated unsaturated carbonyl content of the system concomitant with an increase in the quantity of yellow fluorescent substances; colour development rapidly followed the carbonyl/fluorescence production until the later stages of the browning, when the colour deepened, presumably due to co-polymerization, while the concentrations of the conjugated unsaturated carbonyl compounds and fluorescence intensity diminished. Pre-incubated glucose solutions develop coloration more rapidly with glycine than do the fresh unheated glucose solutions, and fluorescence development was found to occur more rapidly in this case.

There seemed a strong possibility that the fluorescent compounds might be cyclic unsaturated carbonyl compounds, formed by cyclization of the unsaturated compound formed following the initial sugar carbonyl–amino group link up. It was also felt that some of the fluorescent compounds might contain nitrogen. In a semi-quantitative analysis of their impure blue/purple fluorescent fractions from the reaction between 2-*N*-acetyl-L-lysine and glucose, Hannan and Lea[14] found a nitrogen content of 5–10 per cent and a reducing action in ammoniacal silver nitrate. Their products were not furfural derivatives; one of them browned on heating. Cyclization to give a pyridine ring in a simple reaction between ammonia buffers and an unsaturated aldehyde[15,16] is already known to take place. This reaction seems explicable by the same type of mechanism which we believe occurs to give some fluorophores.

We have now examined the development of fluorescence in both sugar/amino-acid and in sugar/amino aqueous systems and have followed its development on heating dry synthetic glycosylamines. The yellow fluorescent compounds can be produced very rapidly by incubating the aqueous solutions at 50° C, and they can be selectively extracted using a range of solvents, for example, ethyl acetate, *cyclo*hexanone, *n*-butanol. From our results it would seem that the first fluorescent compounds formed from ethanolamine are hydroxylic, since they extract into butanol but not into cyclohexanone or ethyl acetate. On further incubation, we obtained yellow fluorescent fractions in each solvent, presumably due to dehydration or combination. The generation process is continuous in the early stages, since on re-incubation over four cycles the extracted aqueous residues produced a similar range of selectively extractable yellow fluorescent compounds. Haas[17] had previously extracted browning mixtures with ethyl acetate and found a multiplicity of carbonyl compounds absorbing in the conjugated unsaturated carbonyl region about 285 mμ. This continuous extraction process retarded the browning of his mixture, but even so it appears from our results that using this solvent he may not have isolated some of the first types of compound formed. Wolfrom and his co-workers had shown that the addition of dimedone removed carbonyl-compounds from glucose- and xylose-/glycine systems and thereby retarded the chromophore development of the residue.

The initial compounds isolated in our investigations on the xylose/ethanolamine system were extracted into butanol, precipitated with hexane, and the product leached with acetone and chloroform. The viscous solid remaining after this treatment was dissolved in methanol and chromatographed on 'Celite' using chloroform/methanol as solvent. The product gave two impure yellow fluorescent compounds (found, N – 15·2 per cent, 9·6 per cent) which on treatment with 2 : 4 dinitrophenylhydrazine gave 2 : 4 dinitrophenylhydrazine derivatives. On attempted acetylation of milligram quantities of the fluorophoric material with warm acetic anhydride and anhydrous sodium acetate the solution immediately darkened and the product, unlike the reactant, was soluble in acetone. However, it still gave carbonyl reactions and a precipitate with 2 : 4 dinitrophenylhydrazine. When acetylation was carried out in the cold no discoloration occurred and the product was soluble in acetone and still carbonylic in nature. The explanation may be that under warm acid conditions dehydration rapidly occurs with the production of vinyl groups, which polymerize rapidly to give a carbonyl polymer whereas in the cold, acetylation of the hydroxyl groups occurs. When the yellow fluorescent substances isolated from aqueous sugar/amino systems or from heated dry glycosylamines (which all gave 2 : 4 dinitrophenylhydrazones the spectral data of which were indicative of αβ unsaturation) were incubated alone in solution, they developed a different fluorescence. When incubated with glucose at 50° C little or no different fluorescence or colour appeared, but when incubated with glycine the fluorescence altered and the solution darkened, brown polymeric material eventually forming on the sides of the vessel. This appears to be the first proof that these fluorescent compounds are directly involved in forming melanoidins.

Shore and Purdee[18] showed that amino-acids can be detected fluorimetrically after resolution by paper chromatography, by spraying with xylose and then heating. The spray contained 1 per cent sodium sulphite to prevent loss of fluorescence and prevent the production of a brown stain. We have found that the presence of sodium metabisulphite slowed down the production of the fluorophores, which might have been expected from our previous work, but the introduction of sulphites also retarded the development of brown soluble compounds and subsequent insoluble polymers from incubates of the fluorophores. This, then, is another point at which sulphites, if present, can retard non-enzymatic browning. In the solid state we found that sodium sulphite, co-precipitated with glycosylamines by drying from an aqueous dispersion *in vacuo* over phosphorus pentoxide, also retarded development of fluorescence and brown polymer formation on heating, and a similar effect was noted when comparing the fluorescence development and browning-rates of glucose anilide and glucose-*p*-toluidide with the corresponding C_1–K sulphonated analogues by heating them in dry state at temperatures from 50° to 100° C.

The proportion of the reactive fluorescent compounds present in the heated sugar-amine compounds at any one time was expected to be low, and brown products were present, so that infra-red readings taken during the heat treatment were not unequivocal. Such indications as were obtained were not inconsistent with the development of unsaturation and cyclic nitrogen compounds.

In foodstuffs the nitrogen functions are often fixed in solid polymers. In order to simulate such a nitrogen functional surface, we incubated 50 ml. glucose/glycine mixture 2·5 M with respect to glucose and 1·0 M with respect to glycine in the presence of 0·5 g of washed, defatted wool. Within 3 days the wool, separated, washed, and dried over phosphorus pentoxide, showed a yellow fluorescence under ultraviolet light. Further 0·5 g portions of wool were admitted to and removed from the solution periodically, giving a range of darkening fluorescence on wool of increasing colour, in order light yellow → golden → cinnamon → brown. There was insufficient wool at each stage to react with all the precursors of fluorescent compounds formed, so that the various stages of the melanoidin reaction were proceeding giving deeper colours on the wool with the passage of time. (Wolfrom's work, as well as our own, showed that even at a late stage the soluble sugar/amino-acid polymers still contained carbonyl functions.)

The control solutions darkened much more rapidly than those from which intermediates had been removed by the wool at each stage, even though there was some extra oxygenation of the latter during opening and filtration.

The solutions were examined visually and measured by means of an EEL photoelectric colorimeter at 460 mμ. Results are given in Table 1.

Table 1. COLOUR DEVELOPED IN A SOLUTION 2·5 M WITH RESPECT TO GLUCOSE AND 1·0 M WITH RESPECT TO GLYCINE, INCUBATED AT 50° C IN THE PRESENCE AND ABSENCE OF DEFATTED WOOL

Incubation period (days)	Colorimeter readings	
	Control solution	Wool-treated solution
3	3·6	2·2
6	19·5	9·1
10	53	33·5
13	86	52

A similar experiment was carried out using successive additions of 2 g portions of amino-cellulose (about 1 per cent nitrogen) in place of wool. The initial solutions (60 ml.) were 1·25 M with respect to glucose and 0·5 M with respect to glycine. Similar results were obtained, though in this experiment there were sufficient amino groups to combine with the early fluorescence precursors and no colour appeared in the treated solutions until a late stage.

After 13 days' incubation the colour readings measured as here were 9·0 for the control and 0·9 for the solution treated with amino-cellulose. Analysis of the washed amino-cellulose gave an initial nitrogen content about 0·93 per cent; 0·9 per cent for the 3-day incubate; 1·4 per cent for the 6-day incubate and 1 per cent for the 10-day incubate.

From the analytical figures it would appear that the initial fluorophores and chromophores are nitrogen-containing carbonyl compounds, and that the colour developing later in the system may be due to co-polymerization with low nitrogen-containing compounds.

When incubated with sugars in the absence of glycine, fluorescence development was slower, and was again suppressed in the early stages of reaction in the solution; then it occurred only on the amino-cellulose. The fluorescent compounds formed on the fibres were not eluted by treatment with organic solvents nor with aqueous/organic solvent mixtures.

It would appear that fluorescent compounds with various side-chains might appear in the course of incubation of a hydroxy carbonyl/amino system, depending under a given set of conditions, on the sugar and on the amino compound used. These intermediate compounds will have differing reactivities which will influence the rate of polymer development. The pentoses develop yellow fluorescence rapidly when incubated with glycine, whereas glyceraldehyde does not and the mixture browns differently. This is probably bound up with a cyclization process since in the absence of aldolization there is a need for at least four carbon atoms from the carbonyl skeleton. Fructose, which develops unsaturation very rapidly on heating and develops fluorescence very rapidly when heated with aqueous ethanolamine, rapidly gives a melanoidin, with loss of fluorescence. This is in contrast with glucose systems, in which fluorescence and early browning occur somewhat later, but the more slowly developing glucose intermediates then produce a darker solution and give a darker polymer. The pentose sugars and fructose rapidly give a precipitate with ethanolamine but not with glycine, which may be due to the greater possibility of a rapid formation of a cross-linking intermediate in the former case. It would seem, too, from our experience with fructose, that the carbonyl function need not be at C_1 in the sugar series for the development of browning. Thus production of a carbonyl function anywhere in the sugar chain following dehydration might lead to melanoidin formation, provided reactive intermediates can be formed. This would appear to us to be the explanation for the rapid development of melanoidins from 2-deoxy-galactose, following a slow utilization of the NH_2 function, reported by Lea and Rhodes[19]. From these considerations one can conclude that the yellow fluorescent substances are in fact fast reacting carbonyl intermediates, active in non-enzymatic browning, and formed as a result of carbonyl-amino reactions.

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HEAT INACTIVATION OF CRYPTIC CATALASE OF YEAST

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ONLY a small fraction of the total catalase activity of the intact, aerobically grown yeast cell can be detected *in situ*[1]; this is the patent, or whole-cell, activity. The latent, or cryptic, activity can be revealed by treating the cell with certain chemical or physical agents[1-3]; we have called this the Euler effect. Such treatment leaves all the enzyme still within the cell, which is now incapable of dividing[3], but causes alteration of certain of the properties of the enzyme.

There are three distinct hypotheses available to account for the phenomenon of enzyme latency.

(1) The interfacial hypothesis pictures all the enzyme of the intact cell adsorbed at an intracellular interface in a condition of low structural specificity, such that its specific activity is low[3]; this hypothesis has had to be abandoned in view of more recent work on the state of catalase in anaerobically grown yeast, where the patent enzyme is essentially the same as that of the aerobic cells, but where there is no cryptic enzyme[4,5].

(2) The permeability hypothesis assumes the existence of only one form of intracellular enzyme and of a barrier to penetration of substrate, such that the enzyme cannot be saturated with substrate; thus, the enzyme activity per cell is low; but there is no real difference between patent and cryptic activities.

(3) The membrane hypothesis supposes that the patent enzyme is bound to some cell membrane at which it is accessible to substrate; the cryptic enzyme is contained within the membrane and its active centre is not accessible to substrate. This hypothesis requires that there exist differences between the location and orientation of the patent and of the cryptic enzymes; it thus suggests the possibility that other enzymatic properties might differ as well.

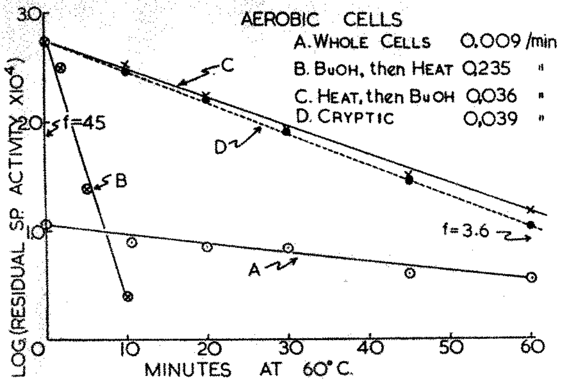

Fig. 1. Rates of heat inactivation of intracellular catalase of aerobic yeast

One property of the cryptic enzyme which can readily be examined and which can serve as a basis of comparison with the patent enzyme is the rate at which it is inactivated by heat.

Suspensions of yeast cells (*S. cerevisiae*) in M/15 phosphate buffer, pH 7·0, were placed in a water bath maintained at 60° ± 0·1° C, and aliquots were withdrawn periodically. The catalase activity of a portion of the sample was determined directly; this was the residual patent activity. The residual total activity was determined by treating the remainder of the aliquot with n-butanol (final concentration 0·8 M), as previously described[3]. The cells were then washed and resuspended in buffer for catalase determination. In some experiments, cells which had previously been treated with butanol and then washed and resuspended in buffer to the original cell density were placed in the water bath alongside the suspension of untreated cells. Enzyme assays were performed by following the decrease in absorption at 240 mμ of 0·013 M hydrogen peroxide, using a Beckman model *DU* spectrophotometer. With this method of assay, the cryptic factor (the ratio of the activity of butanol-treated cells to that of the untreated cells) was about 40–80 in aerobic yeast.

The results of a typical experiment are shown in Fig. 1. Curve *A* represents the decrease in residual patent activity of a suspension of untreated aerobic cells. Curve *C* represents the decrease in total catalase activity of the same suspension, determined after subsequent butanol treatment of the heated cells. The difference between curves *A* and *C* is obviously due to the presence of cryptic enzyme; it is apparent that the cryptic activity was much more rapidly inactivated by heat. During the heating, the cryptic factor diminished from 45 in the unheated cells to 3·6 in those heated for 60 min. Curve *B* demonstrates the high heat lability of catalase in cells treated with butanol and other agents which reveal all of the cryptic activity[2].

It is possible to determine the rate of heat inactivation of the cryptic enzyme itself by subtracting arithmetically the residual patent activities (the logarithms of which are shown in curve *A*) from the residual total activities. Correcting thus for the high heat resistance of the patent enzyme, one sees from curve *D* that the cryptic enzyme has been inactivated at a slightly higher rate than the total activity; the difference is 10 per cent or less, and we may thus take the rate of decrease of total activity (curve *C*) as measuring in effect the heat sensitivity of the cryptic catalase.

During the anaerobic growth of an initially aerobic yeast suspension, the cryptic factor of catalase diminishes with each cell division[5] and approaches unity in about 7 divisions. When the cryptic factor was unity (actually it might vary from 0·8 to 1·8, depending, among other things, on the temperature of assay), post-treatment of heated cells did not significantly change the activity of the suspension: that is, curve *C* coincided with curve *A*. It was of interest to examine the heat-sensitivity of the cryptic enzyme in a culture which had grown anaerobically for several generations, such that the quantity per cell of cryptic enzyme had been greatly reduced, but not eliminated. A series of experiments was performed in which the initial cryptic factor before heating varied from about 3 to 5.

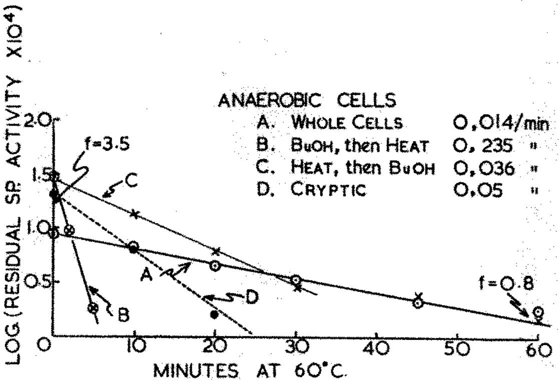

Fig. 2. Rates of heat inactivation of intracellular catalase of an originally aerobic yeast suspension, grown anaerobically for several generations

The results of such an experiment are shown in Fig. 2. The slopes of curves *A*, *B* and *C* are about the same as for the aerobic cells. However, curve *C* shows an abrupt change in slope when all the cryptic catalase has been inactivated, the curve thereafter coinciding with curve *A*. Curve *D* shows the decline in the cryptic activity. Since the difference in slope of curves *C* and *D* was relatively small (10–25 per cent), one may safely take curve *C* to measure the heat sensitivity of the cryptic catalase. The results from experiments of this type are summarized in Table 1, section II. The difference between the rates of inactivation of patent (column *D*) and cryptic (column *F*) activities is highly significant. There is no significant difference between the heat sensitivity of patent, cryptic and butanol-altered enzymes of the aerobic yeast on the one hand, and

Table 1. MEAN RATES OF INACTIVATION OF INTRACELLULAR CATALASE AT 60° C
(Standard errors of the mean are shown)

I. Aerobic cells			II. Anaerobic cells		
A. Whole cells (patent)	*B*. Butanol, then heat (altered)	*C*. Heat, then butanol (cryptic)	*D*. Whole cells (patent)	*E*. Butanol, then heat (altered)	*F*. Heat, then butanol (cryptic)
0·011 ± 0·002 $n = 11$	0·25 ± 0·04 $n = 5$	0·037 ± 0·003 $n = 10$	0·012 ± 0·002 $n = 10$	0·19 ± 0·02 $n = 6$	0·037 ± 0·003 $n = 10$

Differences between means, with standard errors of the difference: *A–C*: 0·026 ± 0·004; $P < 0·01$. *D–F*: 0·025 ± 0·004; $P < 0·01$
A–D, *B–E*, *C–F*, not significantly different.

of the same categories of enzyme in aerobic yeast on the other.

Treatment of the cells at 60° C did not affect the energy of activation of the patent enzyme-substrate system, which remained approximately 9 kcal/mole throughout the experiment.

We may conclude that in the intact aerobic yeast cell the enzyme catalase exists in two distinct forms, cryptic and patent. The cryptic enzyme is three times as rapidly inactivated by heat as the patent enzyme. In cells which have grown anaerobically for several generations, one can eliminate selectively all the cryptic catalase, because of its relatively great sensitivity to heat. Treatment of the cells with butanol reveals the latent activity without removing it from the cell, but alters both the patent and cryptic enzymes to a third form, which is extremely heat labile.

In a subsequent article, it will be shown that during anaerobic growth, where there is inhibition of catalase synthesis, the cryptic catalase is progressively converted into the patent form. In terms of the membrane hypothesis, the cryptic enzyme becomes bound to specific membrane sites during growth under conditions where synthesis of new enzyme is prevented.

This work was supported by grants from the Medical Research Council of Canada.

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ACTION OF SAPONIN ON BIOLOGICAL CELL MEMBRANES

THE tacit assumption made by Dourmashkin, Dougherty and Harris[1] to explain the hexagonal structure of some virus and mammalian cells treated with saponin was that, by removal of certain lipid components, an ordered membrane structure was revealed. Schulman and Rideal[2], however, showed that saponin from an aqueous phase could penetrate into, and complex with, a number of surface-absorbed lipids—particularly cholesterol—to form very insoluble complexes. This suggested to us that the structures revealed by Dourmashkin[1] resulted from addition to, rather than subtraction from, components of the cell membrane.

That the membrane after treatment assumed an ordered structure resembling a hexagonal lattice was, perhaps, to be expected in the light of the work of Luzzati and Husson[3], who have shown recently that binary mixtures of soap and water can exhibit a hexagonal lattice structure as one of a number of characteristic phases if conditions of concentration and temperature were right. They also showed by X-ray diffraction that multi-component mixtures of brain lipids showed a similar structure as a transitional phase between that of a crystalline solid (coagel) and typical laminar structure of a liquid crystal. Stoeckenius[4] has confirmed the existence of this phase by electron microscopy in identical material but fixed with osmium.

To test the hypothesis that the pattern obtained by Dourmashkin[1] represented a transitional phase, of a mixture of saponin–water–membrane lipids (cholesterol constituting approximately 50 per cent of the lipids), we dried down pure solutions of either lecithin, lecithin and cholesterol or cholesterol alone on to carbon-coated cellulose grids. We then placed a drop of an aqueous solution of sodium phosphotungstate 1 per cent containing white saponin 0·005 per cent on the grid and after 30 sec drained the drop away and examined the grid in an Elmiskop I (Siemens) microscope. The result of such a procedure is shown in Fig. 1. The centre-to-centre spacing of the lattice is about 140 Å and the diameter of the hole 80 Å, both values being in good agreement with those found in cell membranes. It is clear that the pattern obtained is remarkably similar to that obtained by Dourmashkin[1] and Stoeckenius[4]. A similar picture can be obtained if a monolayer of cholesterol is lifted on to a grid and afterwards

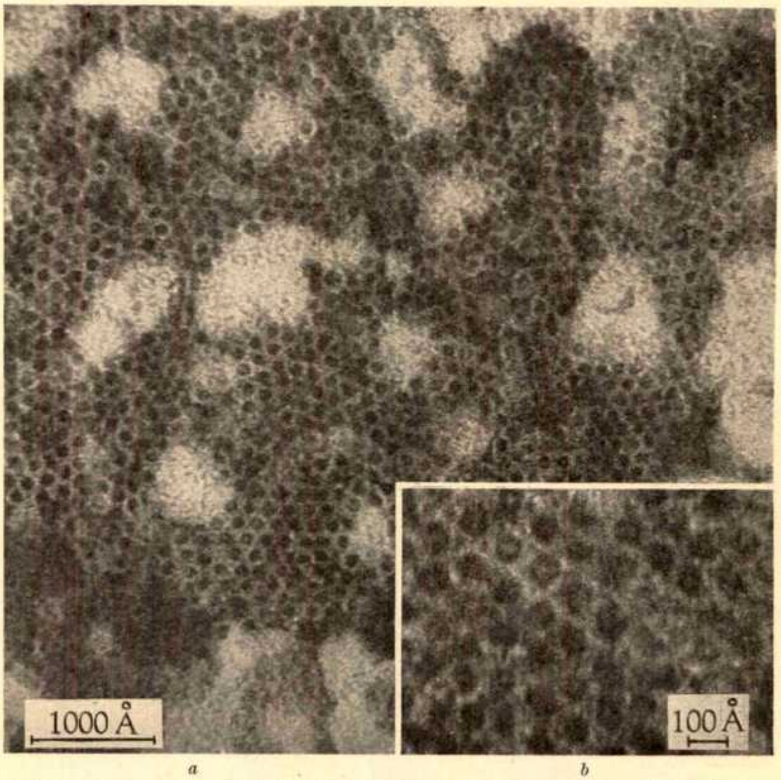

Fig. 1. Electron micrograph showing a negatively stained preparation of cholesterol after treatment with saponin. (*a*) A region at low magnification showing the network interspersed with unreacted material (× *c*.160,000); (*b*) an area at higher magnification showing the hexagonal packing of rings. (× *c*. 500,000)

allowed to react with the drop of saponin containing sodium phosphotungstate. The pattern obtained with equal molecular mixtures of lecithin and cholesterol is more heterogeneous. Many areas show a disruption of the hexagonal lattice with the appearance of free rings of lipid, some of them showing the vestigeal corners from adjacent rings. The dimensions of the free rings are also in good agreement with those observed by Dourmashkin *et al.*[1], although the piling of ring on ring has not yet been seen (Fig. 3). The hexagonal structure was rarely seen after treatment of pure lecithin and, instead, the typical laminar structure of lecithin in water was observed.

The implications of these findings are probably more germane to the functions of lipid membranes in terms of their permeability than to any interpretation as to their structure. It would seem improbable that holes of the size produced by saponin also occur in intact cells following a similar phase change. On the other hand, it is to be expected that this phenomenon will be encountered with a number of compounds known to modify membrane permeability, each producing a characteristic hole-to-surface ratio. Vitamin A alcohol, for example, appears to react with monolayers of lecithin in much the same way as saponin reacts with cholesterol[5]. It is also lytic for red cells[6], mitochondria[7,8] and lysosomes[9]. Furthermore, assuming that there is a selectivity of lipids participating in the phase change generally, or in the composition of the lipids lining the holes in particular, a mechanism for selective permeability is apparent. The role of long-chain cations in preventing extreme degrees of cell permeability may be explained in these terms[10].

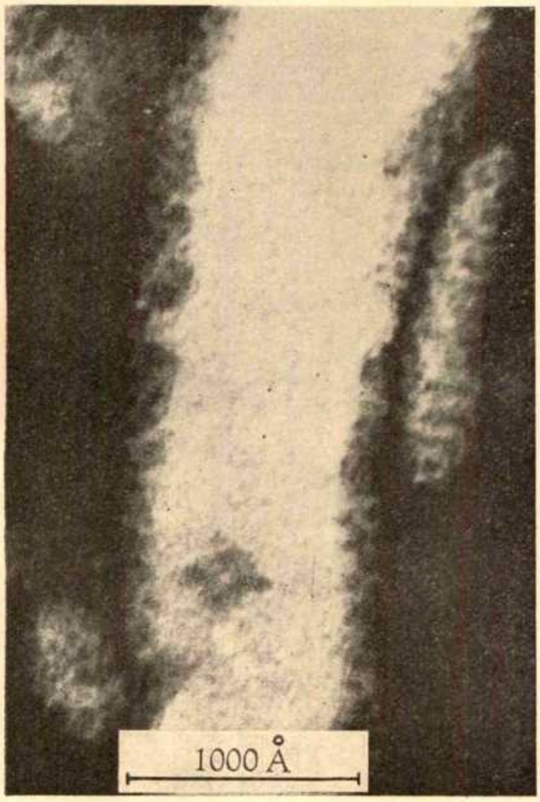

Fig. 2. Shows a relatively thick layer of cholesterol after treatment with saponin revealing the phase change occurring on the surface. (× 300,000)

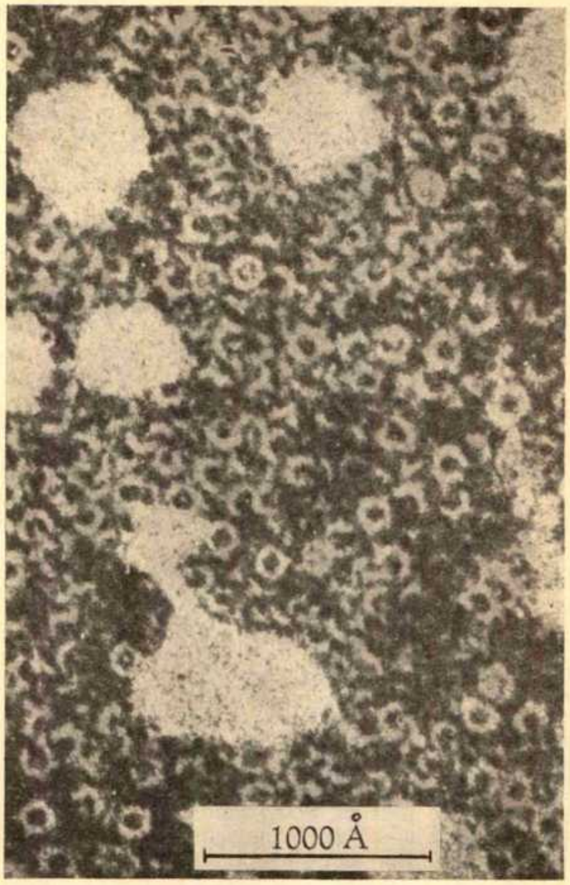

Fig. 3. A preparation of equal parts of lecithin and cholesterol treated with saponin showing separation of ring-like structures. (× 300,000)

Finally, the precise interpretation of Schulman and Rideal's original phase boundary potential and surface expansion data are now subject to some suspicion if the phase structure of a penetrated monolayer is indeed, hexagonal.

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ELEGANT electron micrographs recently published by Dourmashkin, Dougherty and Harris[1] demonstrated the presence of holes or pits in the surface membranes of Rous sarcoma viruses, erythrocytes and tissue cells after treatment with saponin. When a sufficiently high concentration of saponin was used, many holes were produced which were arranged in a hexagonal array. The holes or pits varied in size from 70 to 95 Å in diameter according to the membrane studied, and each was surrounded by a ring some 30–50 Å wide. It was suggested by Dourmashkin *et al.* that "the almost identical appearance of the saponin-treated cell membranes from such disparate species as chicken, guinea pig and man lends credence to a general theory for the structure of the cell membrane". Furthermore, these workers suggested that their observations were in fair agreement with the mosaic structure of lipid and non-lipid areas proposed by Parpart and Ballentine[2] for cell membranes in which cylinders of phospholipid and cholesterol, having a diameter of about 90 Å and a centre-to-centre spacing of approximately 150 Å, were surrounded by a meshwork of protein.

Saponin has the ability to form a complex with cholesterol. The production of pits or holes in a membrane containing cholesterol by treatment with saponin might therefore be thought to result from removal of cholesterol from the membrane by the saponin solution. However, when saponin molecules are injected beneath a monolayer of cholesterol, combination does occur, but in this instance the saponin molecules are incorporated into the structurally orientated monolayer of cholesterol. This is shown by the rapid increase in the surface pressure of the cholesterol film as the saponin molecules penetrate the monolayer[3].

Since treatment of cell membranes with saponin resembles treatment of a cholesterol monolayer with saponin, it seemed possible that the hexagonal pattern seen in saponin-treated membranes represented the molecular orientation of the lipids of the cell membrane after penetration by saponin molecules.

It was therefore of interest to see if the dimensions of saponin molecules bore any relationship to the hexagonal pattern of the treated membranes. Measurements of a model of a saponin-type glycoside showed that the lipid-soluble steroid ring system, or triterpene system, which comprises the 'head-group' of such a glycoside is of the order of 10 Å by 16 Å. The water-soluble chain, assuming it to be composed of five sugar molecules as occurs in many saponins, is approximately 25 Å long. If saponin molecules were arranged in a two-dimensional micellar-type structure in a lipid monolayer with the lipophilic head groups on the outside where they would be associated with the original lipid of the film, the hydrophilic sugar chains of the saponin molecules would be in a central aqueous phase that might appear as a dark hole on negative staining with phosphotungstate.

Close inspection of electron micrographs of saponin-treated Rous sarcoma cell and erythrocyte membranes published by Dourmashkin *et al.*[1] indicated the presence of approximately twenty sub-units in the 30–50 Å wide rings which surround each central dark hole. Arrangement of twenty saponin molecules in a circular structure with a distinguishable space between each lipophilic head-group would produce a ring having a central hydrophilic hole, approximately 80 Å in diameter, that would contain the sugar moieties of the saponin molecules. Interaction of cholesterol molecules and the lipophilic heads of the saponin in a 1 : 1 molar ratio on the outside of the circular structure would then give a ring of approximately 30 Å in width composed of discrete sub-units (Fig. 1). Since the dimensions of this hypothetical structure are similar to those of the holes and rings in saponin-treated membranes, it was decided to treat a simple monolayer of cholesterol with saponin in the absence of protein to see if a similar hexagonal pattern could be produced. We made the test experiment on October 26, 1962; we were unaware of the experiments being made simultaneously by A. D. Bangham and R. W. Horne at Babraham (previous communication).

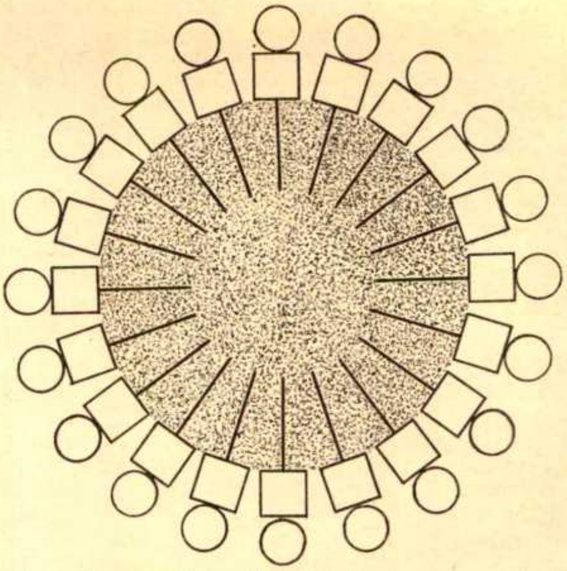

Fig. 1. Diagram of the hypothetical micellar-type arrangement of saponin (□) and cholesterol (○). The central speckled area represents the water phase

A drop of a dilute solution of cholesterol (2 mM) in petroleum ether (b.p. 40°–60°) was placed on the surface of distilled water (60 ml.) contained in a Petri dish. All water used in the experiment had been passed through a sterilizing filter. After the petroleum ether had evaporated from the surface, a 1 per cent aqueous solution of saponin (3 ml.) was injected through the film into the water below the

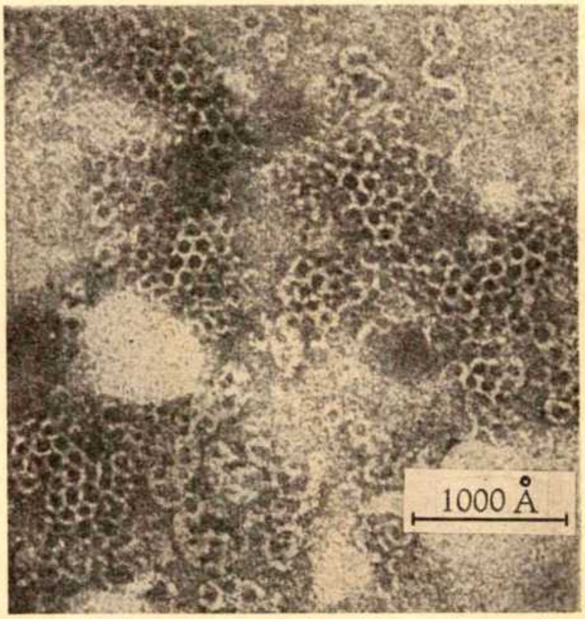

Fig. 2. Electron micrograph of a film of cholesterol treated with saponin and then negatively stained with potassium phosphotungstate. (× 200,000)

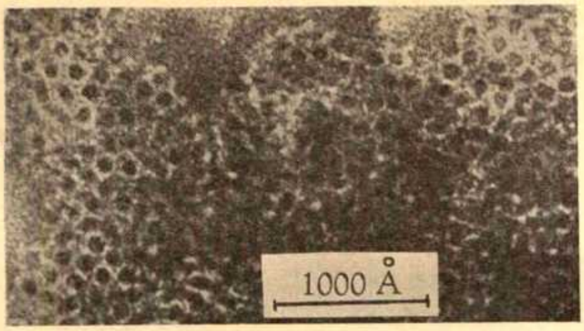

Fig. 3. Electron micrograph of a fragment of a rabbit erythrocyte haemolysed with saponin and then negatively stained with potassium phosphotungstate. (× 200,000)

cholesterol and the mixture stirred by pipetting the bulk phase up and down several times. A portion of the surface film was collected on an electron microscope grid coated with collodion and carbon, by dipping the grid through the film. A drop of a solution of potassium phosphotungstate (0·5 per cent; pH 7) was then placed on the surface of the grid and afterwards withdrawn with filter paper. Micrographs were taken with a Siemens Elmiskop I.

Fig. 2 shows an electron micrograph of a negatively stained preparation of cholesterol after treatment with saponin in the manner described. A hexagonal arrangement of holes can be seen in certain areas; individual holes have a diameter of approximately 80 Å. Rabbit erythrocyte ghosts which had been treated with saponin in this laboratory by the technique of Dourmashkin *et al.*[1] are shown in Fig. 3. A comparison of the two patterns shows that they are very similar. It is suggested therefore that the hexagonal structure that is produced by adding saponin to preparations of cell membranes represents primarily the molecular arrangement of a complex of saponin with cholesterol, and does not represent any pre-existing arrangement of lipid and protein in the cell membrane as inferred by Dourmashkin *et al.*[1].

The findings of Dourmashkin, Dougherty and Harris remain of great interest, however, when interpreted as demonstrating the addition of saponin to the cell membrane rather than a loss of cholesterol from the membrane. Their observations appear to show that extensive re-arrangement of membrane lipids can occur in the presence of suitable agents. The incorporation of saponin into the cell membrane probably forms a structure that is more permeable than the original membrane, since the structure containing saponin has areas 80 Å across that seem to contain water. The production of these holes through the lipid component of the cell membrane probably constitutes the hæmolytic mechanism of saponin. Other compounds of biological significance such as vitamin A which, like saponin, is hæmolytic[4,5], and can penetrate lipid monolayers[6], may produce similar changes *in vivo*, and furthermore, such changes may be reversible. It is possible that reversible alterations in lipid structure of this kind produced in the cell membrane by compounds such as vitamin A may be concerned in controlling the permeability of cells under normal physiological conditions.

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ROLE OF ISOCITRATE LYASE IN SYNTHESIS OF OXALIC ACID IN PLANTS

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BOTH the non-photosynthetic and the green tissues of *Oxalis pes-caprae*, a weed of economic significance in South Australia, contain up to 16 per cent of the dry weight as oxalic acid; the pH of the expressed sap is about 2. With compounds labelled with carbon-14, we[1] have shown that glyoxylic and glycollic acids are immediate precursors of the oxalic acid formed in the white emergent shoots from the starchy bulbs of this plant. These investigations[1] excluded the fixation of carbon dioxide (which occurs in many photosynthetic tissues) and de-amination of glycine (or of serine) as major physiological pathways for formation of glyoxylic and glycollic acids, which appeared to arise directly from the sugar or tricarboxylic acid pools. From the results of Carpenter and Beevers[2], it has hitherto been considered that isocitrate lyase (L_s-isocitrate glyoxalate-lyase, formerly known as isocitritase[3]) occurs only in those plants which actively convert fat to carbohydrate, a condition which does not apply to *Oxalis*. Moreover, since Clagett *et al.*[4] had found glycollate oxidase in green tissues but absent from embryos and etiolated plants, it appeared likely that this enzyme was restricted to photosynthetic tissue.

It has now been shown that the following enzymatic reactions occur in extracts from the white shoots of *Oxalis pes-caprae*:

$$\text{isocitric acid} \rightarrow \text{glyoxylic acid} + \text{succinic acid} \qquad (1)$$

$$\text{glycollic acid} + A \rightarrow \text{glyoxylic acid} + AH_2 \qquad (2)$$

$$\text{glyoxylic acid} + A \rightarrow \text{oxalic acid} + AH_2 \qquad (3)$$

$$\text{glyoxylic acid} + \text{NADP.H}_2 \rightarrow \text{glycollic acid} + \text{NADP} \qquad (4)$$

$$\text{oxalic acid} + \text{NADP.H}_2 \xrightarrow{\text{CoA}} \text{glyoxylic acid} + \text{NADP} \qquad (5)$$

here A is 2 : 6-dichlorophenol indophenol, or oxygen, and NADP and NADP.H_2 are the oxidized and reduced forms of coenzyme II respectively, and CoA is coenzyme A. Reaction (1) is catalysed by isocitrate lyase and reactions (2) and (3) by flavin-linked oxidase (or oxidases). Reaction (4) is catalysed by a glyoxylate reductase, and reaction (5) probably involves the intermediate formation of oxalyl–CoA.

Isocitrate lyase. The enzyme was demonstrated with an extract of an acetone powder of a particulate fraction from an homogenate of white *Oxalis* shoots. Several different methods gave confirmatory results.

(*a*) With reduced glutathione, Mg^{2+} ions, and the enzyme preparation, DL-isocitric acid was converted to a keto-acid as shown spectrophotometrically at 252 mμ by formation of the semi-carbazone. Under the conditions used, keto-acid formation was 0·084 O.D. units per mg of protein per min at *p*H 6·0 and at 25° C. After treatment of the enzyme extract with acid-washed charcoal ('Norite' *SX*-2) to remove nicotinamide nucleotide co-enzymes and so to prevent the possible formation of α-oxoglutaric acid by the action of isocitrate dehydrogenase, the rate of keto-acid formation was 0·055 O.D. units per mg of protein per min.

(*b*) The enzyme preparation was incubated at *p*H 7·5 with DL-isocitric acid, Mg^{2+} ions and cysteine; samples were removed at various intervals after commencement of the reaction and autoclaved at 15 lb. for 15 min with 2 : 4-dinitrophenyl hydrazine to decompose the glyoxylate–cysteine complex[5]. There was a linear increase in extinction at 435 mμ with time. Total keto-acids were assayed according to Friedman[6], and characterized by the absorption spectra of the 2 : 4-dinitrophenyl hydrazones. This method enabled correction for α-oxoglutaric acid initially present in the crude enzyme preparation, and clearly showed that glyoxylic acid was formed enzymically from DL-isocitric acid.

(*c*) The enzyme preparation was incubated at *p*H 6·0 with reduced glutathione, DL-isocitric acid and Mg^{2+} ions; glyoxylic acid formation was measured by the semicarbazide method, and succinic acid formation by specific enzymatic reduction of ferricytochrome *c* catalysed by succinate dehydrogenase[7]. Under the conditions used, 0·13 μmoles of glyoxylic acid and 0·12 μmoles of succinic acid were formed in 53 min, in good agreement with the stoichiometry of the reaction catalysed by isocitrate lyase.

The *in vivo* activity of the enzyme was shown by injecting ^{14}C-labelled isocitric acid into *Oxalis* shoots. Extracts were prepared from the shoots and the compounds containing isotope were separated by paper chromatography and detected by autoradiography[1]. Because of its instability, glyoxylic acid was difficult to detect but succinic and oxalic acids both contained considerable amounts of isotope; citric and α-oxoglutaric acids also contained appreciable amounts of isotope but glycollic and malic acids contained significantly less.

Oxidation of glyoxylic and glycollic acids. When injected into *Oxalis* shoots, isotope from ^{14}C-labelled glycollic acid appeared in about equal amounts in glyoxylic and oxalic acids, and isotope from ^{14}C-labelled glyoxylic acid was equally distributed between glycollic and oxalic acids[1].

At *p*H 7·8, in the presence of added riboflavin phosphate, glycollic and glyoxylic acids were oxidized by an enzymatic extract of *Oxalis* shoots, as demonstrated manometrically by oxygen uptake and spectrophotometrically by reduction of 2 : 6-dichlorophenol indophenol at 600 mμ (reactions (2) and (3)). The initial rate of oxidation of glyoxylic acid, measured spectrophotometrically, was approximately 60 per cent of the rate of oxidation of glycollic acid. These oxidations are probably catalysed by a flavoprotein similar to glycollate oxidase of other plants[8].

Reduction of oxalic and glyoxylic acids. It was apparent from studies with *Oxalis* shoots that isotope from ^{14}C-labelled glyoxylic acid appeared in glycollic acid, and that isotope from ^{14}C-labelled oxalic acid appeared in small amounts in glycollic and glyoxylic acids[1].

Reaction (4) was demonstrated spectrophotometrically at 340 mμ by a glyoxylic acid-dependent oxidation of either NAD.H_2 or of NADP.H_2 at *p*H 7·4 in the presence of an extract of *Oxalis* shoots. The initial rate with NADP.H_2 was about twenty times greater than that with NAD.H_2. The coupled reactions (3) and (4) would account for the dismutation of glyoxylic acid to glycollic and oxalic acids[1].

With a similar extract, at *p*H 8·3, NADP.H_2 was also oxidized in the presence of cysteine, adenosine triphosphate (ATP), Mg^{2+}, succinic acid and oxalic acid. There was no oxidation in the absence of oxalic acid or of any of the co-factors. It is therefore probable that reaction (5) proceeds via oxalyl–CoA. This intermediate has been shown to be formed from oxalic acid, succinic acid, ATP and CoA with an enzyme system of *Pseudomonas oxaliticus*[9].

Metabolism of Oxalis. The metabolic reactions of *Oxalis* related to the synthesis of oxalic acid are shown in Fig. 1.

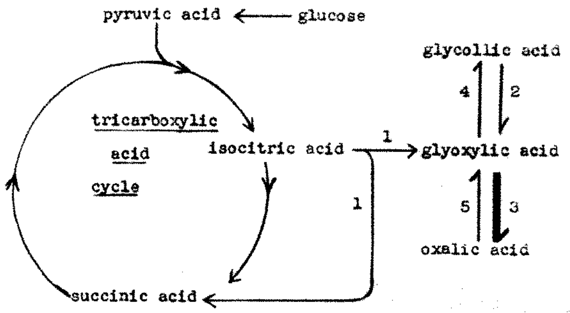

Fig. 1. Pathway of synthesis of oxalic acid in *Oxalis*. The numbers refer to the following enzymes: 1, isocitrate lyase; 2 and 3, flavin-linked oxidase (or oxidases); 4, NADP-linked glyoxylate reductase; 5, an enzymatic system involving formation of oxalyl-CoA

Since formation of oxalic acid appears to be an essential feature of the metabolism of *Oxalis* and is dependent on isocitrate lyase, an enzyme of restricted occurrence in plants[2], specific inhibition of isocitrate lyase offers a possible means of selective control of this and related weeds; work on potential inhibitors is therefore in progress.

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CONTRACTION OF KERATIN FIBRES IN AQUEOUS LITHIUM BROMIDE

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WHEN an α-keratin fibre is immersed in aqueous lithium bromide solution its length may stay unchanged, may decrease reversibly or may decrease irreversibly to a greater degree, the actual behaviour depending on the type of fibre, the concentration of the solution and the temperature[1,2]. These 'super-contraction' phenomena have recently been re-examined by Mandelkern, Halpin, Diorio and Posner[3] in relation to studies of phase transitions in partly crystalline polymers. After confirming the correlation between super-contraction and disappearance of crystallinity (as indicated by X-ray diffraction technique) Mandelkern *et al.* drew an analogy between this situation and melting in crystalline and cross-linked rubber or polyethylene. Thus they represent the fibre in its initial state as a cross-linked two-phase system of crystallites and matrix, in which reversible super-contraction corresponds to the melting of the crystallites, either thermally or by chemical action, and irreversible super-contraction occurs when the cross-links are broken. They further suggest that the introduction of more complex models is unwarranted.

It seems necessary to point out that although the foregoing model is adequate to account for super-contraction in keratin fibres, and was in fact originally used by us in this way[4], it has to be rejected as incorrect when the following properties are considered[5]: (*a*) the longitudinal mechanical behaviour (load-extension, stress-relaxation, creep) of single keratin fibres in water in the hookean, yield and post-yield regions, together with the associated X-ray patterns; (*b*) the relationship between force per unit extension of single keratin fibres in concentrated aqueous lithium bromide at the end of both first and second stages of contraction, and the amount of the respective contractions.

All these properties can be consistently explained by assuming the existence in the keratin structure of long crystalline microfibrils aligned parallel to the fibre axis and embedded in a non-crystalline matrix[6]; along these microfibrils alternate regions (*X*- and *Y*-zones) are postulated, the two types of zones having different mechanical properties[5]. Extension in the yield region and first-stage super-contraction take place in the *X*-zones, while extension in the post-yield region and second stage super-contraction take place in the *Y*-zones.

A detailed discussion of this model and its relationship to electron microscope, infra-red and X-ray results was reported at a recent conference on wool structure[7]. Three points may be emphasized with regard to the structure of wool and hair keratin.

(1) It is now well established from evidence based on a wide variety of experimental techniques[6,7] that α-keratin fibres consist of long. aligned microfibrils of 70–80 Å diameter separated by a matrix. These microfibrils are aligned in the direction of the fibre axis and are identified with the crystalline phase of the wool fibre; the matrix is not crystalline. Electron microscope[8,9] evidence suggests that the microfibrils are long and possibly run the full length of a cortical cell (*c.* 100μ). It follows that the extension of the fibre as a whole corresponds closely to the extension of the microfibrils.

(2) As a wool fibre in water at room temperature is extended from the yield region (about 2–30 per cent extension) into the post-yield region (beyond about 30 per cent extension) there is a rapid stiffening of the fibre, the incremental modulus being an order of magnitude greater than in the yield region (Fig. 1). The high-angle X-ray diffraction[10] pattern shows a continuous decrease of crystalline α-keratin with increase of extension (Fig. 1). The major part of the α-type crystallinity is still present at the turn-over from the yield to post-yield regions and a significant proportion is still present at extensions well into the post-yield region.

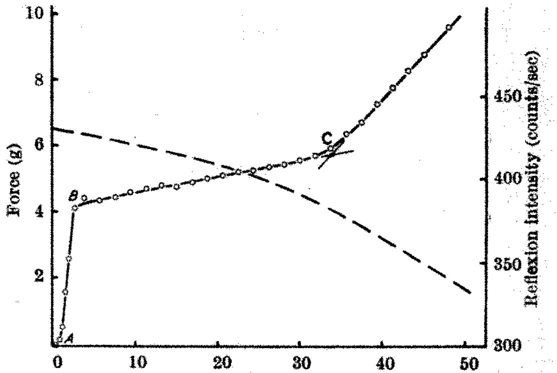

Fig. 1. –○–, The load-extension curve of a Corriedale wool fibre in water at 20° C was obtained by extending in steps indicated by the experimental points, the values of force being measured after the fibre had been held 24 h at each extension. The section *AB* of the curve is the hookean region, *BC* is the yield region, and extensions higher than *C* correspond to the post-yield region; – – –, values of the intensity of the 5·1 Å meridional reflexion of α-keratin fibres at varying extensions, as measured by Bendit (ref. 9)

At the turn-over extension between the yield and post-yield regions, the bonds opposing extension into the post-yield region must be either in the α-keratin crystallites still present or in the matrix immediately surrounding these crystallites. As pointed out elsewhere[7] the latter proposition is favoured. However, in either case these crystallites and their neighbouring matrix form the *Y*-zones that open out in the post-yield region, the *X*-zones having been opened out in the yield regions. Although the introduction of *X* and *Y* zones is in the first instance purely formal, it is quite plausible that real differences in physico-chemical structure should exist; for example, in cystine content or in degree of cross-linking. The essential feature is that each *Y*-zone should be large enough to contain a sufficient number of unit cells of the α-helix structure to preserve the 5·1 Å reflexion after the *X*-zones have extended. Since the bonds controlling the rate of contraction in the second stage for wool fibres in concentrated lithium bromide have been correlated with the bonds controlling the stiffness of the fibres in the post-yield region[7], it follows that contraction must also be considered in terms of the series zone model.

(3) The sharpness of the change of slope of the load-extension curve between the yield and post-yield regions also suggests the existence of two distinct zones rather than a distribution of cross-links and entanglements. In the latter case one would expect a more gradual increase in stiffness from the yield to post-yield regions as the progressive opening-up of the keratin structure is opposed by more and more of the randomly distributed bonds.

Finally, we suggest that the observation by Mandelkern *et al.*[3] that fibres in 12 M aqueous lithium bromide do not contract at 24° C may be an artefact. Keratin fibres placed in concentrated lithium bromide solution dehydrate rapidly to the state corresponding to the relative humidity above the solution. The lithium bromide penetrates as a front into the fibre, the rate of penetration being very dependent on the moisture content of the fibre. For Corriedale wool fibres (diameter *c.* 50μ) the times of penetration[1] at room temperature are 30–45 min in 6·3 M lithium bromide, 5–7 h in 8·3 M lithium bromide, and 7–8 days in 10·4 M lithium bromide. Moreover, no contraction occurs until the fibre is nearly completely penetrated. In 12 M lithium bromide solution moisture equilibrium[11] is reached with a relative humidity of 3–4 per cent and penetration of the lithium bromide into wool would be extremely slow, requiring, at 20° C, weeks and possibly months. For horse-hairs these times would be even longer.

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DERMOCYSTIDIUM GASTEROSTEI n. sp., A PARASITE OF *GASTEROSTEUS ACULEATUS* L. AND *GASTEROSTEUS PUNGITIUS* L.

By DR. E. ELKAN

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THE genus *Dermocystidium* is much ignored, even by parasitologists. Grassé (1953), in his text-book on protozoology, mentions only one species while neither Kudo (1960) nor Hyman (1940) mentions the genus at all. Doflein and Reichenow[1], however, remind us that at that time six different species of *Dermocystidium* had been described. Even their list was, however, incomplete because it ignored *D. percae*, described by Reichenbach-Klinke in 1950[2].

The genus was established by Pérez in 1907[3] from observations on the contents of dermal cysts in newts infected with *Dermocystidium pusula* P. In 1921 Guyénot and Naville[4] described *D. ranae* from *Rana temporaria* and *Rana esculenta* as well as from *Alytes obstetricans*. Léger[5] found *D. branchialis* on the gills of *Trutta fario*; Jirovec[6] described *D. vejdovskyi* from the pike (*Esox lucius* L.).

Authors usually list *Dermocystidium* with the Sporozoa and regard it as a member of the Haplosporidia. This classification of the genus is based on the presence of a lid in the spores. It may not be final because in some species the lid cannot with certainty be demonstrated.

The investigation reported here was undertaken with the object of elucidating the nature of a disease observed among sticklebacks (*Gasterosteus aculeatus* L. and *G. pungitius* L.) in waters around Newdigate, Surrey. After the fairly severe winter of 1961–62 numerous specimens of both species were caught in May presenting the picture as shown in Fig. 1. Spring and early summer remained cold, and specimens similarly afflicted were caught up to the middle of June. With the appearance of warmer weather towards the end of June the gross manifestations of the disease disappeared, but fish with minute white cysts on their dorsal and tail fins could still be found.

Attempts to take swabs from these cysts while the fish were alive failed. Nor was it possible to remove any cysts *in toto* from live specimens. This failure is easily explained by a glance at a section (Fig. 3), which shows that the cysts do not lie on the surface but in the dermis and that many of them—not all—have a thick and resistant cyst wall. The impression that the cysts lie on the surface is derived from the extreme transparency of the epidermis covering them. Smears from cysts contents could only be made by opening them with a fine scalpel and squeezing the contents out on glass slides. Smears and sections were fixed with methyl alcohol, formaldehyde, weak Flemming's solution or Carnoy's solution and stained after Giemsa by McNamara's method as modified by Shortt and Cooper[7], hæmatoxylin–eosin or by the periodic acid–Schiff reaction.

At the height of the epizootic the fish were covered with structures looking at first sight like 'white worms'. Closer examination showed these structures to be cysts, 2–3 mm long and 0·2–0·3 mm wide, either straight or bent, of even thickness or club-shaped. On the tail fin their arrangement was

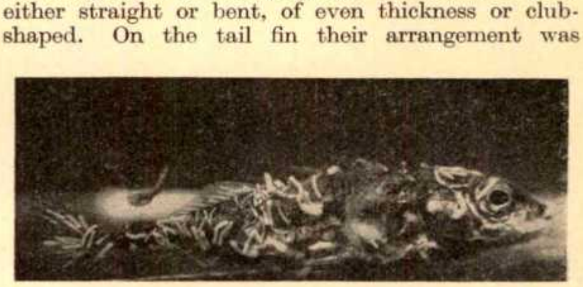

Fig. 1. Male stickleback infected with *Dermocystidium gasterostei* n.sp. Note radial arrangement of cysts on the tail, also one cyst crossing the rt. cornea (× *c.* 1·5)

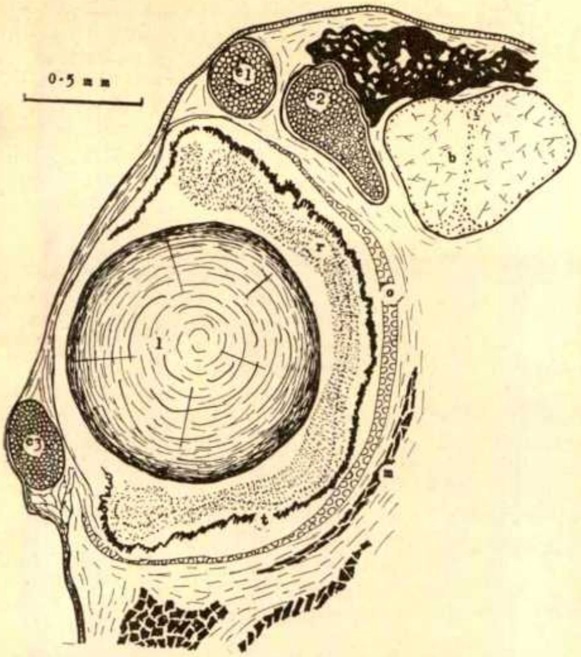

Fig. 2. *Gasterosteus aculeatus*. Transverse section through the skull showing cysts filled with spores of *Dermocystidium gasterostei* n.sp.
b, Brain; c_1, cyst in sub-cutis; c_2, cyst in the angle between frontal bone, brain and orbit; c_3, cyst in the cornea; *l*, lens; *m*, ocular muscle; *o*, orbital cartilage; *r*, retina; *t*, tapetum

fan-shaped, following the direction of the tail bones. No cysts were found on the gills or in the visceral organs. In several specimens cysts were seen in the cornea of the eye, some of them were also found deeply embedded between the skull bones, close to the brain capsule (Fig. 2). Although the cysts were numerous even on small fish, they did not seem to inconvenience the sticklebacks unduly. The fact that some of them died soon after arrival may be explained by the effect of transport; those which survived the first few days were seen to feed quite normally in the laboratory aquarium.

The following sporozoan parasites have previously been found to occur in *Gasterosteus: Glugea anomala* Moniez (= *G. microspora* Thél. in the sub-cutis, *Henneguya media* Thél., *H. brevis* Thél. and *Sphaerospora elegans* Thél. in the kidney and the ovary, and *Coccidium gasterostei* Thél.) in the liver. Of these parasites only *Glugea anomala* M. produces dermal cysts which may be deeply embedded but which are always spherical. The appearance of the diseased fish and of the parasite described here differs widely from any formerly described from *Gasterosteus*.

The contents of the cysts were not uniform. In one and the same fish cysts in various stages of development could be observed. The staining reactions of the cyst wall was always that of the surrounding connective tissue. The cyst wall must therefore be regarded as a product of the host. Yet, in one and the same fish some cysts could be seen with walls of considerable thickness while other agglomerations of spores were either surrounded by an extremely thin cyst wall or by none at all (Fig. 4). Nor can these various developments of the cyst wall be paralleled by the general development of the cyst contents. Some cysts, containing young stages of spores, had well-developed walls, while others, containing mature spores, had thin walls or none at all. The relation of cyst maturity and cyst wall is unpredictable and appears to be haphazard, nor is the development of the wall related to the site in which the cyst happens to develop. Cysts with very differently developed walls could be seen to lie side by side (Fig. 4). The earliest stages of development in this material were represented by cysts filled with an amorphous protoplasmic mass in which chromatinic granules were more or less evenly distributed (Fig. 5*a*). These chromatinic granules gradually coalesce to form nuclei, splitting up the protoplasmic mass in the process (Fig. 5*b*). Gradually the whole protoplasmic mass splits up into spherical portions of between 8–10μ diameter each of which contains several larger and many smaller chromatinic granules (Fig. 5*c*). These spherical or ovoid bodies are surrounded by an extremely thin cell wall. They

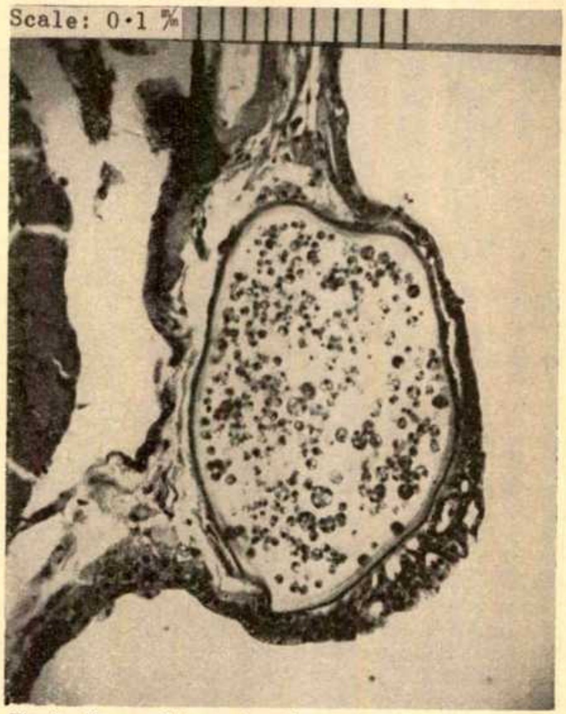

Fig. 3. *Dermocystidium gasterostei* n.sp. Typical cyst with cyst wall and well-developed spores in the sub-cutis of a stickleback. Note the unequal size of the spores

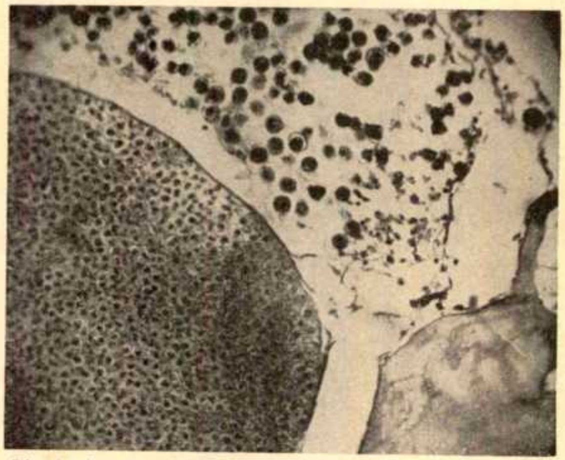

Fig. 4. *Dermocystidium gasterostei* n.sp. Two agglomerations of spores in different stages of development from the head of the stickleback (*Gasterosteus aculeatus* L.). Neither of these 'cysts' is surrounded by the typical cyst wall. (Hæmatoxylin and eosin, × *c*. 35)

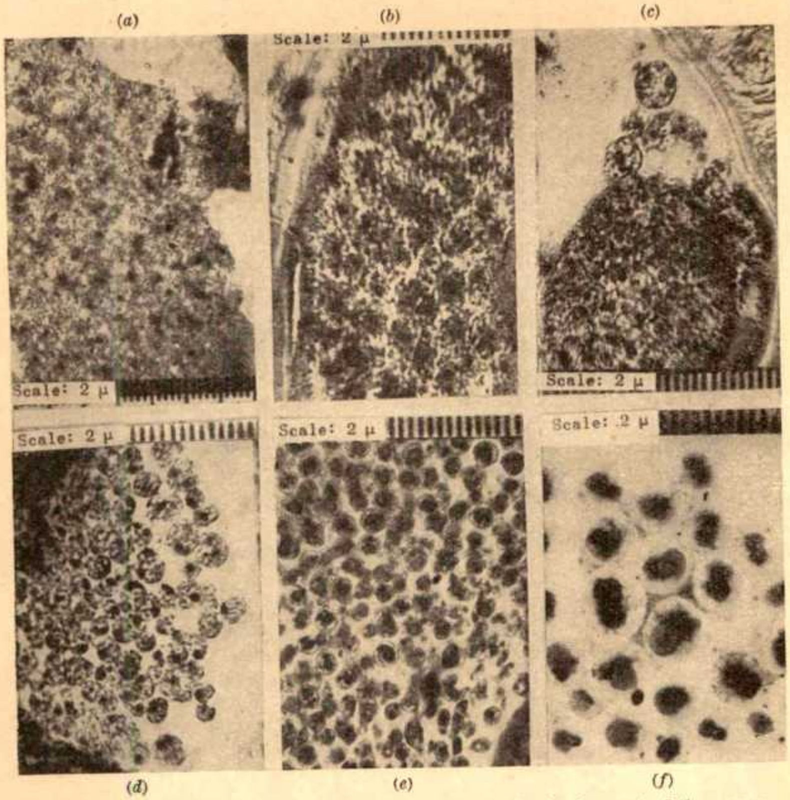

Fig. 5. *Dermocystidium gasterostei* n.sp. Six stages in the development of the spores. (*a*), Plasmodium, first concentration of chromatinic masses; (*b*) concentration of cytoplasm around developing nuclei; (*c*) separation of spherical multinuclear portions (schizonts); (*d*) further division of schizonts which become uninuclear; (*e*) appearance of spore membrane and acidophile inclusion body; (*f*) spores almost mature but adhering to one another by their sticky membrane

divide in turn, transforming themselves gradually into spores of 3–4μ diameter. They are then seen to have a definite cell wall, a nucleus, cytoplasm and an inclusion body which takes up most of the cell content.

The cell wall is, on the outer side, covered with a sticky substance in virtue of which the spores stick to one another and also adhere to the skin of the fish when the cyst is ruptured. The inclusion body is so large that it displaces the cytoplasm and the nucleus to one side towards the periphery of the cell where it can be seen in the shape of a bowl, or, in lateral view, as a crescent. When fully developed, the cell wall gives a positive periodic acid–Schiff reaction. It can be shown, with the aid of this reaction, that the individual spores within one cyst do not all develop at the same rate. Some become completely opaque because of the heavily +ve periodic acid–Schiff-capsule while others maintain graded degrees of transparency. The final size of the spores is not uniform but varies between 3 and 5μ. It has not been possible to demonstrate that these spores have a lid. The development described here follows the course of a primitive asexual reproduction. One could easily label the various stages as plasmodium, sporont, sporoblast and spore analogous to the terms used in the more highly developed sporozoa but little would be gained by applying this nomenclature to a process the different stages of which blend into one another so imperceptively that it would be impossible to say when one is abandoned and the next stage reached. The most characteristic feature of these spores is undoubtedly the very large inclusion body which, like the cell wall, always gives a positive periodic acid–Schiff reaction. It contains presumably nutritive material which can be utilized when the spore has entered a new host.

Several years of observation will be required to show whether the appearance of this parasite is always linked with the early months of the year and whether it is, in Britain, confined to *Gasterosteus*. Regarding the life-cycle of these spores it has been suggested for *Dermocystidium percae*[2] that the spores, after falling to the bottom of the pond and over-wintering there, might be taken up by Copepods and spend an intermediate stage in *Daphnia* or *Cyclops*. An unsuccessful search was made for both these crustaceans in the waters where the sticklebacks were taken. Also, the assumption of an intermediate host does not really seem necessary where sticklebacks are concerned. These are among the most pugnacious of fish, well known to bite each other viciously, particularly during the breeding season. It would be easy for the attacker to rupture a cyst and to fill its mouth with spores. It is, on the other hand, difficult to visualize *Daphnia* or *Cyclops* picking up and swallowing anything as large and sticky as these spores, which, if they fell to the bottom of a pond, would immediately be covered by the mud. It must further be considered that the cyst-covered fish appeared at a time of the year too early for the appearance of Copepods, which only hatch with the advent of warmer weather.

The parasite described here is morphologically very similar to *Dermocystidium percae* R–K, with the difference that the latter was said to have spores with a lid, whereas no lid could be seen in the spores from sticklebacks. Also, the cysts found on perch were all lying on the surface or in the subcutis whereas in *Gasterosteus* some penetrated deeply into the skull. So far the parasite has been found exclusively in two species of *Gasterosteus* and on no other fish, particularly not on perch, although these inhabited the same waters in which the sticklebacks were found.

For these reasons the parasite is regarded as a new species and is named *Dermocystidium gasterostei* n.sp. with the characters described. Host: *Gasterosteus aculeatus* L. and *G. pungitius* L.; locality: Newdigate, Surrey, England; type material in my collection and at the Department of Parasitology, Imperial College of Science and Technology, Sunninghill.

I thank Dr. J. R. Baker, London School of Hygiene and Tropical Medicine, for his advice.

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USE OF 'SEASONAL' REGRESSIONS IN MEASURING FEED INTAKE OF GRAZING ANIMALS

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RELATIONSHIPS established with pen-fed sheep or cattle between herbage digestibility or feed-to-fæces ratio and either fæcal nitrogen or fæcal 'chromogens' are being increasingly used for measuring the feed intake of grazing animals, but the regression lines vary according to the time of the year at which the herbage is cut[1,2].

A series of digestion trials was carried out here in spring 1961 to test the hypothesis that such variation might result from changes in the relative proportions of leaf and stem in the herbage eaten.

The pasture of ryegrass (*Lolium perenne*), *Phalaris tuberosa* and white clover (*Trifolium repens*) was about 6 in. high and predominantly leafy at the start and about 15–18 in. high and in early seed head at the end of the series of four trials.

Pasture was cut every second day at a height of 1–2 in. and chaffed, this material being referred to afterwards as 'whole herbage'. A portion of it was winnowed by dropping it through the air stream from an electric fan in a horizontal tunnel. Grass leaf laminæ tended to be blown out and were collected as the 'leaf' fraction. Stem and leaf-sheath tended to remain and were collected as the 'stem' fraction. Clover stem tended to remain in the 'stem' fraction and clover leaflets were evenly divided between the two fractions. The process thus gave 'stem'-rich and 'leaf'-rich fractions rather than complete separation.

Table 1. MORPHOLOGICAL COMPOSITION OF FEED OFFERED

Composition of feed offered (dry matter basis)*

		Feed type					
		Whole herbage		Leaf		Stem	
Trial	Morphological components	%	Sheep	%	Sheep	%	Sheep
1 (7.10.61–16.10.61)	Grass lamina	62	*A* and *B*	72	*C*	58	*D*
	Clover leaf	17		22		27	
	Stem and leaf sheath	19		6		16	
2 (23.10.61–1.11.61)	Grass lamina	63	*A* and *B*	78	*D*	29	*C*
	Clover leaf	15		14		17	
	Stem and leaf sheath	22		7		54	
3 (6.11.61–15.11.61)	Grass lamina	37	*B*	73	*D*	29	*C*
	Clover leaf	37		12		7	
	Stem and leaf sheath	25		14		64	
4 (8.12.61–17.12.61)	Grass lamina	43	*A* and *B*	63	*C*	10	*D*
	Clover leaf	4		4		0	
	Stem and leaf sheath	34		15		64	

* Extraneous material (weeds, seed heads, etc.) was 1–2 per cent except in trial 4, when it amounted to about 20 per cent.

Four Merino wethers in metabolism cages were used throughout the experiment except in trial 3, when sheep *A* was not used. In each digestion trial, two sheep were fed whole herbage, one was fed the 'stem' and one the 'leaf' fraction. Dried samples of each type of feed were bulked and later hand-sorted into morphological components. Table 1 gives details of the feed offered.

Table 2. FEED INTAKE AND DIGESTIBILITY AND FÆCAL NITROGEN AND 'CHROMOGEN' CONCENTRATIONS

		Trial No.			
	Feed	1	2	3	4
Dry matter eaten (g/day)	Whole herbage	281, 235	495, 476	–, 617	381, 397
	'Leaf'	378	535	862	396
	'Stem'	249	444	740	387
Organic matter digestibility (per cent)	Whole herbage	84·5, 84·3	81·0, 80·5	–, 80·1	68·9, 71·0
	'Leaf'	84·0	79·6	73·8	70·2
	'Stem'	86·9	82·8	75·4	67·6
N in fæces (g/100 g organic matter)	Whole herbage	4·32, 4·75	3·59, 3·71	–, 3·06	2·27, 2·57
	'Leaf'	4·26	3·77	3·16	2·88
	'Stem'	4·14	3·32	3·06	2·01
'Chromogen' concentration*	Whole herbage	1090, 1040	730, 710	–, 420	395, 230
	'Leaf'	1125	840	335	585
	'Stem'	890	790	345	270

* [(Optical density × volume of extract in ml.) divided by (weight organic matter extracted)] × 1/10

Table 2 shows the feed consumption, organic matter digestibility and concentrations of nitrogen (dried fæces) and of 'chromogens' (frozen fæces). Feed rejection was 11 per cent or less in all cases except for sheep *A* trial 1 (28 per cent) and sheep *B* trial 3 (30 per cent), both fed on whole herbage.

In Fig. 1 the relationships of intake factor (feed organic matter divided by fæcal organic matter) to fæcal nitrogen concentration for whole herbage, 'leaf', and 'stem' are shown. The regression lines for 'leaf' and 'stem' differ significantly in elevation. The difference (about 1·7 units of intake factor) exceeds the difference between 'summer' and 'winter' lines (0·7 units) found in this Laboratory[3], and exceeds also the difference between Minson and Kemp's[1] June and November lines (ranging from about 0·7 to 1·7 units of intake factor).

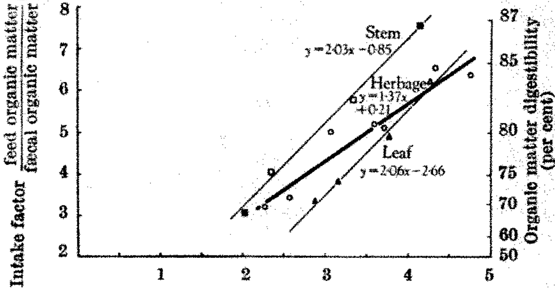

Fig. 1. Regressions of intake factor (feed organic matter + fæcal organic matter) on N per cent of fæcal organic matter for whole herbage (○, sheep *A* and *B*), 'leaf' (△, sheep *C*, ▲, sheep *D*), and 'stem' (□, sheep *C*, ■, sheep *D*). N per cent determined by Kjeldahl digestion on dried samples

Values for whole herbage fell generally between the 'leaf' and 'stem' lines.

In Fig. 2 the relationships between intake factor and fæcal 'chromogens' for whole herbage, 'leaf' and 'stem' are shown. General features similar to Fig. 1 are evident but the difference between 'leaf' and

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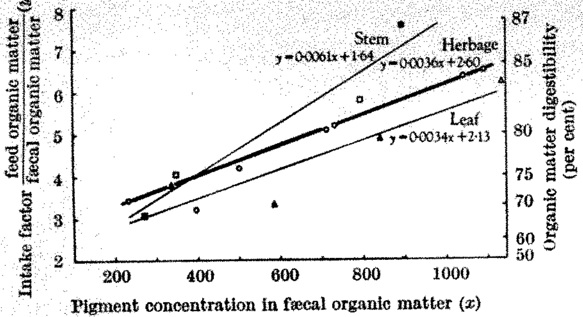

Fig. 2. Regressions of intake factor (feed organic matter + fæcal organic matter) on fæcal pigment concentration ('chromogens') for herbage (○, sheep *A* and *B*), 'leaf' (△, sheep *C*, ▲, sheep *D*), and 'stem' (□, sheep *C*, ■, sheep *D*). Pigments determined as optical density of 85 per cent acetone extract of frozen fæces

'stem' lines is not statistically significant. Whole herbage values again tended to be intermediate.

Changes in the position of regression lines of digestibility (or of feed-to-fæces ratio) on fæcal nitrogen or 'chromogens' have thus been brought about solely by feeding 'leaf'-rich and 'stem'-rich fractions.

The digestible material of 'leaf' was presumably associated with much more chloroplast pigment and protein, and that of 'stem' with relatively greater amounts of soluble and digestible carbohydrates[4] but with less chloroplast material.

In these trials the digestibility of 'stem' was initially higher than that of 'leaf' but fell more rapidly after ear emergence. This confirms and helps to explain the plateau of digestibility observed by Minson, Raymond and Harris[5] despite a big reduction in the percentage of leaf lamina.

Digestion trials with the cattle pasture[6] often yield higher values of intake factor for a given fæcal nitrogen percentage than do trials with shorter, leafy, sheep pasture[3,7,8]. This may perhaps now be ascribed to the higher proportion of 'stem' material in the taller herbage.

These trials clearly indicate that the 'leaf' and 'stem' herbages have intrinsically different digestibility patterns and fæcal index regressions, and that 'seasonal' variation in regressions may result primarily from variations in leaf/stem ratio following the cycle of vegetative and reproductive growth. If this is so, the regression appropriate to a particular grazing experiment cannot be determined by calendar date alone. The prime requirement is rather that the regression should be based on herbage morphologically similar to that eaten in the experiment even if they are not from the same sward or the same month.

There is good evidence[9] that grazing sheep tend to select leafy herbage rather than stem. The more conservative estimates of herbage intake obtained by using the 'leaf' type regressions are therefore preferable for sheep experiments.

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VIRUSES ASSOCIATED WITH A DIE-BACK DISEASE OF CULTIVATED MUSHROOM

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ALTHOUGH conclusive evidence is lacking, viruses have been suggested as the causes of certain abnormalities of fungi from which no visible pathogens could be isolated, such as the transmissible disease[1] of *Helminthosporium victoriae* Meehan and Murphy, and of several Agaricaceae[2]. Jinks[3], however, considered the 'vegetative death' in *Aspergillus glaucus* to be caused by lethal suppressive cytoplasm.

There is a group of cultivated mushroom (*Agaricus bisporus* (J. Lange) Pilát 1951) disorders that includes watery stipe, La France, brown, mummy and *X*-diseases, the cause of which is uncertain and the symptoms of which have in common the degeneration of the mycelium, various sporophore abnormalities and, often, waterlogging of the tissues, especially the stipe[4].

Although waterlogged stipes can result from cultural conditions[5], Gandy demonstrated that they are sometimes associated with a transmissible disease[6] the characteristic symptom of which is complete mycelial degeneration. This disease was transmitted to healthy mushrooms not only by adding infected mushroom compost or agar culture of diseased mycelium to trays containing healthy spawn but also *in vitro*, when disks cut from agar cultures of normal mycelium were inoculated by dipping in a suspension of macerated diseased cultures containing viable cells[7]. Moreover, when infected agar cultures in Petri dishes were grown at 33° C for two weeks, and hyphal tips then transferred to new medium at 25° C, normal growth resulted. Similar cultures maintained throughout at 25° C remained diseased[7].

In the absence of a visible pathogen, these results suggested a virus disease, and preliminary experiments[8] showed that purified preparations from diseased, but not from healthy, sporophores contained virus-like 'spherical' particles (approximately 25 mμ diameter), produced a characteristic light-scattering zone in density gradient centrifugation, and caused infection when injected by hypodermic needle into developing sporophores at the first flush stage. Infection of the mycelium in inoculated trays was later confirmed by transmission tests in Petri dish cultures, and the disease was reproduced by further inoculation of healthy mushrooms in trays. The most characteristic symptom of the disease was not the waterlogging of the stipes but the rapid mycelial degeneration, and the way cropping ceased; the name 'die-back' was therefore given to distinguish it from other conditions.

In the present work, cell-free preparations of mushroom tissues have been examined for infectivity, serological activity, formation of zones in density gradient centrifugation, and the presence of virus-like particles. Material for study was at first collected from outbreaks of disease on mushroom farms, but was later obtained by routine inoculation of trays of healthy mushroom spawn with infective compost. Infection developed much more rapidly in mushroom trays containing healthy spawn when inoculated with infective mycelium or compost than when purified virus preparations were injected into the first-formed sporophores. Thus, preparations from sporophores 13 days after inoculation of the tray with infective mycelium had a high virus content, whereas those from trays inoculated by injection of the sporophores at the 'button' stage yielded no detectable virus in a similar time. Inocula were checked for infectivity and standard reaction by the Petri dish culture test[7].

Initially, virus purification was attempted by mincing sporophores showing typical symptoms of watery stipe in 1·25 × wt./vol. of 0·05 M buffer ($Na_2HPO_4 - KH_2PO_4$, *p*H 7·6) containing 0·1 per cent thioglycollic acid. *n*-Butanol was added drop by drop to 8·5 per cent final concentration, and coagulated matter removed by slow-speed centrifugation. The supernatant fluid was filtered and centrifuged to sediment the virus (30,000 r.p.m. for 120 min in the *R*.30 rotor of a Spinco model *L* preparative centrifuge), the pellets re-suspended in one-twentieth original volume of 0·03 M phosphate buffer, and insoluble matter removed by brief centrifugation. Although such preparations were infective and contained characteristic virus-like particles, they still contained much unwanted mushroom material, which produced two non-specific zones in density gradient centrifugation: a broad upper layer, intense creamy white in colour, extending from 10 to 22 per cent, and a fainter creamy grey zone at 31–36 per cent levels, in sucrose gradient columns[9] after centrifugation for 120 min at 25,000 r.p.m. in the Spinco *R* 25 rotor. Preparations from both healthy and diseased mushrooms gave these two zones, but a specific, faint bluish-grey zone at the 28–30 per cent sucrose-level occurred only with diseased material, and the typical 'spherical' particles, 25 mμ diameter, were concentrated here.

Serum obtained after infective preparations had been injected into rabbits reacted as well with healthy as with diseased mushroom preparations; the double reaction lines in gel-diffusion slide tests[10] were derived from antigens that were confined to the top, creamy-white zone in the gradient tubes. The addition of formaldehyde to 0·2 per cent to the antigen preparations before injection made no apparent difference to the performance of the serum, although this treatment had enabled a specific serum to be prepared against a virus of celery[11].

Different kinds and concentrations of buffer solutions were then compared in efforts to improve the virus purification. No benefit was obtained by using chelating agents, such as ethylenediamine tetraacetic acid, but substitution of borate buffer ($H_3BO_3 - Na_2B_4O_7$, *p*H 7·6) containing thioglycollic acid for the phosphate buffer gave final preparations containing more virus and without the unwanted mushroom constituents. With other plant viruses, the intensity of specific zones in density gradients has given a measure of the virus concentration, and at first provided the only means of assay of mushroom preparations. Later, the serological titre was used. A concentration of 0·05 M borate buffer was better than either 0·5 M or 0·1 M, and preparations made at laboratory temperatures (about 18° C) were better than those made at about 4° C. Using 0·05 M buffer with the butanol extraction method previously described, mushroom stipes were a much better source of virus than the pileus, and the symptomless sporophores first produced after infection better than later-formed ones showing typical symptoms. Older sporophores not only contained less virus, but also more of the unwanted substances that hampered virus purification. Re-suspension of the final pellets in 0·03 M phosphate buffer was better than in equivalent borate buffer. The virus obtained from 50 g of infected sporophore stipes was normally dispersed in 1 ml. of buffer; 0·25–0·5 ml. of a good preparation gave a clearly defined zone in density gradient centrifugation, and had a titre of 1/64 in serological gel-diffusion slide tests. The yield of virus from different batches of material varied considerably.

Petri dish cultures of diseased mycelium grow extremely slowly, and the small quantity of material produced has never been adequate for virus purification. Attempts to precipitate virus from clarified mushroom juice by ammonium sulphate added to one-third saturation were also unsatisfactory, because copious amounts of mushroom constituents remained; nevertheless, virus could be detected serologically in gel-diffusion tests with such preparations.

For electron microscope examination, virus preparations were stained with potassium phosphotungstate; the measured particle sizes are therefore smaller than would be obtained with shadowed mounts. The routine supply of material contained not only a few 'spherical' particles of the type first seen (25 mμ diameter), but also more numerous, larger particles of approximately 29 mμ diameter, and many elongated particles with rounded ends (size about 19 × 50 mμ) which can be described as 'sausage shaped' or 'bacilliform' (Fig. 1). The shape of the particles is of interest: the 29 mμ ones are angular, usually hexagonal, and resemble some of the soil-borne ringspot

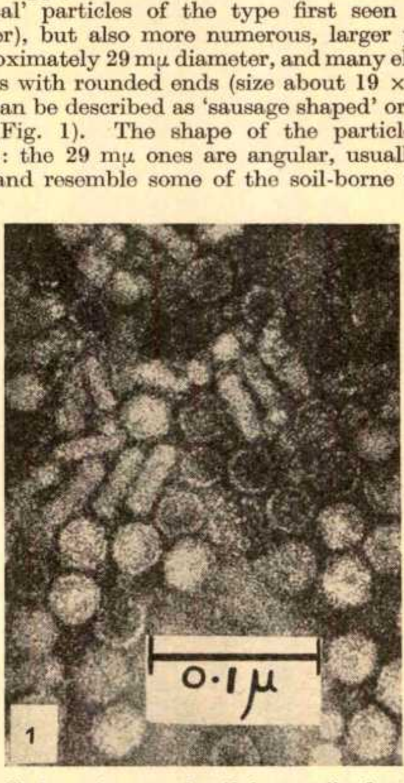

Fig. 1. Electron micrograph of purified preparation from infected mushroom

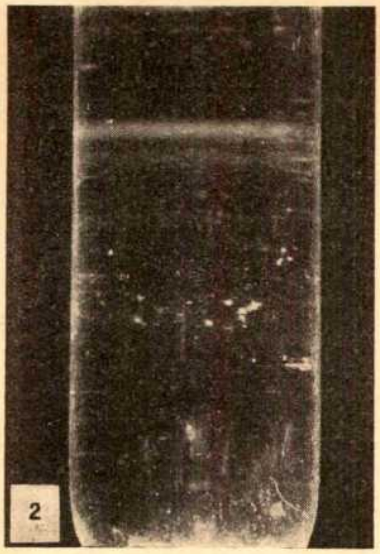

Fig. 2. Specific light scattering zones produced by mushroom virus preparations in density gradient centrifugation approaching equilibrium

viruses of similar size. The only other plant virus with a bacilliform particle known at present is lucerne mosaic[12], where particles 18 mμ diameter occurred in lengths of 36, 48 and 58 mμ.

For some weeks, virus was found only in sporophores from inoculated trays, preparations from uninoculated trays giving negative reactions in all tests. Later, however, infection spread to one of the uninoculated trays, for preparations from symptomless sporophores in this tray produced characteristic zones in density gradient centrifugation, and reacted strongly with a specific antiserum. Electron micrograms, however, showed only the 29 mμ hexagonal particles, suggesting that only one virus had been transferred. Unfortunately, the tray concerned had by this time ceased cropping and been destroyed.

When examined in the Spinco model *E* analytical centrifuge, preparations containing the three types of virus particle gave 3 peaks, whereas those containing only the 29 mμ particles had only 2 peaks—S_{20} = 130 and 74—so that the other peak ($S_{20} = 96$) probably represents the elongated particles, rather than the very few 25 mμ 'spheres'. The peak at 74 may consist of 'empty' particles of the 29 mμ virus, for such 'empty' particles were seen in electron micrograms of that preparation. I do not know whether the elongated particles were present in my early material and were lost in the purification, or what part each type of particle plays in the disease symptoms. The ultra-violet absorption spectrum of preparations containing only the 29 mμ particles showed a maximum at 260 mμ, with the ratio of 1·32 for the optical density at 260/280 mμ.

Density gradient centrifugation of virus preparations, in either sucrose/0·03 M borate or sucrose/0·03 M phosphate buffer, *p*H 7·6, gave either a single or a double zone, of the characteristic blue colour seen with many plant viruses; the unspecific cream-coloured zones produced with phosphate buffer preparations were absent from those made with the borate buffer method (Fig. 2).

After 30 min centrifugation at 25,000 r.p.m. in the *R* 25 rotor in rate-zone[13] gradients (4, 7, 7 and 7 ml. respectively of 10, 20, 30 and 40 per cent sucrose solutions in 0·03 M phosphate buffer), the characteristic zone was at the 14–15 or 15–16 per cent sucrose-level, depending on variation in shape of individual centrifuge tubes. A second, less intense zone sometimes occurred at 12–13 per cent level. There was no difference between the levels of the zones formed in sucrose/borate buffer and sucrose/phosphate buffer gradients, although the reaction between sucrose and borate buffer is stated to decrease the viscosity of the mixture. The 29 mμ and the elongated particles were both present in the specific zones in density gradient tubes; although the two zones sometimes produced were carefully withdrawn by hypodermic needle inserted through the tube wall while the tube was strongly illuminated from above, both upper and lower zones contained the two types of particle. The sedimentation coefficient of a solvated particle is a function of its size, shape and density[14], and because the calculated volumes of the 29 mμ hexagonal particle and the elongated particle are similar (12,770 and 12,435 $m\mu^3$ respectively), they might be expected to sediment at similar rates in rate-zonal centrifugation, and thus not be readily separable in this way.

The formation of double zones is of interest, but none of the explanations so far put forward is entirely satisfactory. Electron microscope examination of the zones did not support the possibility that the upper zone was composed of 'empty' particles, similar to the 'top component' of turnip yellow mosaic virus[15], for empty particles occurred in both zones. Matthews[16] reported multiple zones when apparently homogeneous preparations of turnip yellow mosaic virus were centrifuged in gradients of cæsium chloride, and suggested that these zones comprised virus particles in different stages of assembly. However, some preparations of mushroom virus have consistently given two zones; others, which gave one zone in tests made shortly after purification, produced two zones when they had been stored at 0° C for about 3 weeks. Nor is the suggestion that some particles might aggregate into dimers satisfactory; this might explain two zones in rate-zonal centrifugation, where sedimentation is influenced by the effective size of the particle, but not two zones in sedimentation approaching equilibrium. That the two zones are formed by different types of virus particle is also improbable, for electron microscopy showed that each zone contained the 29 mμ and the elongated particles. Even allowing for failure to withdraw the zones from the gradient column completely separate from each other, preparations containing more than one type of particle have given one zone on some occasions and two zones at other times. Also, the preparations from the naturally infected tray contained only 29 mμ particles, yet gave two zones.

An apparently similar problem has arisen in work with some plant viruses such as pelargonium leaf curl[17], where 2, 3 or 4 zones may occur with different preparations from the same source of virus, and in which results suggest combination of the virus particle during or after purification with some substance that increases the sedimentation-rate and effective density, and impairs the ability of the virus to multiply; for it is the lower zones with pelargonium leaf curl virus, unlike turnip yellow mosaic virus, which behave abnormally in infectivity tests, although serologically indistinguishable from the typical form of the virus.

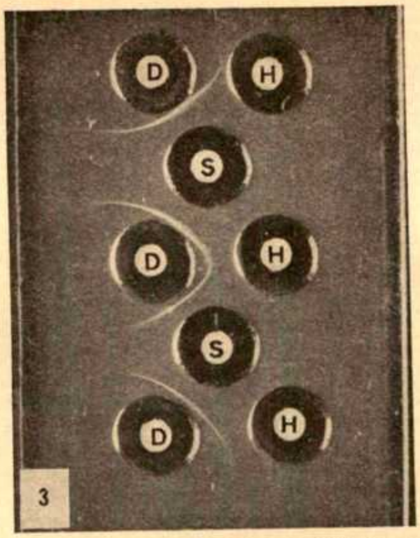

Fig. 3. Gel-diffusion slide serological test. Wells *D* contain preparation from diseased, wells *H* from healthy, mushroom. Virus antiserum in wells *S*

Preparations containing numerous virus particles, but freed from the antigenic mushroom substances, could consistently be produced by the borate buffer–butanol method, and were used to prepare antisera. 1 ml. aliquots of infective preparations were emulsified with 1 ml. of Freund's complete adjuvant (Bacto) and injected intramuscularly into rabbits. Two injections were given, three weeks apart, and serum obtained 2½ weeks after the last injection. This serum reacted specifically with preparations from diseased, but not from healthy, mushrooms (Fig. 3). The antigen preparations contained all three types of particle, but these preparations gave only a single reaction line in gel-diffusion tests. This line was identical and continuous with that formed by the preparations containing only the 29-mμ particles. There is thus no evidence that the other particles were antigenic, although the 25-mμ particles were probably too few to elicit a detectable response in rabbits or in serological tests. The possibility cannot be excluded that the 25-mμ particles are a damaged or breakdown product of the 29-mμ ones, although there is no evidence for this.

The 19 × 50-mμ particles resemble those of lucerne mosaic virus, and tests were made for other similarities between them. However, the mushroom virus preparations did not react with antiserum to lucerne mosaic virus, nor did mushroom virus antiserum react with purified preparations of lucerne mosaic virus. Also, the density gradient zones obtained with preparations of lucerne mosaic virus differ from those of mushroom viruses. Inoculation by rubbing mushroom preparations on standard test plants susceptible to many plant viruses (including lucerne mosaic and the soil-borne ringspot viruses), such as *Chenopodium amaranticolor* Coste and Reyn., *Gomphrena globosa* L., *Nicotiana clevelandii* Gray, *N. glutinosa* L., *N. tabacum* L., *Phaseolus vulgaris* L. and *Tetragonia expansa* Murr., produced no symptoms.

The results reported here show that die-back disease of mushrooms is caused by one or more viruses, and can be transmitted by injection of cell-free preparations into healthy mushroom sporophores. Efforts to separate the viruses, the identification of the disease symptoms with each virus or viruses, and studies of the means of natural spread and epidemiology of the disease continue as part of the programme of this Institute.

I thank Dr. F. T. Last and Miss D. G. Gandy for growing and inoculating the mushrooms, and Mr. H. L. Nixon, of Rothamsted Experimental Station, for the electron micrograms and the sedimentation constants.

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APOCRINE SWEAT GLANDS OF THE SCROTUM OF THE RAM

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EVAPORATION from the skin of sheep is believed to be of secondary thermo-regulatory importance when compared with evaporative cooling from the respiratory tract[1-3]. Although this may be true for the densely fleeced body, it is possible that the more lightly covered skin of the extremities plays an important part in temperature regulation, as the considerable regional differences in the number and volume of the sweat glands in Merino rams would suggest. The glands in the skin of the peripheral parts of the legs are larger and more numerous than in mid-side skin, while in the scrotum they are of considerably greater volume than anywhere else examined (Fig. 1). The sweat glands in the scrotum of rams are of comparable volume with the sweat glands of the ox[4,5] in which sweating is thought to be a major channel of heat loss.

The functional properties of these large apocrine sweat glands were examined by enclosing the scrotum of Merino and Border Leicester × Merino rams in an

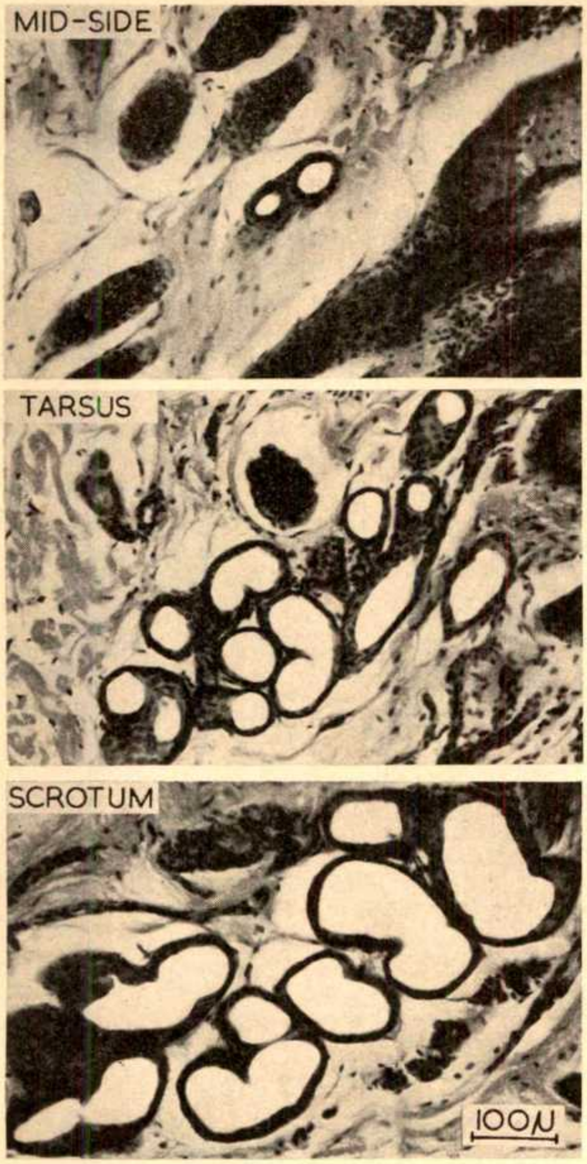

Fig. 1. Sections (20µ thick) of skin cut at right angles to the surface from three regions of a Merino ram, showing marked differences in sweat gland size. (Hæmatoxylin and eosin: × 118)

Figures in table obtained from three Merino rams and demonstrate the considerable individual and regional variation in gland volume

Sweat glands	
No./mm²	Mean vol. mm³
2·9	0·0020
1·8	0·0025
2·1	0·0116
7·1	0·0049
5·9	0·0055
—	—
2·3	0·029
2·5	0·038
2·5	0·043

insulated chamber, the wall temperature of which could be changed and through which dried air at controlled temperatures was circulated in a closed circuit. When the air and wall temperatures were raised from 20° C to 42° C, the temperature of the scrotal skin, recorded by 38 s.w.g. thermocouples, rose rapidly from 30° to 34° C into the range 35°–37° C. The first expulsion of sweat then occurred and was indicated by a sudden increase in the humidity of the air leaving the chamber and passing over a recording dew cell (Fig. 2). A wool-covered patch of scrotal skin was warmer than adjacent bare skin when the air was cool but the temperature of bare skin rose above that of wool-covered skin when the air was heated. This is presumably due to the insulating properties of wool and also to the protection it gives against radiant heat[6] emitted by the chamber wall. Quick rises of up to 1·5° C in the temperature of wool-covered skin coincided with the appearance of water vapour in the chamber air and were followed by more prolonged cooling of the skin. The warming was presumably due to an exothermic reaction of sweat with wool[7,8], for this was not observed when the skin was shaved. Instead, bare skin always quickly cooled by up to 2·6° C, the first expulsion of sweat usually producing the greatest reduction in temperature. Thus, as a result of the same sweat discharge, a patch of wool-covered skin on the scrotum could be warming while an adjacent area of shaved skin was cooling (Fig. 2). Even so, the temperature of skin under wool never rose above that of adjacent bare skin during heating. As the skin dried following each discharge, surface temperature rose again; the warming-up was delayed and slower when wool was present (Fig. 2).

All or a constant proportion of the glands appear to take part in each discharge because thermocouples applied to different areas of the scrotum record similar changes of skin temperature. Mass spontaneous discharges of the sweat glands of mid-side skin of Welsh Mountain sheep have been described when the whole animal was exposed to heat (42° C), but never more frequently than once every ¾–1 h (ref. 7). The expulsion of sweat by the glands of the scrotum occurred at 2–17-min intervals in seven rams, but in one ram the intervals were characteristically longer.

When the scrotum of fully fleeced rams is locally warmed to above 36° C, respiratory rate increases and hypothermia usually follows as a result of the increased evaporative cooling[9,10]. In the experiments reported here, temperatures in the rectum and in free-flowing blood in the carotid artery either remained unaltered or, more usually, fell by up to 1·4° C/h when the scrotum was warmed (Fig. 2). The sweat discharges continued whether body temperatures were falling or steady. The reflex polypnoea was often interrupted during a sweat gland discharge (Fig. 2) and the reduction in breathing rate was often completed before the skin started to cool. As similar changes in respiratory rate were observed in fleece-covered sheep when an exothermic rise of skin temperature occurred on sweating[7], it appears that the effect is unrelated to skin temperature and could be central in origin. All sweat gland discharges by the scrotum in response to heating were abolished by locally blocking the superior perineal nerves which carry sensory fibres from the scrotum. Thus, although central hyperthermia may facilitate sweating in sheep as in other species, cutaneous afferent stimulation alone is sufficient to bring about sweating from the scrotum.

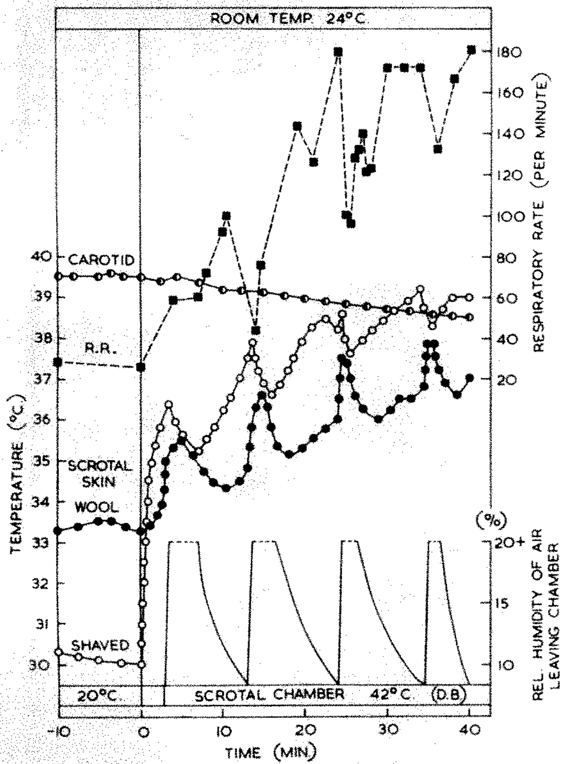

Fig. 2. Record of four spontaneous discharges of sweat from the scrotum of a ram in response to local warming and their effect on the temperature of shaved (○) and wool-covered (●) scrotal skin and on respiratory rate (■). Carotid record from a thermocouple inserted through a hypodermic needle into the artery in a fold of skin

The discharges could be provoked by single intravenous injections of adrenaline and 1-noradrenaline (1–2 μg/kg) into rams in which the scrotal skin was warmed to a temperature just below that at which spontaneous discharges occur. The glands responded to a second dose within 8 min of the first, unlike the mid-side glands, which have been shown to be refractory to adrenaline for at least ½ h (ref. 7). Bilateral adrenalectomy with supporting injections of cortisone acetate (0·5 mg/kg) and deoxycorticosterone acetate (0·05–0·10 mg/kg) did not abolish the sweat discharges in response to a heat stimulus. However, after adrenalectomy the interval between the application of heat to the scrotum and the first discharge was increased and this interval was again reduced by an intravenous infusion of 1 μg adrenaline/kg/min. It is clear that the expulsion of sweat by the glands of the scrotum in response to a local heat stimulus is under neural control although the appearance of the normal pattern of discharges appears to require normal adrenal function.

The fluid that evaporated from the scrotum during the experiments was collected by drying the circulating air in cylinders containing calcium chloride. There was always approximately a three-fold increase in fluid produced by scrotal skin when air temperature was raised from 20°–26° C to 40°–45° C. Both basal and maximal values obtained from individual rams were greater when the tests were made in the summer months (October–April) than in the winter (June–September), and must represent a seasonal variation in both insensible and sensible fluid loss from the scrotum. Maximal fluid production in the winter was 72–153 $g/m^2/h$ and increased to 134–208 $g/m^2/h$ during the summer. This agrees with histological evidence that sweat glands in body skin are larger in summer months[11].

The evidence presented supports the view that, in sheep, sweating is important to the regulation of the temperature of peripheral regions, especially the scrotum, and provides information about the mechanism of sweat released by apocrine glands, about which little is known[12].

We thank Mr. W. H. Clarke and the Histology Section of this Laboratory for assistance with the preparation and staining of the skin samples.

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PROTEIN BODIES AND PROTEIN SYNTHESIS IN DEVELOPING WHEAT ENDOSPERM

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THE type and amount of protein have a marked influence on the baking quality of wheat flour. Hence we have examined several aspects of protein synthesis in developing grain of *Triticum vulgare* (varieties Gabo and Insignia) and of *Triticum durum* (variety Dural) grown under field conditions in Adelaide. This work has involved: (*a*) cytological studies with the electron microscope to determine the site of synthesis and the intra-cellular localization of the 'storage proteins'; (*b*) development of methods for the separation and identification of each of the major proteins of endosperm; (*c*) biochemical investigations related to the mechanism of protein synthesis.

These investigations were carried out with grain harvested at selected intervals from approximately the 5th to the 42nd day after the mean flowering date and immediately stored at − 15° C. Endosperm was obtained from the frozen grain either by hand dissection or by mechanical rolling. Mature grain was milled in a Bühler laboratory mill to 68 per cent extraction. For the cytological study, fresh grain or endosperm dissected out at room temperature was used as soon as possible after harvesting.

During development, both the fresh and dry weight per grain increase from about the 5th to the 35th day, after which the fresh weight declines, and the dry weight increases only slightly, until maturity at about the 45th day. During the period of rapid increase of protein in the endosperm there is a related increase in the amount of ribonucleic acid per grain, but there is little change in the amount of deoxyribonucleic acid per grain after about the 14th day. Thus development is mainly due to increase in dry weight of previously formed cells, and not due to cell division.

Electron microscopy of thin sections of developing wheat grain stained with either osmium tetroxide or potassium permanganate has been carried out at each of the times selected for chemical analyses, except that after about the 25th day so much starch is present that thin sections cannot be prepared. In addition to starch granules formed inside a distinctive 'plastid', the endosperm cells contain relatively large osmophilic bodies. Fig. 1 is typical, and shows that the bodies are contained within a definite lipoprotein membrane. In early stages of development there is a single body contained in the one membrane, but at later stages there are often four or more bodies. The bodies have now been isolated from wheat endosperm; due to their fragility the bodies are difficult to isolate in a relatively pure state, but good preparations contain about 85 per cent protein. Starch-gel electrophoresis patterns of these isolated bodies show that they contain predominantly slow-moving protein components; these patterns closely resemble those obtained for the acetic acid soluble proteins extracted from intact endosperm[1]. Radioisotope investigations have shown, even in short-term experiments, that accumulation of protein occurs in the bodies. As they increase in size and number during development, and as there is a rapid increase in the acetic acid soluble proteins during maturation of the endosperm[1], there is now good evidence that these bodies represent the 'storage proteins' of the wheat endosperm. Other workers[2-4] using light microscopy have described organelles in maize, sorghum and peanut, which on the basis of histochemical tests they have termed protein granules or protein bodies.

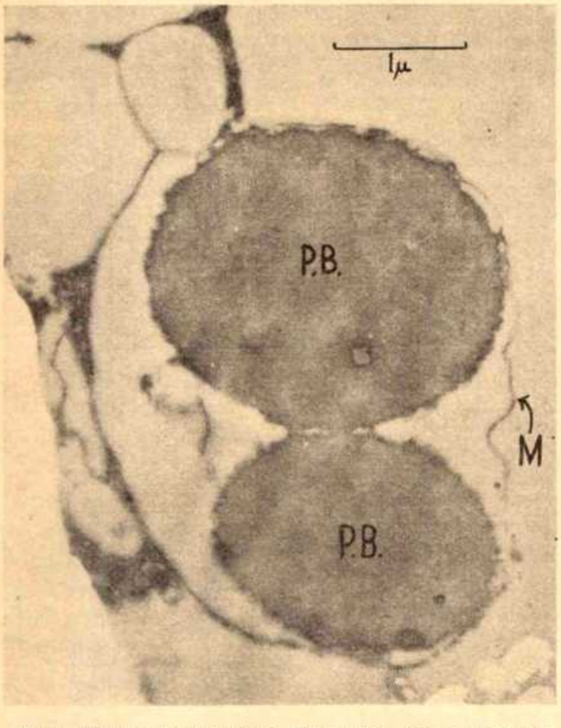

Fig. 1. Electron micrograph of a thin section of endosperm cells of grain of *Triticum durum* (variety Dural). The material was fixed and stained with osmium tetroxide for 1 h at *p*H 7·4 and embedded in methacrylate. Protein bodies (*PB*) and lipoprotein membrane of vacuolar structure enclosing bodies (*M*) are clearly shown

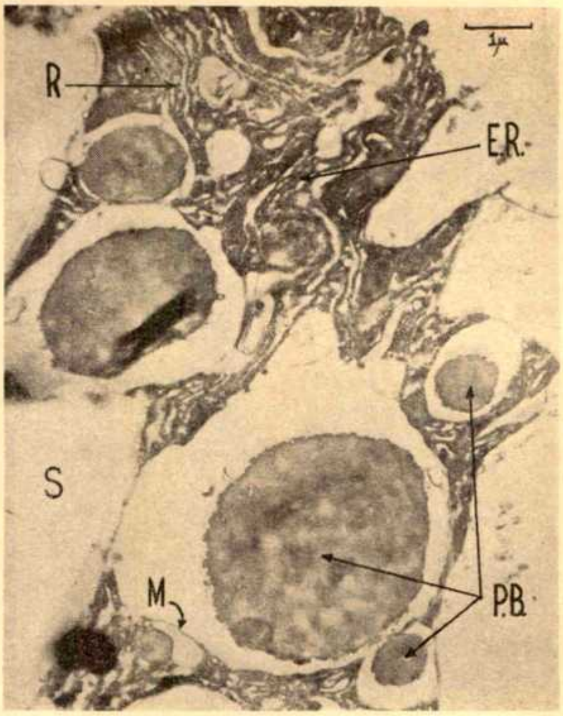

Fig. 2. Electron micrograph showing endoplasmic reticulum (*ER*), ribosomes (*R*) and starch (*S*). Otherwise the conditions are the same as in Fig. 1

In the grain endosperm, in contrast to the developing wheat root[5], the osmophilic ribosomes are arranged in an orderly fashion on parallel arrays of the lipoprotein membranes of the endoplasmic reticulum (Fig. 2). The structure of the endoplasmic reticulum is strikingly similar to that of the mammary gland which synthesizes and secretes casein particles. The 'storage proteins' are almost certainly formed on the ribosomes of the endoplasmic reticulum and afterwards they are secreted, not externally as in the mammary gland, but internally. It is thought that the formed proteins aggregate into the protein bodies within the surrounding lipoprotein membrane and are thus isolated from the remainder of the intracellular structures. It is therefore proposed that this process in plants be called 'internal secretion'. The 'protein bodies' thus appear to be localized in vacuolar structures of the endosperm cell. With increasing development these vacuolar structures containing protein bodies, and somewhat similar structures containing starch granules, occupy most of the expanding endosperm cell. The protein bodies themselves do not have a true limiting membrane but rather appear to be aggregates (as are casein particles), although, like nucleoli, protein bodies have a definite external boundary, the rupture of which may release the internal contents. However, they may be attached to the outer membrane of the vacuolar structure within which they are contained by a fine reticular network, seen in some electron micrographs. Quite frequently some of the vacuolar membrane is observed wrapped around the isolated bodies.

In addition to the biochemical studies of the isolated bodies extensive investigations have been carried out on the protein composition of the whole endosperm. This work has involved extraction of the proteins, fractionation of the extracts on modified cellulose columns[1], and characterization of the proteins by starch-gel electrophoresis. Results have also been obtained for the incorporation of radio isotopes (carbon-14 and sulphur-35) into individual protein components separated by starch-gel electrophoresis. Changes in protein composition during development of the endosperm have been shown to be quantitative rather than qualitative, while the differences in protein composition between the three wheat varieties investigated are present throughout development. Moreover, using starch-gel electrophoresis, it is possible to show a relationship between the protein components of similar genotypes in both wheat and barley. It is suggested that this may provide a sensitive analytical tool for cereal breeding work.

Separation of the protein components by starch-gel electrophoresis has shown that Osborne's procedure[6], based purely on solubility, does not give a true qualitative separation of wheat flour proteins. Since there is no qualitative distinction between proteins extracted by different solvents it is considered that in order to obtain a clear understanding of protein synthesis in wheat endosperm it is necessary to carry out biochemical studies on isolated morphological components. Ribosomes, amino-acid acceptor-RNA and the amino-acid activating enzymes have been prepared from immature endosperm, and synthesis of endosperm proteins *in vitro* may now be envisaged.

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CONVERGENCE AND PROGRESSION IN *EUCALYPTUS* AND *SYMPHYOMYRTUS*

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IN a previous communication[1] we showed that the eucalypts can be divided into two groups on the basis of perianth characters. The divisions thus proposed are supported by vegetative characters such as stellate hairs and by differences in characters of the inflorescence. It was suggested that the group of species in which the operculum is developed from a single perianth whorl and in which the sepals are absent or persist on the fruit as teeth constitutes a sub-genus, for which we coined the informal name '*Monocalyptus*'. We directed attention to the fact that Gauba and Pryor[2] had established that the seeds of the species of the Renantherae (the nomenclature adopted throughout is that of Blakely[3]), which constitute a large part of this sub-genus, have testas formed from both integuments. They also found that *E. jacksoni*, placed by us in *Monocalyptus*, has this type of seed-coat. Since then the same authors[4] have shown that the seeds of members of the series Eudesmieae (also in *Monocalyptus*) have double seed-coats and that the seeds of the remainder of the eucalypts studied by them have a testa formed from the outer integument only.

We have investigated the other species of *Monocalyptus*, namely, those of the series Miniatae as well as *E. pressiana*, *E. megacarpa* and *E. gamophylla*, and the group of species, *E. pachyloma*, *E. diversifolia*, *E. santalifolia*, *E. todtiana* and *E. patens*, which constitute the section Renantheroideae and find that all have seeds with double seed-coats. This character, then, is constant in *Monocalyptus*. The constancy of the characters of the seeds and the perianth can therefore now be adduced, together with the fact that, although many cases of hybridity have been recognized in the eucalypts[5], there is no authenticated record of a hybrid between those species with a seed-coat formed from both integuments and those without this character[6] as evidence for the view that the old genus *Eucalyptus* really consists of two separate but remarkably convergent genera.

We now propose to abandon the informal name *Monocalyptus*. Instead we have elsewhere[7] proposed to designate the mono-operculate species with a double seed-coat as *Eucalyptus* '*sensu stricto*', segregating them from the rest of the eucalypts, which we propose to call *Symphyomyrtus*. This nomenclature follows from the fact that the type species, *E. obliqua* L'Hérit., is mono-operculate and that *Symphyomyrtus* (*S. lehmannii* Schau. syn. *E. lehmannii* (Schau.) Benth.) is the only available synonym for any of the other species. The chief characters of these two genera, of which a full and valid description has already been provided[7], is as follows: *Eucalyptus* L'Hérit. emend Carr and Carr: sepals absent or, if present, alternating with the petals and consisting of four, rarely five, teeth, persistent on the fruit. Stamens forming a continuous ring, or grouped on staminophores opposite the petals. Inflorescence buds with an involucre rarely of more than six

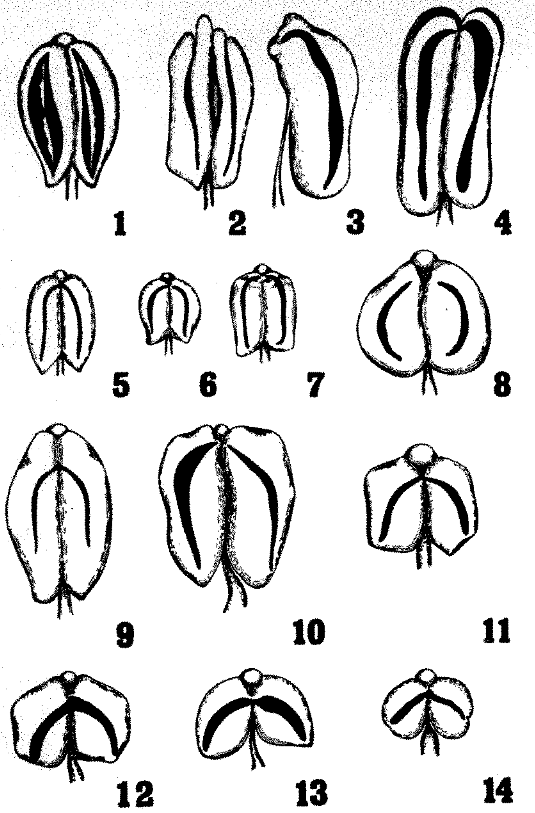

Fig. 1. Drawings of stamens of species of *Eucalyptus*

1, *E. erythrocorys*; 2 and 3, *E. phoenicea* (Miniatae) (3 is a side view); 4, *E. mitrata* ('Megacarpae'); 5, *E. curtisii*; 6, *E. gamophylla*; 7, *E. baileyana* (1, 5, 6 and 7, Eudesmieae); 8, *E. planchoniana*; 9, *E. sepulcralis* (8 and 9, Renantherae); 10, *E. pachyloma* (Renantheroideae); 11, *E. macrorrhyncha*; 12, *E. obliqua*; 13, *E. pauciflora*; 14, *E. coccifera* (11–14, Renantherae)

bracts. The testa of the seed is the product of two integuments. *Symphyomyrtus* Schau. emend Carr and Carr: sepals present, rarely completely free, usually partly or wholly united and caducous either before or at anthesis. Stamens forming a continuous ring. Inflorescence buds with an involucre of bracts always related in number to the number of flowers. Seeds with a testa formed from one integument.

According to this arrangement, the genus *Eucalyptus* would comprise the series Eudesmieae (12 species), with the exception of *E. lirata* but with the inclusion of *E. gamophylla*, the series Miniatae (2 species), *E. jacksoni*, *E. preissiana*, *E. megacarpa* and *E. mitrata* (see following), the section Renantheroideae (5 species) and the Renantherae (104 species) (except *E. guilfoylei*, *E. microcorys* and the series Myrtiformes). We shall use the name *Eucalyptus* only in this restricted sense. *Symphyomyrtus* would contain all the other eucalypts recognized by Blakely. The common name 'eucalypt' will be used for members of both genera. Since the relationships within the genus *Eucalyptus* are the more clearly defined, our attention has been directed principally to it rather than to *Symphyomyrtus*, in which relationships are more complex and of which many species are imperfectly known. The new arrangement cuts right across that based on anthers (shapes and modes of dehiscence) which has been in use since it was originated by Bentham[8]. It now seems abundantly clear that the classification based on anthers was of very limited usefulness, and, as Bentham realized, by its insistence on a single character, inevitably led to many anomalies (see also Blake[9]). The species of Eudesmieae, Renantheroideae and Renantherae can be arranged, for example, to show a continuous gradation of anther forms, ranging from those in which the anther cells are parallel and dehiscence is by parallel, longitudinal slits to those in which the anther cells are divergent and the slits are confluent across the face of the anther. Because this gradation has been overlooked in the past, certain species with intermediate anther shapes (for example, *E. stricta*, *E. alpina*, *E. baileyana*, *E. tenuipes*) have undergone several taxonomic removes[5]. Transitions are also found in the size of the sepal-teeth and the degree of fusion of involucral bracts and the association between these characters and the presence of stellate hairs and the grouping of the stamens on staminophores.

Recent work on the ovules and placentas has confirmed and extended this new view of the taxonomy of the eucalypts. The occurrence on the placentas of many members of Myrtaceae of congenitally sterile ovule-like structures is well known, but no special term has been applied to them. They have been referred to as 'sterile ovules', but this term is ambiguous. As we shall refer to these structures many times here and in the extended account which is in preparation[11] we propose the term 'ovulode' (ovulodium, pl. ovulodia). The numbers of rows of ovules and ovulodes and the disposition of these structures on the placenta, as well as certain other characters of the gynœcium, are of considerable taxonomic value.

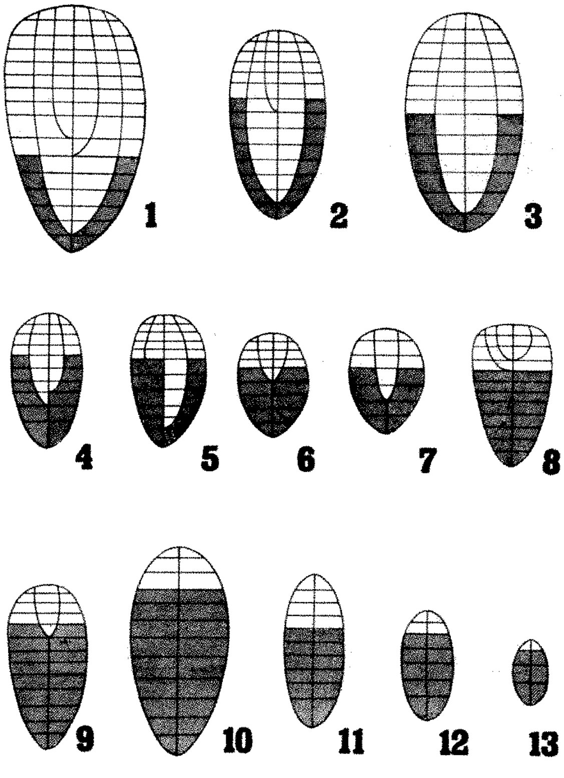

Fig. 2. Diagrams of the disposition of ovules and ovulodes on the placentas of species of *Eucalyptus*. Each 'cell' of each diagram represents an ovule (shaded) or an ovulode (unshaded)

1, *E. erythrocorys*; 2, *E. tetrodonta*; 3, *E. tetragona*; 4 and 5, *E. eudesmioides*; 6, *E. gamophylla*; 7, *E. odontocarpa* (1–7 Eudesmieae); 8 and 9, *E. patens* (Renantheroideae); 10, *E. preissiana* ('Megacarpae'); 11, *E. sepulcralis*; 12, *E. kybeanensis*; 13, *E. yangoura* (11–13, Renantherae)

This may best be shown by a consideration of the genus *Eucalyptus*. In *E. erythrocorys* (Eudesmieae) the ovules and ovulodes are arranged in approximately 20 transverse rows on each placenta and at its broadest part are in 6 longitudinal rows. The ovules occupy the margins of the lower part of the placenta and are in two rows separated by two rows of ovulodes (Fig. 2). Other members of the Eudesmieae exemplify a transition to a situation in which the lower part of the placenta is occupied exclusively by two longitudinal rows of ovules and the upper part by three longitudinal rows of ovulodes (Fig. 2). There is a corresponding reduction in the number of transverse rows as well. There is some correlation between the number of transverse rows and the length of the flower bud, but little between the number of longitudinal rows and the bud length. In the section Renantheroideae the ovules and ovulodes are, with one exception, in two longitudinal rows. In *E. patens* the ovules are in two rows, the ovulodes at the top of the placenta in three to five rows (Fig. 4, c.f. *E. gamophylla* and *E. odontocarpa*). In the Renantherae, also, the ovules and ovulodes are in two longitudinal rows (Fig. 5). This facilitates the recognition of their fruits and seeds. The scars of attachment of the ovules and ovulodes can be seen easily with a hand lens in fruits of all stages of maturity.

This should prove to be an invaluable field and herbarium character for the recognition of the members of this large group, since the two-rowed condition is not found in any other group of eucalypts. Grose and Zimmer[10] examined the shapes and other characters of the seeds and 'chaff' (ovulodes and infertile ovules) of the Victorian species and point out that the seeds and 'chaff' of renantherous species are generally D-shaped. This is in conformity with the fact that the ovules and ovulodes are in two rows and the seeds and 'chaff' have straight edges from mutual pressure along the mid-line of the placenta, while their outer surfaces are curved in conformity with the curve of the loculus they fill. These facts have enabled us to recognize *E. mitrata* as a member of *Eucalyptus*. Blakely places it with *E. preissiana* and *E. megacarpa*. Gardner (personal communication) also stresses its affinity with these species, already recognized as members of *Eucalyptus* in the strict sense. It has not proved possible to obtain sufficiently young flower buds of *E. mitrata* to ascertain whether it has only one perianth whorl. Nevertheless, the fact that its ovules and ovulodes are in two rows, taken together with the other resemblances to *E. preissiana* and *E. megacarpa*, and especially the fact that its seeds have a double seed-coat, warrants its inclusion in *Eucalyptus*.

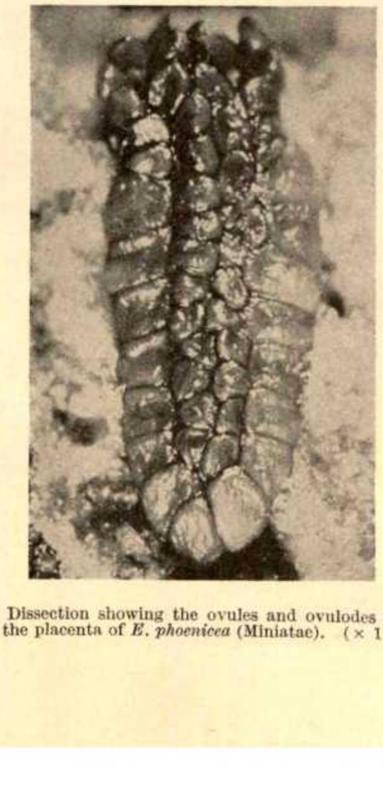

Fig. 3. Dissection showing the ovules and ovulodes *in situ* on the placenta of *E. phoenicea* (Miniatae). (× 17)

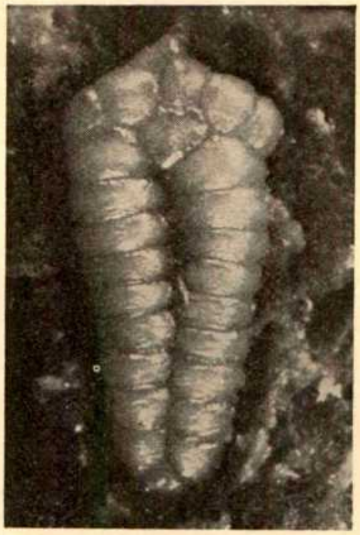

Fig. 4. Dissection of *E. patens* (Renantheroideae). (× 18)

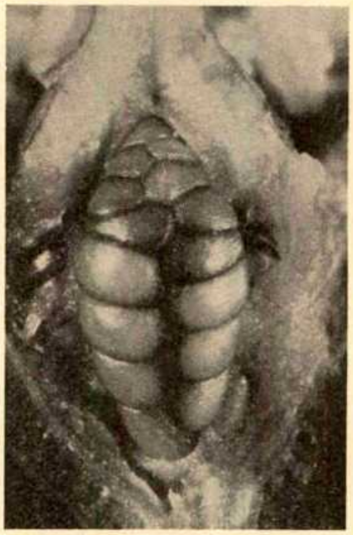

Fig. 5. Dissection of *E. marginata* (Renantherae). (× 19·5)

There is a progressive reduction in the number of longitudinal rows of ovules from six to two and a corresponding reduction in the number of transverse rows in the order Eudesmieae–Renantheroideae–Renantherae. Although each species has its characteristic pattern of arrangement of ovules and ovulodes, there is sufficient variation within certain species (for example, *E. tetrodonta*, *E. eudesmioides* and *E. patens*) to illustrate steps in this reduction at an intraspecific level (Fig. 2). The ratio of the number of ovules to ovulodes increases in the same order, from less than one in the Eudesmieae to more than one in the other sections. The shape of the anther changes in the same order from the so-called 'macrantherous' anthers of some Eudesmieae and of the Miniatae, and those of the 'Megacarpae' (*E. preissiana* and its allies) in which the large anthers have parallel cells but the dehiscence slits are convergent or confluent, to the intermediate condition of the anthers of Renantheroideae and, finally, to the fully 'renantherous' condition in the Renantherae (Fig. 1). Variations and trends in the patterns of arrangement of the ovules and ovulodes, similar to those in *Eucalyptus*, are found also in *Symphyomyrtus*, but the number of longitudinal rows in the latter is never less than four. The similarities between the two genera are as striking in this respect as in many others and testify to their unusual degree of convergence. We shall elsewhere[11] deal more fully with *Symphyomyrtus*.

An examination of ovule and ovulode development[11] has shown that in species in which there are only two longitudinal rows on the placenta, the first primordia to be initiated are those at the top, and the last those at the bottom of the placenta. In species in which there are more than two rows the first primordia are those at the centre of the uppermost transverse row. These are followed by primordia occupying the outer positions on the same transverse row and the central positions of the transverse row below. The last to be initiated are those of the lowest and outermost rows on the placenta. Reference to Fig. 2 shows that the first-formed primordia always develop as ovulodes and that the last-formed are always ovules. There is thus a close parallel with the development of the first-formed stamen primordia to staminodia (lacking anthers) and of the later stamen primordia to functional stamens[11] which is a feature of many eucalypts. Staminodia are found in a few of the Eudesmieae but are rare, if they occur at all, in the other species of *Eucalyptus*. Thus in the order Eudesmieae–Renantheroideae–Renantherae the sterility of the first-formed sporangia is increasingly suppressed.

Among the 12 species of Eudesmieae there is a much greater diversity in the characters of the perianth, anthers and arrangement of the ovules and ovulodes than in the other sections of *Eucalyptus*. *E. curtisii* and *E. tenuipes*, two somewhat anomalous members of Eudesmieae, for example, have many similarities and have long been recognized as being closely related, but they differ considerably in the arrangement of their ovules and ovulodes. In *E. curtisii* the very small ovules and ovulodes are arranged in six longitudinal rows, the ovules occupying the whole of the lower part of the placenta, the ovulodes, unusual in being scarcely distinguishable from the ovules, the upper half. In *E. tenuipes* the ovules again occupy the whole of the lower half of the placenta, but they are in four longitudinal rows and the ovulodes are in only two rows. The Miniatae, at present constituted by the two species *E. miniata* and *E. phoenicea*, may be considered as closely allied to the Eudesmieae. The number of rows of ovules and ovulodes and the pattern of their arrangement (Fig. 3) is remarkably similar in both species and resembles that in *E. erythrocorys*. It has been suggested by Blake[9] that, on the basis of other resemblances, *E. similis* might be placed with the Miniatae. It is therefore of considerable interest that the pattern of ovule and ovulode arrangement in *E. similis* is very like that of the Miniatae.

When the species of *Eucalyptus* are arranged in the order Eudesmieae–Renantheroideae–Renantherae progressions in many characters, both reproductive and vegetative, suggest themselves. For example, *E. erythrocorys*, a typical member of the Eudesmieae, has sepals, staminophores, large numbers of ovulodes and a small ratio of ovules to ovulodes, large flowers in groups of three with two free bracts. It attains a height of 9 m, has stellate hairs and a lignotuber. At the other end of the scale, *E. regnans*, a typical member of Renantherae, has no sepals or staminophores, few ovulodes, and a ratio of ovules to ovulodes greater than one, small flowers in clusters of seven to fifteen, with a mean number of eleven, with six fused bracts. It attains a height of 90 m and has neither stellate hairs nor a lignotuber. Nevertheless, the characters of these two species represent the extremes of morphological series in each of which every step can be exemplified by species of *Eucalyptus*. In all these series the progression is from the Eudesmieae to the Renantheroideae and the Renantherae. We venture to suggest that these progressions in morphological characters, discussed here and elsewhere[11], indicate that the Renantherae and Renantheroideae have evolved from a eudesmioid complex, the few present-day representatives of which betray the degree of variation one might expect in the remnants of an older group from which have sprung the new but comparatively uniform groups of species which constitute the rest of the genus *Eucalyptus*. We have suggested[7] that in the evolution of *Eucalyptus* selection has operated to increase the overall growth-rate, the life-span having been less affected. The progressive reduction in the size of the sepals which is exemplified by some of the Eudesmieae, leading finally to their complete absence in most members of *Eucalyptus*, has been attributed[7] to a shift in timing of sepal and petal growth and in the allometry of sepal and petal growth as by-products of the general trend in the genus towards higher growth rates.

The Miniatae can be considered as an offshoot linked through *E. similis* with the Eudesmieae proper, and comparable in degree of evolution of inflorescence and perianth characters, although not those of stamens or ovules, with the Renantheroideae and Renantherae. *E. curtisii* and *E. tenuipes* are perhaps the remnants of an eastern eudesmioid group, a suggestion which is strengthened by their geographical isolation (they occur close together in southern Queensland) from the rest of the Eudesmieae.

We thank Mrs. W. A. Ross, Hamel, Western Australia, for information and material; Mr. A. K. Cameron, Melbourne, for the loan of his herbarium; and Mr. E. J. Sonenberg, University of Melbourne, for technical assistance.

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LETTERS TO THE EDITOR

GEOPHYSICS

Earth's Central Density

In a paper to be published elsewhere, I have derived the formula:

$$\eta = \frac{\mathrm{d}k}{\mathrm{d}p} - g^{-1}\frac{\mathrm{d}\varphi}{\mathrm{d}z} \qquad (1)$$

where η is a measure of departure from chemical homogeneity at depth z in the Earth (here treated as spherically symmetrical); k, p and g denote the adiabatic incompressibility, pressure and intensity of gravitational attraction, and φ is a function of the P and S seismic velocities. The index η is equal to unity where the Earth is chemically homogeneous, in accordance with a simpler formula[1] developed in 1949; η exceeds unity where the chemical composition is varying with the depth z. An important property of η is that it is equal to the ratio of the actual value of $\mathrm{d}\rho/\mathrm{d}z$ (where ρ denotes density) to the value $\mathrm{d}\rho/\mathrm{d}z$ would take if the composition were unvarying.

Since η depends only on $\mathrm{d}k/\mathrm{d}p$, g and $\mathrm{d}\varphi/\mathrm{d}z$, it is possible to set bounds to η throughout most of the Earth, and so set bounds to the possible density gradients. Physical evidence indicates that $\mathrm{d}k/\mathrm{d}p$ is of the order of 3–5 units in the Earth's deep interior, g is determined in the Earth within uncertainties which reach a maximum of 30 per cent and are mostly much less, while $\mathrm{d}\varphi/\mathrm{d}z$ is directly yielded from the seismic velocity distributions. More complete numerical details on the derivation and use of equation (1) are being set down elsewhere.

Equation (1) acquires some immediate special interest through Bolt's revised P velocity distribution[2] in the Earth's core. The P velocity distribution previously obtained by Jeffreys[3] gave $\mathrm{d}\varphi/\mathrm{d}z$ negative and appreciable in magnitude in the region F. By equation (1), the Jeffreys distribution is found to entail a value of η of order 30 units in F, and thence, in agreement with my Earth model B calculations[4], a large density increase (of about 3 g/cm^3) in this region, resulting in an estimated density of order 18 g/cm^3 at the Earth's centre.

One of the features in Bolt's new distribution is the reduction of $|\mathrm{d}\varphi/\mathrm{d}z|$ to zero in F. By equation (1), this reduces η from about 30 units to the value of $\mathrm{d}k/\mathrm{d}p$, expected to be 4–5 units in this part of the Earth. Thus the value of $\mathrm{d}\rho/\mathrm{d}z$ in F is reduced to one-sixth or less of the value which corresponds to the Jeffreys distribution.

Much detailed analysis, to be presented in a later paper, is required to work out fine details of the density revision in the core required by Bolt's new seismic velocities, since g itself depends on the density distribution. There are also other aspects of the new velocity distribution to be taken into account. But equation (1) has the value of providing a useful first approximation which shows at a glance that the new velocity distribution can permit a substantially reduced estimate of the Earth's central density. The indicated reduction is of the order of 2 g/cm^3 at least.

This is a result of considerable significance to the physics of the Earth's core. Many investigators have found difficulty in accepting a central density as high as 18 g/cm^3. So long as the Jeffreys velocity distribution is used, equation (1) shows immediately that it is difficult to reduce this figure. But with Bolt's distribution, which, incidentally, is compatible with the travel-time data used by Jeffreys, and in fact includes the latter, a central density of order 16 g/cm^3 (possibly a little less) becomes not only possible but also the preferred figure. Thus the revision is in the direction of reconciling previously discordant views on the properties of the Earth's core.

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Cape Town Anomaly and Auroral Emission

It has been recognized since the time of the *Argus* experiments[1,2] that the anomalously low value of the Earth's total magnetic field in the South Atlantic region must have a profound effect on the particles trapped in the radiation belts. The most convincing illustrations of this are the counting-rates of charged particles observed at relatively low altitudes with the Russian cosmic ships[3,4] and recent American satellites in the *Discoverer* series[5]. The maps prepared by both groups show two regions of high radiation intensity in the area of the South Atlantic Ocean, at heights of 250–500 km, where the counting-rates exceed those at corresponding geomagnetic latitudes elsewhere by factors of the order of 1,000 or more. One of these regions, coinciding well with the centre of the magnetic anomaly off the coast of Brazil, is attributed to the inner radiation belt. The other, referred to as the 'south radiation anomaly', lying between Cape Town and Antarctica, is thought to be due to the outer belt. These assignments are supported by the observation that the *Argus* belts of particles lay in the region between the two natural radiation belts[1], corresponding to the positions of the detonation sites between the two recently discovered anomalous radiation zones.

It is the purpose of this communication to point out that the particle flux into the south radiation anomaly is probably large enough to produce observable auroral effects.

Fig. 1 shows the south radiation anomaly as observed by the Russian second cosmic ship[3] and the traces on the Earth's surface of the mirror points of particles entering the zone, estimated with the aid of the longitudinal integral invariant curves published by Vestine and Sibley[6]. Both the northern and southern traces are shown.

O'Brien *et al.*[7,8] have recently reported data telemetered from the satellite *Injun I*, giving electron energies, fluxes and pitch angles at heights of the order of 1,000 km in the vicinity of Iowa City, which is indicated by the point C in Fig. 1. It is evident that these data may be used as a reasonable sample of the

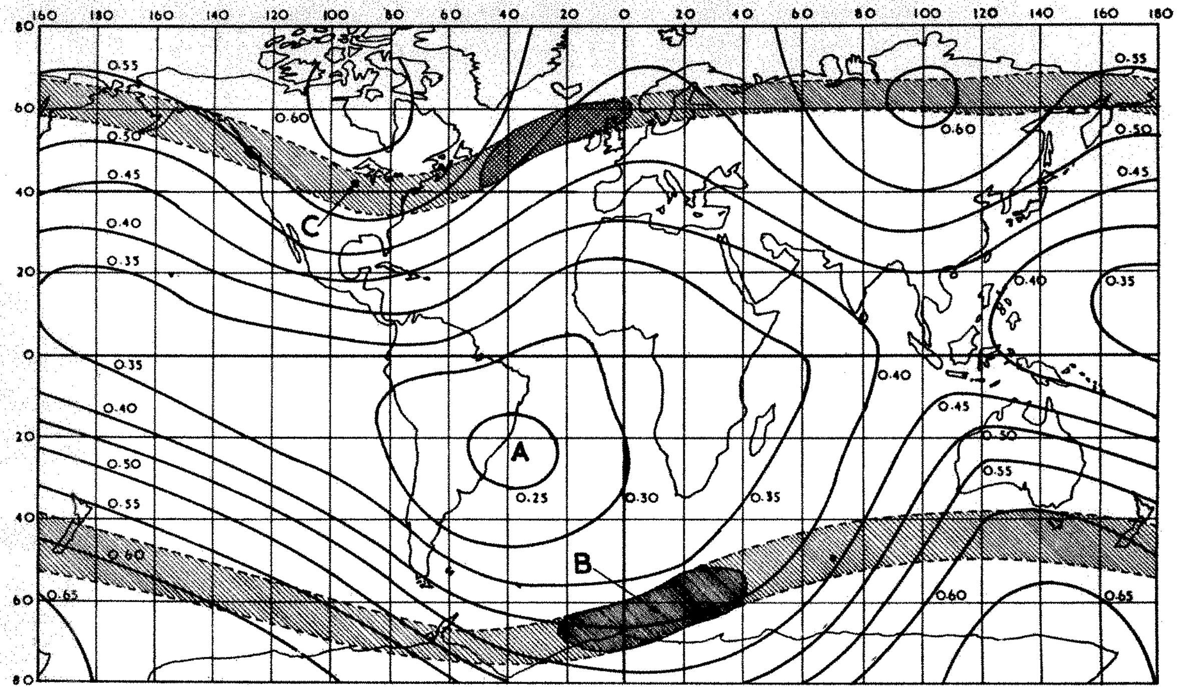

Fig. 1. The geomagnetic anomaly (*A*), the south radiation anomaly (*B*) and traces of the mirror points of electrons falling into the latter. *C* indicates the position of Iowa City

properties of electrons entering the south radiation anomaly. Taking 0·36 gauss as a representative value of the magnetic field in the region sampled[7], it is possible to compute the mirror heights of the trapped particles at other points in their orbits. Fig. 2 was prepared in this way, assuming an inverse cube field and taking the values of the magnetic field at the Earth's surface from Fig. 1. It shows very clearly the pronounced effect of the magnetic anomaly in lowering the mirror points and demonstrates that all the electrons seen by *Injun I* at 1,000 km over Iowa will penetrate to heights below 150 km in the south radiation anomaly, that is, below the F-2 region of the ionosphere. Related graphs have been prepared for other latitudes by other workers[2,9,10] and lead to similar conclusions.

We therefore base our estimates on an assumed energy flux of the order of that found by *Injun I* at 1,000 km over Iowa, namely 100 ergs (cm^2 sec)$^{-1}$, about 90 per cent of this being carried by electrons in the energy range 1–40 keV[7].

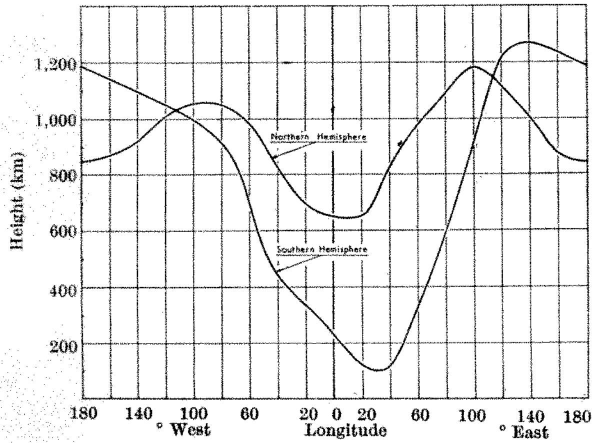

Fig. 2. Mirror point heights for electrons mirroring at 1,000 km over Iowa City

McIlwain[11] has reported measurements of the incident energy flux and electron energy spectrum responsible for two visible auroral displays, obtained by firing instrumented rockets into the upper atmosphere at Fort Churchill, Canada. For "a faint, quiescent auroral glow" he found the electrons to have energies most probably in the range 3–30 keV, with an incident energy flux of 20 ergs (cm^2 sec)$^{-1}$. In a "bright auroral arc" he found almost monoenergetic electrons at about 6 keV to be responsible, the maximum incident flux being then 2,000 ergs (cm^2 sec)$^{-1}$. The intensities of the two auroræ, measured at the ground, were about 0·08 and 0·4 erg (cm^2 sec)$^{-1}$ respectively.

The greater part of the flux which we have estimated to be incident on the south radiation anomaly consists of electrons in just this energy range, and its numerical value lies between those observed in the two auroræ. We may thus expect to find a more or less permanent aurora, possibly even of visible intensity, in this region. The intensity as measured at the ground should be at least 0·1 erg (cm^2 sec)$^{-1}$, or 30 kilorayleighs. Since the normal airglow intensity at these latitudes is a few hundred rayleighs[12] this should be readily detectable. Both McIlwain's observed flux and our assumed one refer to the incident particles, so that the foregoing estimate is presumably valid regardless of what actual fraction of the incident energy is absorbed or converted to light, since the conditions are very similar.

One may wonder why, if there is a permanent glow, faint but nevertheless of visible intensity, it has not been reported by the whalers and expeditions passing through the region. The explanation may well lie in the absence of a background for comparison, so that although the glow is sufficiently intense to be seen, it is so uniform over the visible sky that its presence goes unnoticed. Sandford[13] has discussed this phenomenon in respect of the polar glow, which, even though it may reach "such a brightness that observation of the Milky Way is difficult", may nevertheless be "so uniform over the whole sky that it is not possible, unless the sky appears coloured, for an observer to say for certain that there is an aurora".

By kind permission of the Department of Transport of the South African Government, facilities have been made available for the observation of auroral and other geophysical phenomena from the research ship *R.S.A.* when she is operating in the regions of interest.

We thank Prof. F. D. Seward for making his maps of radiation intensities observed with *Discoverer XXXI* available to us before publication.

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OCEANOGRAPHY

Preliminary Heat-Flow Profile across the Atlantic

DURING the 1962 *Zephyrus* expedition of the Scripps Institution of Oceanography, University of California, fourteen heat-flow measurements were made from Martinique to the Canary Islands. The measurements are on a line except on the crest of the Mid-Atlantic Ridge, where an area was sampled. The instrumentation has been discussed previously[1,2], and our preliminary values are presented in Table 1 and in Fig. 1 with bathymetry.

The results indicate a variation by a factor of about twenty, from 0·3 to $6 \cdot 5 \times 10^{-6}$ cal/cm² sec. High values occur in a belt 200 km wide on the crest of the Mid-Atlantic Ridge and low values are found on its flanks, forming a heat-flow pattern similar to that of the East Pacific Rise[3].

Table 1. HEAT-FLOW VALUES AND LOCATIONS

Station No.	Latitude (N.)	Longitude (W.)	Depth (m)	Thermal conductivity 10^{-3} cal/° C cm sec	Heat-flow 10^{-6} cal/cm² sec
9	16° 24′	57° 39′	4,660	1·8	0·7
11	19° 10′	52° 03′	5,350	1·7	1·4
12	20° 12′	49° 01′	4,640	1·5	0·5
13	21° 06′	46° 30′	3,920	1·7	0·3
14	21° 04′	44° 57′	3,257	2·2	1·8
15	21° 56′	45° 46′	3,380	2·0	6·5
16	23° 06′	45° 39′	3,984	2·0	3·0
17	23° 34′	44° 14′	4,960	2·0	1·6
18	23° 57′	44° 59′	3,490	2·1	2·8
19	23° 36′	42° 28′	4,120	2·2	0·5
20	24° 16′	39° 06′	5,440	1·9	0·4
22	25° 05′	34° 13′	5,610	2·0	0·7
23	26° 14′	26° 27′	5,280	2·0	1·2
25	26° 57′	19° 58′	4,300	2·1	1·0

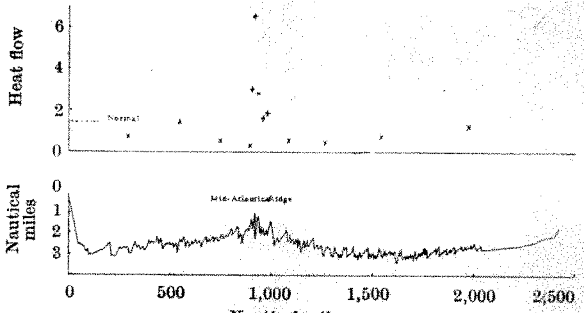

Fig. 1. Heat flow and bathymetry on *Zephyrus* expedition profile. ×, Station in line of bathymetric profile; +, station interpolated into profile

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ASTRONOMY

Dimensional Correlation of Lunar Maria and Terrestrial Ocean Basins

IN a previous paper[1] I have shown that the correlation of diameter versus depth for lunar craters of different apparent ages can be understood in terms of the former presence of a lunar hydrosphere lasting some milliards of years, affecting the relative dimensions at crater formation. Furthermore, this correlation of dimensions included the lunar maria, and was extended later[2] to include the terrestrial ocean basins (corrected for isostasy from the mensuration of present basins). The purpose of this communication is to correct a difficulty in the correlation curves for craters formed explosively in water.

The correlations obtained between the diameter D and depth d for terrestrial explosion and meteoritic craters on land, and for lunar craters of Classes *IH* and *IS* (the youngest), are represented by the lowest curves (continuous) in Fig. 1, given by:

$$D = a_1 d[1 + (d/a_2)] \qquad (1)$$

with a_1 and a_2 constants. To the eye, it appears that an extension of the continuous curves for craters on land might intersect the points for the anisostatic ocean basins. This conclusion is incorrect, as shown by the analytic extrapolations (dot-dashed) from equation (1). However, a different difficulty appears, since both curves in question eventually will intersect the extrapolations (shown dot-dashed) of the curves for craters formed in water. The latter curves were presumed to correspond to craters from surface explosions in water of depth δ, where the parameter $\mu = \delta/d$ has the constant value of unity. It is obvious that two curves of D versus d for the same rock type but differing values of μ cannot intersect, since the depth of water δ must be single-valued. A sufficient condition that two curves of form (1) do not intersect is that a_1 be different but a_2 be the same. In Fig. 1, revised curves of D versus d for $\mu = 1$ are shown (continuous) for hard and soft rock, constructed by taking a_2 equal to the value for craters on land, so that no intersection of corresponding curves for $\mu = 1$ and $\mu = 0$ is possible. The values of a_1 for $\mu = 1$ were fixed from the dimensions of two craters in hard and soft rock, Glasstone (H) and Glasstone (S), respectively, corresponding to nuclear explosions in water[3]; the values are insensitive to μ so long as it lies in the range 1/2–2 (and not 1/12–2, given previously)[1,2].

One notes from Fig. 1 that the points for the larger maria show a general progression away from the revised curve for hard rock with $\mu = 1$, as d increases, in the direction of the corresponding curves for craters on land. Further, the points for the anisostatic ocean basins no longer lie on the locus for $\mu = 1$, as they did in the former case. These points tend to fall on a line segment lying closer to the curves for $\mu = 0$ than the one for which $\mu = 1$, and show a steady progression with increasing d in the direction of the former loci.

It is asserted that the progression of the points for the lunar maria and anisostatic ocean basins from the curve for $\mu = 1$ toward the curves for $\mu = 0$ represents an effect of lessening water depth relative to crater depth, corresponding to the circumstances of the meteoritic explosion. Mean depths of water determined[1,2] previously have been used to fix μ for the maria and ocean basins; some values appear in Fig. 1. On the basis of the limits $1/2 < \mu < 2$ from Glasstone's data, the points for Humboldtianum and the Arctic should lie on and near, respectively, the curve for $\mu = 1$, as is true. For the remaining maria and ocean basins, the values of μ require that the points should be displaced away from the curve for $\mu = 1$ toward the curves for $\mu = 0$, in a progression varying monotonically with μ. The observed progression is monotone, in general, up to the point for the South Pacific, which lies close to the loci for $\mu = 0$ because of the small value (1/13) of μ.

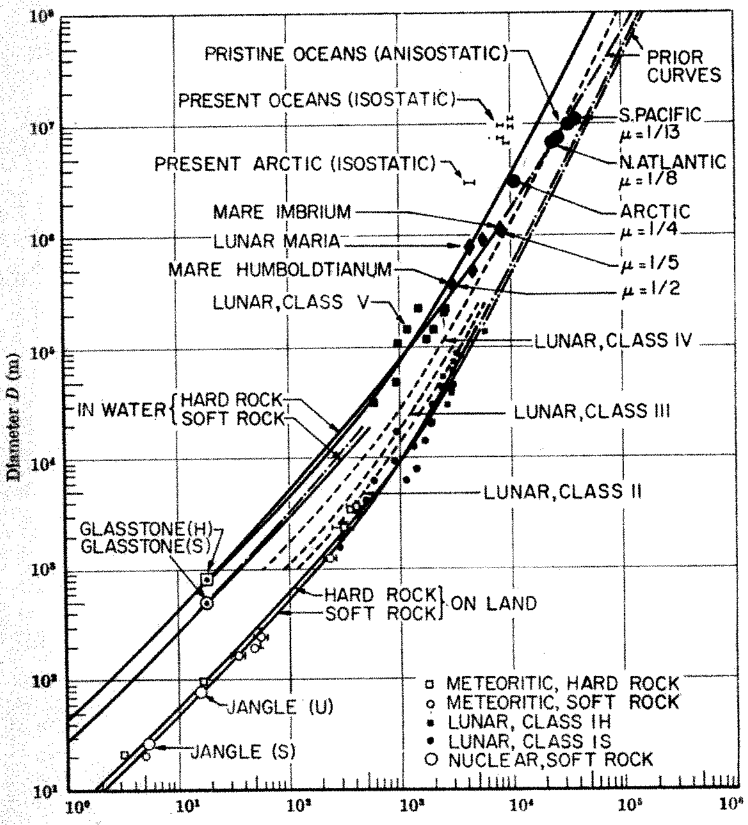

Fig. 1. Correlation curves of diameter versus depth from the present work compared with previous work, for the present and pristine oceans, the lunar maria, and various classes of craters

With this revision of the curves, the representative points for the lunar maria and terrestrial ocean basins show a progression as a function of μ, precisely analogous to that with ordinal number of the class for the points corresponding to the older lunar craters of Classes II, III, and IV, as indicated by the correlation curves shown dashed in Fig. 1.

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PHYSICS

Possible Hydrodynamical Character of Moving and Stationary Striations

THE striated column of the glow discharge has long been one of the most tantalizing phenomena in Nature. Although many theories have been advanced to account for this effect, none has yet met acceptance except perhaps in limited and specialized situations. The extensive and well-known literature is too voluminous for discussion here. Only a few typical references will be cited[1-4]. It is the purpose of this communication to direct attention to a mechanism which can give a unifying framework for the basic phenomena of these striations. The salient features of this new hypothesis will be outlined here; but the details will be reserved for a future report, which is now in process of preparation.

An ordinary fluid is capable of two (irrotational) modes of action: flow motion and wave propagation. When the fluid is compressible, the wave propagation proceeds as a series of compressions and rarefactions (sound waves) if the amplitude is small; but the compressions steepen into shock waves because of the non-linearities in the laws of compressible hydrodynamics when the amplitude is even moderately large. Weak shock waves travel with nearly the same speed as sound (Mach number of order unity); but strong shocks move with supersonic speed. When flow motion and wave motion are both present, the wave moves with the combined speed of both motions. It may seem from laboratory co-ordinates to advance at high speed, to stand still or even to drift backwards depending on the relative directions and magnitudes of the flow velocity and sound velocity. When the wave in question is a shock wave, a moving or stationary discontinuity rides on the flow. In water waves (where the free surface simulates compressibility) these effects are well known and are called 'bores' or 'hydraulic jumps'.

Plasmas display hydrodynamical behaviour and can generate both acoustical waves[5] and shock waves[6]. They are the result of disturbances in which the electron pressure and the ionic mass combine to determine a wave speed $c = (\gamma k T_e/M_i)^{1/2}$ which we will call the plasma-acoustic speed. Here γ is the specific heat ratio of the ideal electron gas, that is, 5/3.

The hypothesis presented here is that striations in the majority of cases are plasma-acoustic shock waves of moderate strength moving relative to the electron current of the column. Striations are known to move both directions in the column at the same time. Those moving from cathode to anode are always fast. Those moving from anode to cathode are slow, and may stop or even drift backward.

For very weak shock waves bordering on the acoustic, the speeds are linearly additive and the forward and backward speeds with respect to the laboratory system are:

$$U_{\pm} = c \pm U_d$$

where U_d is the drift velocity of the electron current. For strong shock waves great complications arise which require the solution of the entire problem of the structure of a single striation, since flow speeds must differ on the two sides of the shock, yet the pattern must repeat from striation to striation. Nevertheless, the condition that shock speed is equal to incoming flow speed can be fulfilled through the Hugoniot relation.

It is probable that the wave-length of the striations is determined by organpipe theory, as has been suggested by Coulter *et al.*[7], and the compressive region in each wave-length should then steepen into a shock across which there will be a potential step. This step has in fact been observed by Boyd[8] in the striations, and elsewhere by Hales and Josephson[9] as a normal concomitant of plasma shock fronts. A striation is thus a unit diffusion cell bounded by two shocks and the wall. Though the electron temperature T_e in a striated column can be described by an average value it is the extra excitation by fast electrons which have moved through this step with a consequent small rise in T_e in the head of a single striation which both produces the extra luminosity which renders the slow striations visible and furnishes the ionization needed to maintain ion balance against diffusion losses despite the low average energy of the bulk of the electrons. The number of electrons accelerated depends on the rate at which the striation advances in the laboratory frame. The energy balance condition in each striation cell is probably that the admixture of these fast and slow electrons equilibrates to a temperature at which the diffusion processes can dispose of the extra electrons produced by the fast component. Observations show that whenever a fast striation collides with a slow striation, luminosity is suppressed, and both striations momentarily stall. This would be because the potential steps, being in opposite sense, cancel each other and require some time to be restored.

Donahue and Dieke have given two points for which both the positive and negative striation velocities have been measured. They find in a 15-mm diameter tube at 3·82 m.amp and 2·1 mm mercury in argon 170 m/sec and 2,300 m/sec respectively; at 20 m.amp. and 12 mm mercury they find 50 m/sec and 1,500 m/sec. From these data we conclude that flow-speed and wave-speed are very closely adjusted to one another. It is only possible to set bounds on such quantities as the electron temperature until the complete theory is furnished. The range in which shock theory says the solution must lie gives a T_e between bounds of $1{\cdot}3 \times 10^3$ and $2{\cdot}1 \times 10^4$ °K for the first case, and between 5×10^2 and 8×10^3 °K in the second. The higher values are the more reasonable ones to expect and agree with the observations of Boyd.

The foregoing hypothesis is directed to the propagation mechanism in long columns only. The excitation of striations probably depends on instabilities at the cathode, recognized by Emeleus[10], while a diffusion theory, such as that of Wojaczek[11], will be needed to describe the structure of a given striation. Instabilities at the anode may only be reflexions of fast negative striations. Whether they have an independent existence should be studied further.

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Electron Drift Velocity in Liquid Argon and Argon–Nitrogen Mixtures

THE very large negative ion mobility observed by Malkin and Schultz[1] and by Williams[2] in liquid argon (about 10 cm²/V.sec) has been interpreted as indicating the existence of free electrons in this liquid. Estimates of the elastic collision cross-section by these authors on a basis of classical kinetic theory appeared to indicate that the liquid could not be considered as a compressed gas, but a comparative study of electron drift velocity data for mixtures of gaseous argon and small concentrations of molecular impurities showed that these earlier interpretations may be in error[3].

It was clear that further investigation was necessary, and this note presents some preliminary results obtained for the drift velocity of electrons in liquid argon and in liquid argon with nitrogen in solution. The experimental method was essentially the same as that of previous investigators[1,2]. Ions were produced close to the cathode surface of a parallel electrode gap by α-particles emitted from a deposit of plutonium-239. By using electrode spacings large in comparison with the length of the α-particle track any range effects could be neglected. The time-constant of the input circuit to the amplifier was made to be very long in comparison with the transit time of the electrons, and under these conditions the integrated

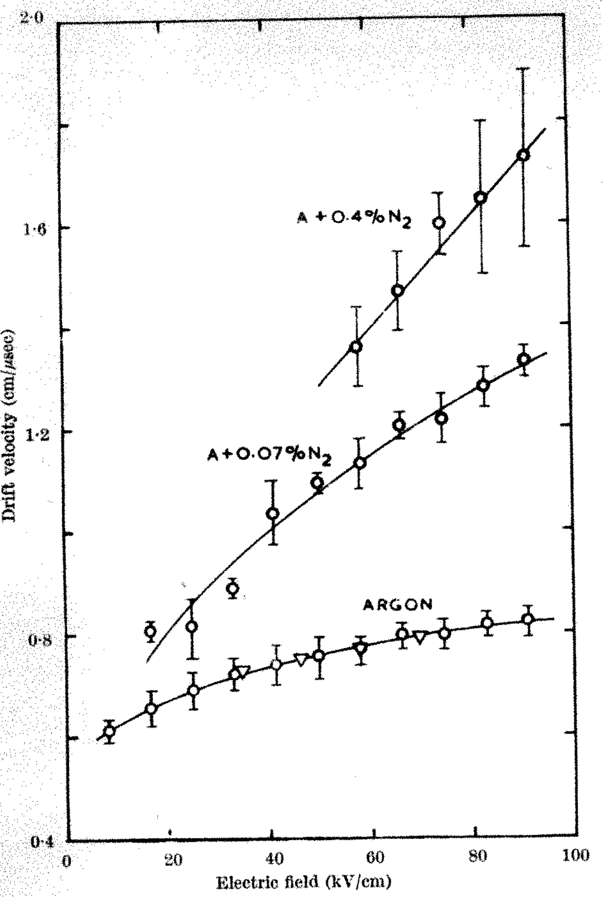

Fig. 1. Electron drift velocity in liquid argon and argon–nitrogen mixtures. Electrode spacing, ○, 1·2 mm; ▽, 1·72 mm

current pulse produced by the electrons in motion was observed using wide-band amplifiers and a high-speed oscilloscope. Individual traces were photographed and enlarged before analysis.

The argon used was of commercial grade, supplied by British Oxygen Gases and stated to be 99·995 per cent argon with nitrogen the principal impurity. When it was desired to mix nitrogen with the argon a known quantity of this impurity gas was admitted to the system first and argon was then condensed until a fixed volume of liquid was in the system. The percentage impurity was computed by assuming that all the nitrogen went into solution.

Fig. 1 shows the electron drift velocity as measured in the commercial grade argon and in argon–nitrogen mixtures for field strengths up to 100 kV/cm. It is seen that the addition of nitrogen increased the drift velocity considerably. With the larger nitrogen concentrations increased recombination in the α-particle track reduced the observed pulse height and measurements at low fields became more difficult. The drift velocity was found to be independent of electrode spacing within the experimental error.

The sensitivity of the electron mobility to small quantities of nitrogen in the argon suggests that the appreciable difference in drift velocity observed by previous investigators was probably due to variations in purity of the argon samples. The mobility-field relationship may be expressed in the form $\mu = AE^{-n}$, where μ is the mobility, E field and A a constant, and using this equation Williams's data yield a value of $n \approx 1$, while Malkin–Schultz found that $n = 0{\cdot}5$. For the three experimental curves given here, n is found to vary from 1·06 for the commercial argon to about 0·6 for the mixture with 0·4 per cent nitrogen.

There is also a very marked similarity in the behaviour of electrons in liquid and gaseous argon, as already suggested by me[3]. The momentum transfer cross-section of argon exhibits the Ramsauer minimum at about 1 eV, and because of this any reduction in mean electron energy will result in a decrease of the cross-section. While there are no inelastic collisions in argon below the first excitation potential, addition of a small quantity of nitrogen will introduce inelastic scattering at energies as low as 1 eV through the excitation of vibrational and rotational modes. The influence of the impurity, therefore, is to reduce the mean energy of the electrons, and as a consequence, to reduce the mean momentum transfer cross-section also. From kinetic theory the drift velocity at a given value of the ratio of field to density is inversely proportional to the momentum transfer cross-section and to the square root of the mean energy, and since both are reduced by the impurity the drift velocity increases. This effect has been fully studied for gaseous argon both experimentally[4] and theoretically[5].

In the liquid state the proximity of neighbouring atoms might be expected to modify the momentum transfer cross-section considerably, in which case the influence of impurities could be very different, but the results presented herein indicate that argon in the liquid and in the gaseous state behave in a similar way. These results suggest that in the liquid state the momentum transfer cross-section exhibits a Ramsauer type minimum as in the gaseous phase. However, the Ramsauer minimum arises from the form of the scattering potential assumed for the heavier of the rare gases, and the 'polarization correction' to the Hartree field which was introduced by Holtsmark in order to give good agreement between theory and experiment extends to well over 4 Å from the atom. In liquid argon the nearest neighbour to any atom can approach within 3·05 Å, while the mean separation between two atoms is 3·86 Å[6], and thus there would be considerable overlap and distortion of the scattering field. In view of this it is rather surprising that the drift velocity data presented here suggest that there is very little interaction between neighbouring atoms.

The results given for commercial grade argon do not by any means represent those for pure argon, and further experiments will be carried out using purified argon. The effect of other gases dissolved in argon will also be examined, and information gained on the electron trapping properties of the impurity molecules. Full accounts of this work will be published elsewhere.

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Polygonization in Silver Chloride

Jones and Mitchell[1] showed that etch pits were produced on surfaces of silver chloride crystals close to {100} by a 3 N solution of sodium thiosulphate, and demonstrated further that in many crystals (depending on the "precise crystallographic orientation of the surface and upon particular properties of individual crystals") there was a one-to-one correspondence between the etch pits and dislocation lines emerging at the surface. This technique has been used here to examine the dislocation arrangement in bent single crystals of silver chloride grown from the melt.

In the specimens used the surface normal to the axis of bending was close to {100}, deformation was by single slip and the dislocations introduced into the crystal by bending clearly lay on slip planes. Silver chloride deforms by pencil glide[2] and hence a surface showing straight slip traces presumably makes a small angle with the slip direction, and slip dislocations the lines of which are normal to it must be of predominantly edge character. Single crystal bars (about 1 × 3 × 40 mm) were polished in a solution of four parts of 3 N sodium thiosulphate and one part of ammonium hydroxide and then annealed *in vacuo* and allowed to cool slowly in the furnace, so that they appeared completely dark when viewed between crossed nicols. This indicated the absence of any long-range internal stresses. Etching in a solution of 3 N sodium thiosulphate for 1 or 2 sec at this stage showed that most of the 'grown-in' dislocations were, after annealing, arranged in low-angle boundaries. The crystals were then repolished, bent (plastically) at room temperature to a radius of 5 cm by means of a former and immediately etched.

This etching revealed the grown-in dislocations and also those introduced into the crystal by bending, and by comparison with the etch-pit pattern on the annealed crystal showed that the dislocations in the sub-boundaries play practically no part in the plastic deformation of the crystal (except possibly in the very narrow regions close to the surface where poor viewing conditions prevented observations being made). In the specimens used the dislocations produced by bending were arranged on slip planes as in Fig. 1, where the slip plane traces are parallel to the arrow. It can be seen that some of the dislocations have, without heat treatment, arranged themselves into small segments of low-angle boundaries approximately normal to the glide planes, a process termed 'glide polygonization'[3] or 'mechanical polygonization'[4].

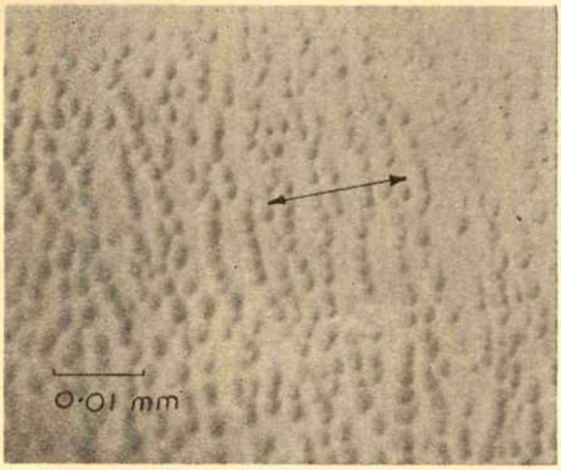

Fig. 1

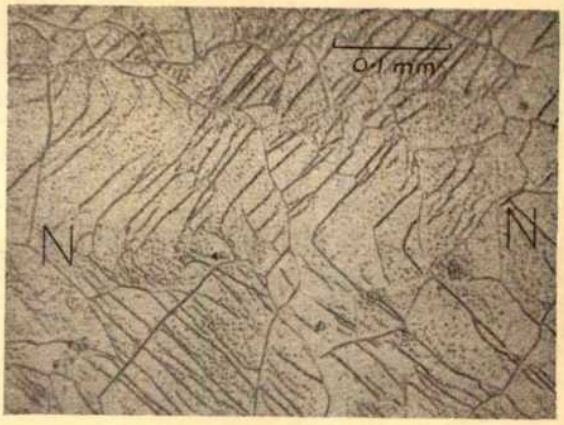

Fig. 2

The crystals were then repolished and annealed *in vacuo* and allowed to cool slowly in the furnace so that they appeared dark between crossed nicols. As a result of heating, the dislocations climbed into continuous low-angle boundaries normal to the slip planes (that is, thermal polygonization took place), to give Fig. 2, where *NN* is the neutral plane of bending. The crystal shown had deformed on different glide planes on opposite sides of the neutral plane and the dislocation climb on annealing produced the chevron pattern seen.

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ENGINEERING

Electron and Ion Density Hysteresis Effects in a Low-Pressure Mercury-Vapour Plasma carrying a Current of Varying Instantaneous Value

An experimental single-anode mercury arc valve was constructed containing a low-heat-resistance pool cathode of the molybdenum-cup type[1] to anchor the cathode spot and a plasma probe inserted with its tungsten tip at the axis of the valve at a distance about one-third from anode to cathode. The valve was operated in a resistance-loaded single-phase half-wave rectifier circuit, thus carrying a half-period sinusoidal current at a frequency of 50 c/s. The cathode temperature of the valve, and hence its saturated vapour pressure, was adjusted by a control of air flow from a fan below the cathode, and the pressure was thus varied over a range of 2·5–17·5 μm mercury. The crest value of current through the valve was altered by changing the load resistance. Further on, a modified version of plasmograph[2] was constructed to obtain on a cathode-ray tube screen a trace in semi-logarithmic form of the probe characteristic at any chosen instant of the wave-shape of the current pulses passing through the valve. Probe characteristics were obtained to construct sets of characteristics of the plasma parameters as functions of the instantaneous values of current i_a for different values of load resistance at a fixed cathode temperature, and for different values of cathode temperature at a fixed value of load resistance.

Fig. 1 shows the typical shape of a dynamic characteristic of electron density, n_e, as a function of the instantaneous value of i_a, and it also indicates the general tendency for the shape of ion density, n_i. Thus $n_e = n_e' + \Delta n_e$ and $n_i = n_i' + \Delta n_i$ where $n_e' \neq n_i'$ each depending only on the instantaneous value of i_a, and where Δn_e and Δn_i are each dependent both on i_a as well as on the magnitude and sign of its rate of change di_a/dt. The condition $di_a/dt > 0$ causes Δn_e and Δn_i to have negative values, that is, in Fig. 1 $n_e < n_e'$ and $n_i < n_i'$ for rising instantaneous values of current, and the condition $di_a/dt < 0$ causes Δn_e and Δn_i to be positive, that is, in Fig. 1, $n_e > n_e'$ and $n_i > n_i'$ for falling instantaneous values of current. The absolute values in both cases are throughout $|\Delta n_i| > |\Delta n_e|$. Thus the hysteresis effect which occurs for each density is greater for the ion density than for the electron density. At this stage no mechanism is proposed to explain the cause of the two effects apart from suggesting the possibility of some influence derived from delayed ionization as a result of collisions of pairs of metastable excited atoms[3].

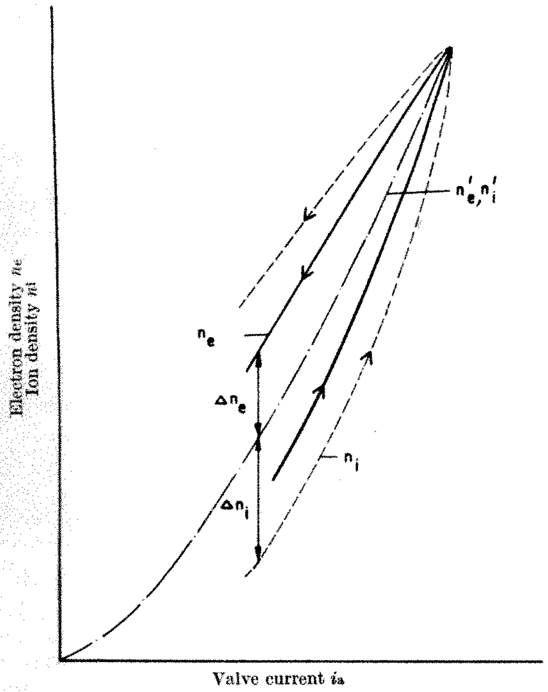

Fig. 1. Typical shapes of the dynamic characteristics for the electron and ion densities

This work was carried out in the Electrical Engineering Department of the Manchester College of Science and Technology. We thank Dr. H. Adam for providing essential parts for the construction of the experimental valve.

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Dry Flotation

A factor of major importance in the concentration of fine mineral particles by the process of froth flotation is the high moisture content of the final vacuum-filtered product. This is especially the case for coal flotation concentrates which contain more than 20 per cent of moisture. Potential savings in thermal efficiency and freight initiated research in this field.

Since successful flotation of selected minerals and coal was possible following dry conditioning (private communication from the Department of Mineral Technology, Imperial College of Science and Technology, London), it appeared reasonable to consider attempting flotation without proceeding to wet the hydrophobic surface induced by conditioning. Of the methods so far investigated the most successful has been the use of hydrocarbons having a low boiling point.

Samples of coal were conditioned with pentane and after pulping fed into a flotation cell. The cell was heated, externally, causing vaporization (a form of spontaneous aeration) of the pentane. Since this vaporization occurred at the surface of the coal adhesion or contact occurred and the composite of coal and hydrocarbon vapour rose to the surface, forming an agglomeration of particles similar to the froth obtained in normal flotation operations. Particles of impurity, such as shale, were released from the bubbles of vaporized pentane which occurred at their surface and, since adhesion did not take place, the shale remained in the pulp body of the cell.

The froth concentrate was collected and filtered and as a result of the non-wetting of the particles the moisture content of the product was less than 10 per cent, an appreciable improvement over that achieved by conventional flotation–filtration procedure. Subsequent tests were made using low-octane petrol as a substitute for pentane. Comparable results were obtained.

To overcome the necessity of heating the pulp body, an alternative procedure was to induce vaporization of the hydrocarbon by pressure reduction. Following conditioning, samples were floated by inducing vaporization in a vacuum type flotation cell. Results were similar to those obtained using the pulp-heating process.

Initial experiments indicated that similarly successful flotation was obtainable for the mineral galena provided that the process consisted of collector coating followed by conditioning with hydrocarbon and vacuum or elevated temperature flotation.

Samples of coal concentrate obtained by conventional flotation were vacuum filtered and washed with pentane. The final moisture content was less than that obtained by normal processes but greater than for hydrocarbon vacuum flotation. Work on samples of galena gave similar results.

It would appear that, following activation, conditioning with hydrocarbon prevents the particle from becoming water wetted and permits bubble attachment to a relatively dry particle. The gaseous film reduces subsequent wetting during froth collection and filtration. The alternative of replacing the water film during filtration was less successful.

The process as discussed permits an increased volume of bubbles to be obtained in vacuum flotation giving better aeration of the cell volume. Recovery and re-use of the hydrocarbon used is possible by collection of the vapour from the flotation cell and filters and liquefying.

Work completed so far must be considered as an initial approach to the problem of lower moisture contents in concentrates. Since comminution and conditioning may be achieved efficiently in the dry state it seems desirable to control flotation so that the economic mineral is not water wetted, only the gangue minerals being so affected.

Further work is being planned to examine this process and to assess its potentialities.

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METALLURGY

High-Frequency Chromizing

In recent years new processes involving the use of a medium containing 75 per cent chromium with 25 per cent cryolite and high-frequency heating have been developed for the chromizing of ferrous and non-ferrous metals[1–4]. Generators operating with frequencies of 3–3·5 Mc/sec have been used, and it has been claimed that coatings which would require the treatment of mild steel for at least 30 min at 1,200° C can be produced in 60–80 sec with high-frequency heating. Under these conditions the estimated temperature at the surface of the specimen during inter-diffusion was 1,250° C. Enhanced diffusion-rates under high-frequency heating conditions have been claimed and it has been suggested that there may be some 'high-frequency effect' in such processes. Specimen surface temperatures have been measured during recent investigations, and the results shed some light on this problem.

Twelve mild-steel specimens 0·125 in. in diameter and 2 in. long were placed in the chromium–cryolite medium contained in the annulus between two concentric cylinders (Fig. 1). Both ends of the annulus were sealed with refractory cement and the apparatus placed in the high-frequency coil. This apparatus allowed the preparation of sound chromized coatings and gave reproducible results. Specimen surface temperatures were measured during chromizing with the following arrangements of cylinders (Fig. 1): (*a*) both cylinders of refractory material; (*b*) outer cylinder of refractory, inner mild steel; (*c*) both cylinders of mild steel.

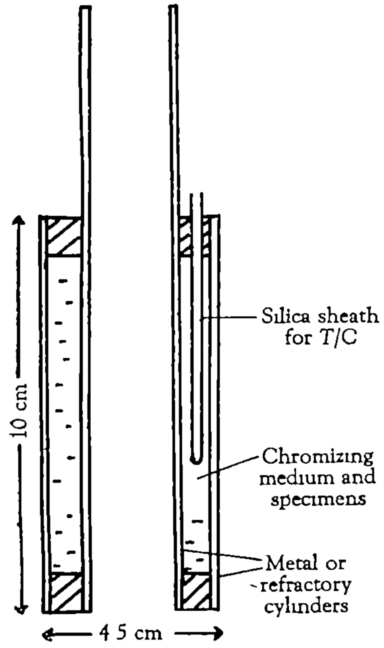
10 cm
Silica sheath for T/C
Chromizing medium and specimens
Metal or refractory cylinders
4 5 cm

Fig. 1. Concentric cylindrical apparatus developed for chromizing

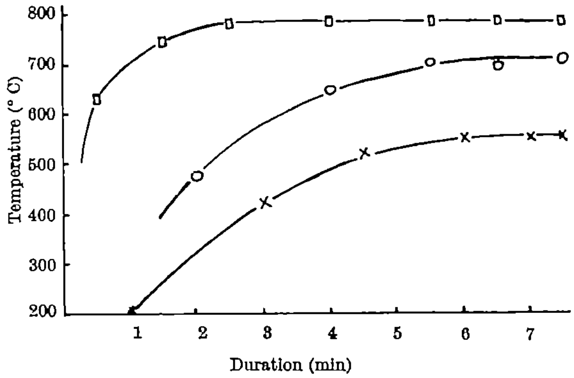

Fig. 2. Temperature distribution as a function of duration of heating (using two silica cylinders). ×, Unprotected *T/C* in the medium; □, specimen *T/C*; ○, sheathed *T/C*

Platinum–platinum rhodium thermocouples were sited in the annulus as follows: (1) unprotected in the chromizing medium approximately 0·2 in. from the nearest specimen; (2) unprotected, welded to 0·125 in.-diameter specimen; (3) protected by a thin silica sheath placed in the medium.

A typical set of heating curves for arrangement (*a*), that is, the all-refractory system, is given in Fig. 2. Here, even with twelve specimens in position the maximum surface temperature achieved was about 790° C. It is of significance, however, that this temperature was ~230° C greater than the temperature of the chromium–cryolite medium. With arrangement (*b*), the inner wall of the steel cylinder reached the greatest temperature, 1,150° C, closely followed by that of the specimen surface. The maximum temperature in the medium was approximately 1,060° C. There was no significant difference in temperature between that of the inner cylinder, the specimen surface and the medium when arrangement (*c*) was used. A temperature of 1,400° C was obtained with this arrangement.

These experiments indicate that when specimens are heated by induced eddy currents, arrangement (*a*), the true specimen surface temperature can be at least 230° C higher than that of the chromizing medium. In the case of arrangement (*c*) the specimens will be heated mainly by conduction of heat from the walls of the steel cylinders through the medium, and here the temperatures of the medium and of the specimen surfaces are the same. This corresponds more closely to the conditions encountered in conventional chromizing processes. Since chromizing temperatures in high-frequency chromizing processes have, hitherto, been measured with optical pyrometers[1,3] it seems likely that such temperatures will correspond more closely to those of the medium than those of the specimen surfaces. This is illustrated in Fig. 3, in which diffusion coefficients calculated from the data of Minkevitch and Ulybin for the chromizing of low

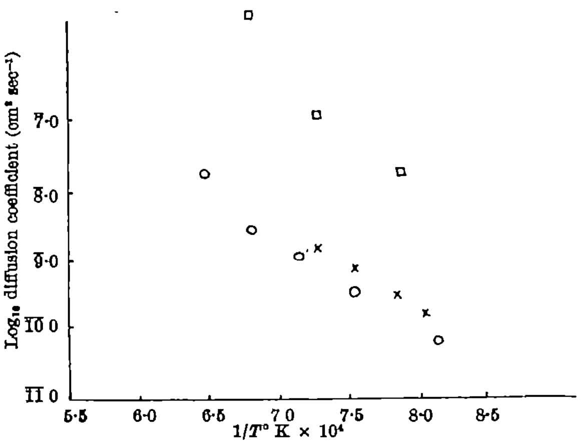

Fig. 3. Comparison of the diffusion coefficients for the chromizing of low carbon steels by conventional and high-frequency techniques
☐, High-frequency, techniques 0 2 per cent carbon steel (ref. 3);
×, conventional techniques with 0 1 per cent carbon steel (ref. 6);
○, present work using cylindrical arrangement (c) on 0·16 per cent carbon steel

carbon steels under high-frequency conditions (3·5 Mc/sec) are compared with values obtained in recent work[5] using arrangement (c) and those of other authors[6] using conventional techniques. The values of the diffusion coefficient from the results of Minkevitch and Ulybin at 1,000° C correspond to values obtained in recent work at ~1,260° C. This suggests that the temperatures at the specimen surfaces in the high-frequency process were about 260° C higher than the nominal chromizing temperature. This is much greater than the value of 100°–125° C suggested by Minkevitch and Ulybin and corresponds more closely to the value of 230° C found in the present work in which a generator of lower frequency (1 Mc/sec) was used.

This investigation strongly suggests that the apparently high inter-diffusion coefficients obtained during high-frequency chromizing arise because of unsuspectedly high temperatures at the specimen surfaces. This alone would be sufficient to create the required additional vacancies in the alloy lattice and would obviate the need to ascribe enhanced diffusion-rates to some unknown 'high-frequency effect' as has been suggested[3].

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RADIATION CHEMISTRY

Annealing Behaviour of Phosphorus-32 Recoils in Potassium Chloride Crystals

THE behaviour of radioactive species, formed by neutron capture in crystals, as a function of post-irradiation annealing treatment has been extensively studied for central atoms in oxy-anions[1,2]. So far as we are aware, in only one case have annealing curves shown a lowering of the valency of the activated atom in the species resulting from dissolution of the crystal, namely in the case of the $^{75}As(n,\gamma)^{76}As$ reaction in As_2O_3 and $NaAsO_2$ (ref. 3). In most cases

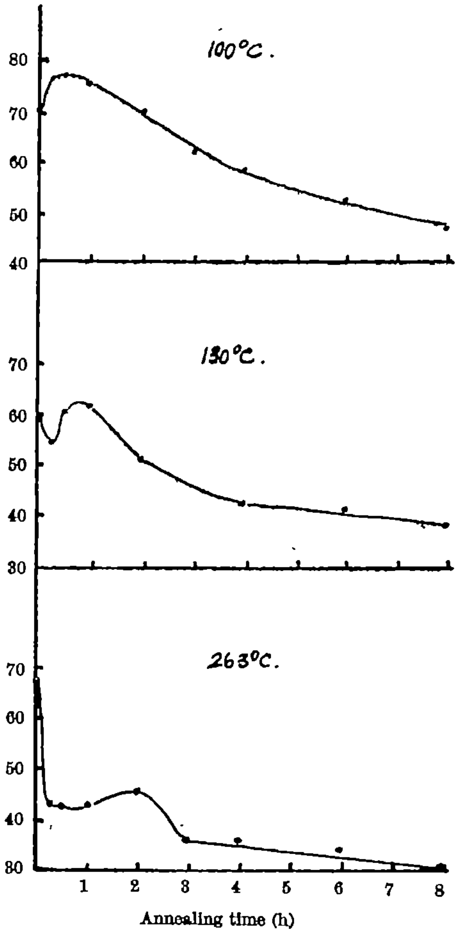

Fig. 1. Percentage of lower-valency phosphorus-32 as a function of time in pile-irradiated crystals of potassium chloride at annealing temperatures of 100° C, 130° C and 263° C

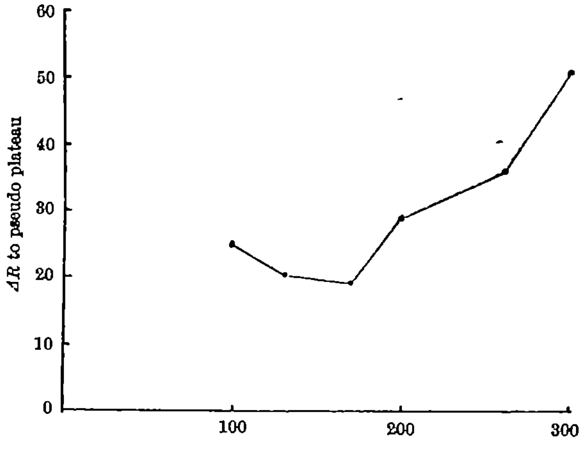

Fig. 2. Change in percentage lower-valency phosphorus-32, ΔR, from non-annealed value to pseudo-plateau value, as a function of annealing temperature

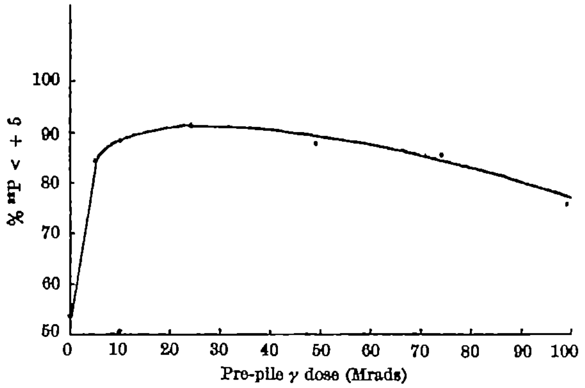

Fig. 3. The effect of γ-irradiation of potassium chloride targets, prior to pile irradiation, on percentage of lower-valency phosphorus-32

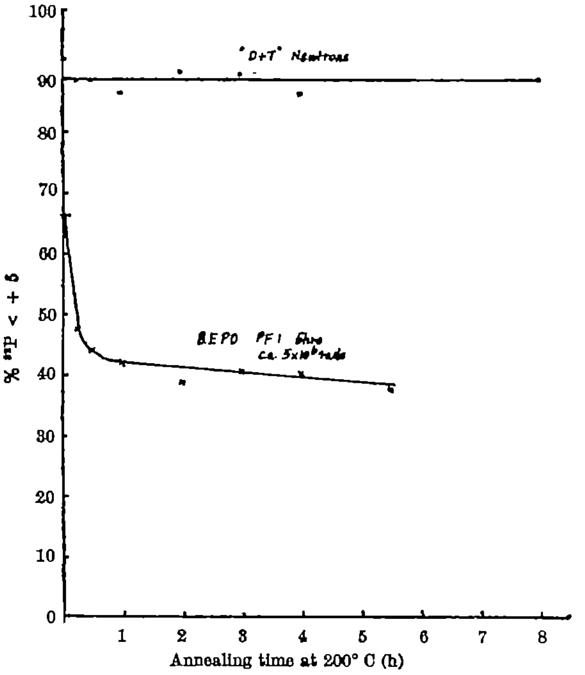

Fig. 4. Percentage lower-valency phosphorus-32 as a function of annealing time in potassium chloride crystals irradiated with pile neutrons and 14·1 MeV D + T neutrons

there is a rapid rise in oxidation state of the radioactive species followed by a further slow rise or pseudo-plateau. Süe and Caillat[4] found that heating neutron-irradiated sodium chloride for a few minutes to 350° C caused a significant increase in the proportion of lower-valency phosphorus-32. Since the colour of the irradiated crystals was simultaneously discharged they suggested that electrons from *F* centres might be effecting the reduction of phosphorus-32 + 5 species.

We have found evidence for a reductive reaction involving phosphorus-32 recoils during the thermal annealing of pile-irradiated potassium chloride crystals. In this work we have simply separated ^{32}P $\leqslant +3$ from higher valency forms by magnesium ammonium phosphate precipitation. Fig. 1 shows that superimposed on the normal fast and slow oxidative reactions there is a fairly fast reduction reaction, appearing as a hump on the curves. Fig. 2 shows a plot of the change in percentage lower-valency phosphorus-32 from the non-annealed value to the value at the pseudo-plateau plotted against the annealing temperature. A linear relationship is often obtained for this plot when $1/T$ is used, interpreted by the Maddock–de Maine annealing model[1–5]. In the present case, however, the curve is fairly flat up to 170° C after which it begins to rise, suggesting that in the region up to 170° C a reducing reaction involving the recoils is competing with the more normally observed oxidation reactions. Kobayashi[6] concludes from his work on proton-irradiated sodium chloride crystals that *F* centre discharge commences at about 100° C and is occurring extensively in the range 150°–170° C. It thus seems likely that electrons from *F* centres are reducing phosphorus-32 species in the lattice which would otherwise yield P + 5 compounds on solution of the crystal.

It has recently been reported[2] that irradiation of chromate crystals with 1·8-MeV electrons prior to pile irradiation significantly increases the proportion of chromium-51 atoms in lower valency states. Fig. 3 shows the results of irradiating potassium chloride crystals with varying doses of γ-rays from spent reactor fuel in the Harwell irradiation pond prior to a pile irradiation in *Bepo* involving a ~5 Mrads additional γ-dose. It can be seen that a pre-pile dose as small as 5 Mrads can significantly increase the proportion of lower valency phosphorus-32. This pre-pile irradiation effect appears to have very nearly reached saturation with a dose of 5 Mrad. There is some evidence from this figure that there is a slight rise to maximum lower valency phosphorus-32 at a γ-dose of ~24 Mrads, followed by a small decline. The pre-pile γ-radiation lowering of phosphorus-32 valency state is probably due to encountering of *F* centres during the recoil or to discharge of *F* centres in the 'hot zone' formed in the terminal region of the recoil track[7].

Fig. 4 shows the effects of thermal annealing of potassium chloride irradiated with neutrons from the pile and from a D + T source. In the case of the virtually γ-free D + T neutrons, there is very little annealing, while in the case of the pile irradiation a normal two-phase oxidative annealing reaction occurs. This finding lends rather striking support to the recent suggestion that lattice defects may be responsible for annealing reactions[3]. The interesting feature of the present case, however, is that the defects or radicals generated along the recoil track are not able to take part in oxidative annealing, whereas defects induced by radiation in the bulk of the crystal can allow such reactions to occur. There seems to be a difference in behaviour between recoil-induced defects and radiation-induced defects in this system.

Phosphorus-32 recoils in potassium chloride crystals would seem to provide useful material for studying oxidation-reduction reactions between polyvalent atoms and lattice defects.

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CHEMISTRY

Substrate Cleavage Point with Glucamylase

THE glycosidic link can, theoretically, be hydrolysed by two alternative routes, proceeding with the fission of the bond between (*a*) the anomeric carbon atom and the glycosidic oxygen or (*b*) the aglycon group and the glycosidic oxygen. For a number of glycosides the mechanism of the acidic and/or enzymatic hydrolysis has been investigated[1-8]. Since it became possible to prepare highly purified glucamylase it was interesting to investigate the cleavage point when this enzyme acts on maltose.

An aqueous extract (8 ml.)[9] of ruptured *Aspergillus niger* '152' cells was fractionated on a column (29 × 1·5 cm) of diethyl-amino-ethyl cellulose (Whatman *DE* 50) by the method of Pazur and Ando[10], using the same citric acid phosphate buffer but a smaller *p*H gradient for elution. Elution was commenced with buffer solution (100 ml.) of *p*H 7·5 and followed successively with the same volume of buffer solutions of *p*H 7·0 and 6·5. Fractions of the eluate (5 ml.) were analysed for transglucosylase and glucamylase activity by incubating portions in digests containing (*a*) amylose and (*b*) maltose. Transglucosylase activity was found in fractions No. 13–16 and glucamylase activity in fractions No. 14–20. Fractions No. 17–20 contained glucamylase activity only. The iodine-absorption value reducing power curve[11] of the pure glucamylase fraction was found to be identical with that of glucamylase purified by acid treatment[9] and remained unaltered after such treatment.

The point of cleavage of the α-1 : 4-glucosidic link when glucamylase acts on maltose was investigated by conducting the hydrolysis in $H_2{}^{18}O$.

The enzymatically produced glucose was isolated as its phenylosazone. Thus, fission of the $C_{(1)}$-O bond, route (*a*), would lead to a glucosazone, all oxygen atoms of which would be isotopically normal, whereas fission of the $C_{(4)}$–O bond, route (*b*), would give a glucosazone, one oxygen atom of which would be isotopically half-enriched.

A digest containing maltose (112 mg) in $H_2{}^{18}O$ (1·5 ml.; oxygen-18 enrichment 4·2 per cent), M-acetate buffer (*p*H 4·0; 0·1 ml.) and glucamylase solution (0·4 ml.) was incubated at 50° C for 16 h, when all the maltose had been hydrolysed to glucose. This was isolated as its phenylosazone with m.p. 204°–205° C which was not depressed on admixture with authentic glucosazone. A control experiment was carried out in normal water. Samples of glucosazone were pyrolysed by a method similar to that described by Oita and Conway[12]. Mass-spectrometric analysis of the carbon dioxide obtained showed no enrichment in oxygen-18.

The results show that the hydrolysis of maltose by glucamylase proceeds almost entirely by the fission of the bond between the anomeric carbon atom and the glycosidic oxygen. This is in contrast to the acidic hydrolysis of maltose where 78 per cent of the reaction apparently proceeds by this route[8]. On the other hand, with few exceptions such as the hydrolysis of α-D-glucose-1-phosphate by prostatic acid phosphatase[4], enzymatic reactions studied seem to proceed by route (*a*). Thus, the hydrolysis of maltose by glucamylase follows a general pattern for hydrolases and transglycosylases.

We thank Prof. E. J. Bourne for his interest, the Department of Scientific and Industrial Research for financial assistance, Dr. C. A. Bunton for a gift of oxygen-18-enriched water, and Dr. G. Majer for assistance with isotopic analysis.

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'Filterscan'; a Novel Technique in X-ray Fluorescence Analysis

NOTABLE successes in recent years have attended the development of X-ray fluorescence in the field of spectrochemical analysis[1]. The versatile and non-destructive methods which have ensued are conventionally based on the dispersive principle in spectrometers which are equipped with crystal monochromators.

The non-dispersive modification proposed here, however, not only offers marked simplification by avoidance of the analysing crystal but also a straightforward resolution of elements of adjacent atomic number. The underlying principle[2] is based on the fact that radiation from an element of atomic number Z passes much more readily through a filter composed of element Z–1 than through a filter Z–2. For example, copper ($Z = 29$) $K\alpha$-radiation passes very strongly through nickel ($Z = 28$) foil, but weakly through cobalt ($Z = 27$) foil (Fig. 2).

The procedure is to place filters successively in the path of the specimen's excited fluorescent X-rays. Measurements of the transmitted radiation intensity result effectively in a scan of the absorption edges of the incident radiation, providing dispersion in a manner alternative to that of the crystal spectrometer or pulse-height analyser[3].

Since a sequence of interchangeable filters is a feature which, so far as I am aware, lends novelty to this method, the term 'Filterscan' is proposed for its description.

Hitherto, non-dispersive methods[4] have been capable of resolving elements of adjacent atomic number but only at the expense of elaborate and costly electronic discriminating circuitry. The 'Filterscan' method involves relatively inexpensive equipment. Moreover, the technique proposed here differs from the majority of previous absorption methods[5,6] in that the absorption process is divorced from the sample and complete control over the absorption is thereby achieved. In addition, because a radioactive source is used, there is no problem associated with source instability, the exponential decay factor being readily assessable.

The arrangement shown in Fig. 1 has been found to be practicable, in which *A* is a radioactive X-ray source chosen to give sufficient energy to excite the elements of interest in the sample at *B*. Fluorescent radiation excited in the sample is detected by the end-window Geiger–Müller counter *GM* after passing through the interchangeable filter *F*. The filters consist of elements of suitable consecutive atomic numbers and are similar in their absorption properties to conventional β-filters as used in X-ray diffraction.

By plotting the filter used against transmitted intensity a discontinuity or step is revealed as each absorption edge is passed. Fig. 2 illustrates this effect in the case of solid copper and yellow-brass samples (approximately 70 per cent copper, 30 per cent zinc) where the two steps due to the copper *Kα*-radiation are seen to be in approximately the correct ratio.

Aqueous solutions of manganese dichloride, examined over the range from 21·0 to 0·66 per cent w/v manganese, yield a satisfactory working curve (Fig. 3).

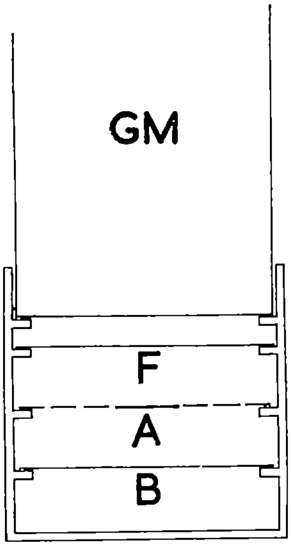

Fig. 1. 'Filterscan' housing showing radioactive source *A*; specimen *B*; interchangeable filter *F*; end-window Geiger–Muller counter *GM*

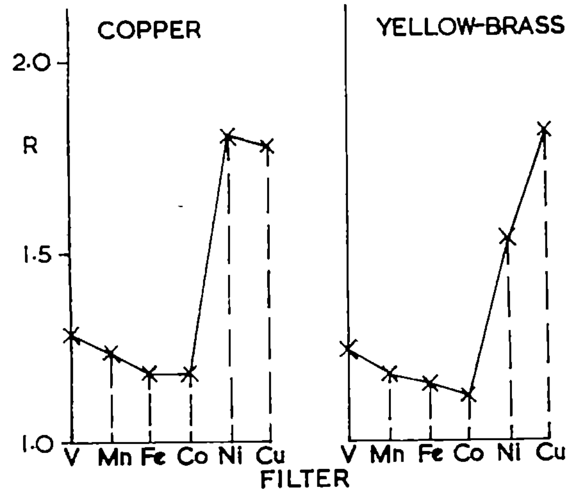

Fig. 2. Scans produced from copper and yellow-brass specimens.

$$R = \frac{\text{counts/10 sec (sample)}}{\text{counts/10 sec (Al blank)}}$$

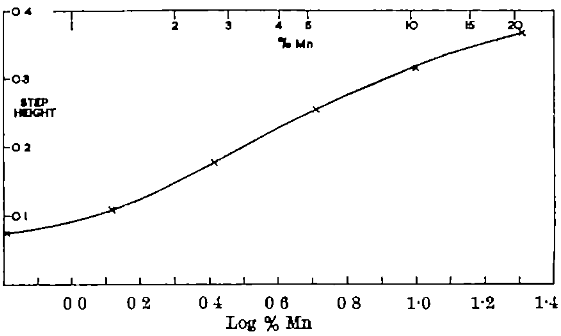

Fig. 3. Working curve for aqueous manganese dichloride solutions

Step height = *P* (Mn) – *P*(V)

$$P = \frac{\text{time for 4,000 counts (Al blank)}}{\text{time for 4,000 counts (sample)}}$$

Further refinement will be possible by using a scintillation counter in place of the end-window Geiger-Müller counter. It is anticipated that greatly improved sensitivity will be thereby achieved.

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High-resolution Nuclear Magnetic Resonance Spectrum of a Methanol Lignin

A SAMPLE of methanol lignin from *Eucalyptus regnans*[1], purified by counter-current distribution and possessing the empirical formula (on the basis of a C_9 unit) of $C_9H_{8\cdot1}O_{2\cdot70}(OCH_3)_{1\cdot86}$, was examined by means of the high-resolution nuclear magnetic resonance technique. This lignin was shown to contain approximately equal numbers of guaiacyl and syringyl units and to have been methylated to the extent of 0·41 methoxyl groups per C_9 unit[1]. The spectrum (Fig. 1) of an approximately 10 per cent solution in deuterochloroform was recorded on a Varian model *A*60 spectrometer operating at 60 Mc/s and was calibrated in parts per million from tetramethyl silane as internal reference[2]. The feature centred at 6·62 p.p.m. can be attributed to protons linked directly to aromatic nuclei and that centred at 3·85 p.p.m. to protons in methoxyl groups. When recorded under the same conditions the spectrum of vanillin showed the methoxyl feature at 3·88 p.p.m. and that of syringaldehyde at 3·93 p.p.m. In the foregoing empirical formula, hydrogen present as methoxyl accounts for about 41 per cent of the total, whereas the feature at 4·98–3·45 p.p.m. equals 46 per cent of the total. The remaining features are most likely to be mainly associated with protons attached directly to oxygen (hydroxyl groups) or to carbon atoms bearing negative substituents. Protons resonating at corresponding frequencies may be found in the spectra of lignans[3,4]. There is a striking lack of features upfield from 2 p.p.m. where most aliphatic and alicyclic methyl, methylene and methine protons would be expected to occur[5]. In general, the proton

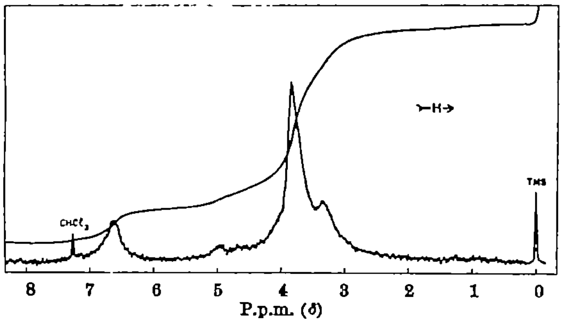

Fig. 1. Nuclear magnetic resonance spectrum of methanol lignin

magnetic resonance spectrum appears to be in agreement with the type of structure predicted by Freudenberg[6].

The relative proportions of protons in different environment were estimated by integration and the aromatic protons were found to account for 13·4 per cent of the total. With half the units of the guaiacyl and half the syringyl type in the empirical formula quoted here, the aromatic protons should have accounted for 18·3 per cent of the total if no coupling directly to the aromatic nuclei had occurred. This discrepancy can be interpreted as signifying that approximately 27 per cent of the units present are coupled in this manner. This figure then represents the total number of aromatic nuclei involved in 'condensed units', that is, aryl–aryl and aryl–alkyl linkages. As these linkages are believed to occur ortho to the phenolic group the syringyl nuclei could not take part. This result suggests that about 54 per cent of the guaiacyl nuclei are so linked. This appears to be in agreement with the type of lignin structure proposed by Adler[7] for softwood lignin. The remaining protons are distributed in the following way (region in p.p.m.): per cent of total protium—(6·0–4·98): 18, (3·45–2·12): 20, (2·12—10): 2·6. Part of the signal contributing to the pronounced feature at 3·35 p.p.m. may be due to hydroxyl protons, as it was affected by treatment with deuterium oxide[8].

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BIOPHYSICS

Efflux of Chloride Ions during the Action Potential of *Nitella*

This communication is prompted by a report of Spyropoulos, Tasaki and Haywood[1] that they were unable to confirm the findings of Gaffey and Mullins[2] that in *Chara* there is an efflux of chloride ions during an action potential. It would appear, however, that the experimental conditions of Spyropoulos *et al.* were not such as to make a demonstration possible. This is shown by the following calculations. A *Nitella* cell, 0·06 cm in diameter by 4 cm in length, has a volume of 0·011 cm^3 and very nearly 0·66 cm^2 of surface area. The volume of the cell is nearly all sap and if its [Cl] is taken as 100 µmole/cm^3 then a cell contains 1·1 µmole Cl^-. With a membrane capacity of 1 µF/cm^2 and an action potential of 100 mV, 1×10^{-12} mole of Cl^- must be lost to produce the potential change, per cm^2 or 0·66 pmole for the *Nitella* cell. The cells used by Spyropoulos *et al.* had 1,000 c.p.m. chlorine-36 and 1 µmole Cl^-, so that each c.p.m. represented 10^{-9} mole Cl^- and the expected loss for an action potential is about 1/1,000th of a c.p.m. Forty stimuli were delivered—this would increase the efflux but the efflux was collected in 20 fractions so that the total efflux to be expected for the experiment would be 2/1,000ths of a c.p.m. in each fraction. In the experiments with *Chara*[2] the quantities of Cl^- lost were 500–5,000 times greater than that calculated above and were equivalent to K^+ loss, while the action potential was 200 mV, or considerably greater than that in *Nitella*. The K^+ loss demonstrated by Spyropoulos *et al.* ought to equal Cl^- loss, but in the absence of information on the specific activity of their potassium-42 in the cell no calculation is possible. The specific activities of potassium-42 and chlorine-36 as supplied by the Atomic Energy Commission, Oak Ridge, are as 2,000 : 1, and for equal loading times the intracellular potassium would be preferentially labelled. What does seem clear is that the extent to which cells were labelled with chlorine-36 was inadequate to detect an extra efflux of activity (over the resting Cl^- efflux and over background).

Two other reports[3,4] suggest that in *Chara* and *Nitella* the action potential is brought about by an inward movement of Ca^{++}. There is no evidence from isotope studies that this is so, and the authors argue from the point of view of the bioelectric effects of Ca^{++}. They are both apparently unaware of the work of Frankenhaeuser and Hodgkin[5] on the squid axon and of much other work showing that increases in $[Ca]_0$ act like increases in membrane potential in: (*a*) diminishing K^+ conductance at rest; (*b*) in making available a greater passive movement of Na^+ during a subsequent depolarization. In *Chara*, hyperpolarization clearly increases spike height, and the observation of Hope that increases in $[Ca]_0$ enhance spike height are not a convincing demonstration that calcium ions carry the depolarization current. The observations of Findlay are with *Nitella* under voltage clamp conditions. These show precisely the sort of effects one might expect if the action of Ca^{++} were to activate a greater fraction of the mechanism responsible for Cl^- movement subsequent to depolarization. Two different Cl^- concentrations were used, 0·1 and 1·0 mM, presumably as a test of the extent to which the chloride equilibrium potential would be shifted by such a concentration change. It is important to note that such evidence as there is suggests that the equilibrium potential of an ion is not a very useful concept when the concentration ratio across the membrane exceeds perhaps 50 because the contributions of current from other ions tend to act as shunts in the system. With $[Cl]_i = 100$ mM, and a clamped depolarization to zero membrane potential, the chloride current would be expected to be proportional to 100–1 and 100–0·1 for the two concentrations used. One must conclude, therefore, that the two currents responsible for depolarization and repolarization in

Nitella have not been resolved in this voltage clamp study.

Because Cl^- efflux during an action potential has not been demonstrated in an ecorticate *Nitella* cell, it seemed valuable to make such measurements. Internodal cells of *Nitella clavata* (strain *XO*-33) from cultures maintained in the laboratory under controlled conditions of light and temperature (I am indebted to Dr. Charles Barr for the cells used) were obtained and were loaded for 30 days with chlorine-36. The loading solution contained sodium chloride 15 mM, potassium chloride 0·1 mM, and calcium chloride 0·1 mM, and its specific activity was 0·3 mc./g chloride. After loading, the cell sap had a specific activity of 0·3 that of the loading solution. Such cells were transferred to 1 litre of a solution containing 1 mM sodium chloride, 0·1 mM potassium chloride and 0·1 mM calcium chloride. Cells were washed for 24 h in 2 changes of this solution and were then mounted in a glass experimental chamber containing 4 gold electrodes (2 for stimulating and 2 for recording), and 5 ml. of the 1 mM sodium chloride solution. Exhaustive tests were made for any evidence that the metal electrodes might in any way alter either the electrical properties of the cells or the resting efflux of chlorine-36. These tests showed that both threshold and spike amplitude were unchanged after cells had spent 48 h in the chamber; similarly, chlorine-36 efflux was unchanged after a similar period of time. As a final check, sub-threshold stimulation with 0·1 msec shocks at 1/min failed to alter the resting chloride efflux. On the other hand, a single conducted action potential sufficed to double the rate constant for chlorine-36 efflux during a 15-min collecting period. Resting effluxes were of the order of 2–4 c.p.m. per hour of efflux and all counts were made in an automatic sample-changing flow gas counter with an anti-coincidence guard. Background was 2 c.p.m. and at least 1,000 counts over background were obtained on each sample measured. Some results obtained are shown in Fig. 1, where the rate constant for loss of chlorine-36 is plotted against time. In all experiments there was a 1 h resting efflux collection before stimulation and in one case the cell was maintained in the chamber for 24 h after stimulation and efflux was measured again. Stimulation was effected with 0·1 msec square wave pulses, with an amplitude roughly twice threshold; usually 15–17 stimuli were given during 15–20 min. It is clear that stimulation leads to a large increase in the rate constant for loss of chlorine-36 from the cells and that this increased rate of loss subsides to resting values within 30 min after stimulation. The delay in efflux of chlorine-36 from the cell may be because the cell wall is a cation exchange system with a low anion permeability. The absolute values of efflux for the 5 cells used are shown in Table 1. Values for resting efflux are within the range of values previously given for *Chara*[2] while values for the stimulated efflux, although large, are somewhat smaller than those for *Chara*. The difference may be due to the rather different duration of the action potential (10 sec for *Chara*, 2·5 sec for the species of *Nitella* used in this investigation). What is important is the clear demonstration of an efflux of chlorine-36 about 1,000-fold greater than that necessary to bring about the bioelectric activity that is observed.

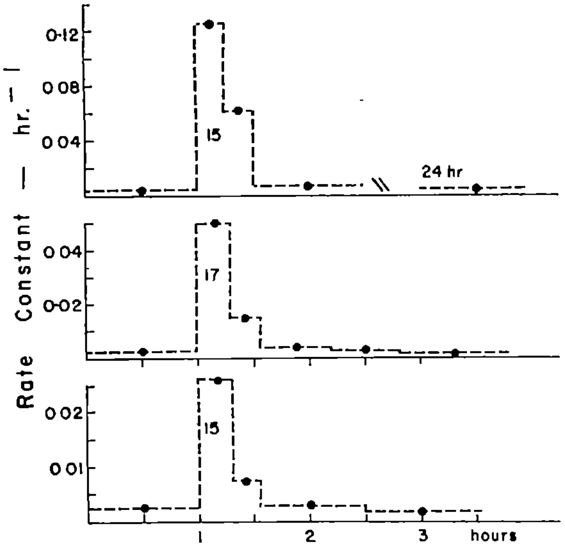

Fig. 1. The rate constant for efflux of chlorine-36 is shown as a function of time for three cells. All cells had a 1-h rest period and were then stimulated; the numbers on the graph indicate the number of such stimuli. In the chart at the top, the cell remained in the chamber for 24 h after the last sampling and the rate constant for efflux during this period is shown

Table 1. Efflux of Chlorine-36 from *Nitella* at Rest and during Stimulation

Cell No.	Resting efflux (pmole/cm² sec)	Stimulated efflux (pmole/cm² impulse)	Ratio: rate constant during stimulation/ rate constant at rest
1	1·0	8,000	41
2	1·2	1,350	31
3	1·0	1,220	25
4	1·0	540	15
5	3·1	870	20

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BIOCHEMISTRY

A Rapid Enzymatic Assay for Glycerol

Enzyme methods for the assay of glycerol using glycerol dehydrogenase[1] or glycerol kinase with L-glycerol 1-phosphate dehydrogenase[2] and based on the reduction of NAD to $NADH_2$ (measured by change in extinction at 340 mμ) have the disadvantage that the reaction time is comparatively long (about 30 min). An alternative and more rapid method has been developed which is based on the following reactions:

glycerol + ATP ⇌ L-glycerol 1-phosphate + ADP (glycerol kinase)

ADP + phosphoenolpyruvate ⇌ ATP + pyruvate (pyruvate kinase)

pyruvate + $NADH_2$ ⇌ lactate + NAD (lactate dehydrogenase)

The phosphorylation of glycerol is coupled with the oxidation of $NADH_2$ which may be followed spectrophotometrically at 340 mμ. At *p*H 7·6 the forward reaction is favoured in each case and hence rapid and complete assay of glycerol may be made. Under the conditions to be described the assay reaction for glycerol (in the range 0·005–0·05 μmole) is complete in 3–5 min.

The reagents required for assay were obtained from C. F. Boehringer und Soehne through Courtin and Warner, Ltd., Lewes. It was convenient to prepare stock solutions of 75 mM ATP disodium salt; 3 mM NADH$_2$; and 0·1 M triethanolamine hydrochloride (adjusted to *p*H 7·6 with saturated NaOH) containing 6 mM magnesium sulphate, 2 mM potassium chloride, and 7 mM phosphoenolpyruvate tri*cyclo*hexylammonium salt. These solutions may be stored at − 15°. An assay mixture is prepared by adding 0·25 ml. of ATP solution, 500 μg of pyruvate kinase and 200 μg of lactate dehydrogenase to 10 ml. of triethanolamine buffer, magnesium sulphate, potassium chloride. phosphoenolpyruvate solution. In general sufficient NADH$_2$ was added to give an extinction of 0·8 at 340 mμ in a 1-cm light path when the assay mixture is read against a water blank (1 ml. of stock NADH$_2$ solution added to above assay mixture). The quantity of NADH$_2$ may need to be increased if the sample for glycerol assay contains much pyruvate or ADP. Samples of blood plasma or incubation media or tissue extracts for glycerol assay may be deproteinized with an approximately equal volume of 10 per cent trichloroacetic acid and the latter removed by extracting thrice with a five-fold excess of ether and the ether removed by suction and *in vacuo*. The ether extraction concentrates glycerol and other ether insoluble components by a factor of 1·15 due to the removal of water in the ether phase, and this can be allowed for in calculating the results. Extracts deproteinized with perchloric acid were found to be unsuitable for assay of glycerol since they inhibited pyruvate kinase.

Assays were performed in quartz cells with a 1-cm light path. To 1 ml. of assay mixture is added 1 ml. of sample for glycerol assay (containing 0·01–0·1 μmole glycerol) and the change in extinction due to reaction of pyruvate or ADP in the sample allowed to complete. The change in extinction due to glycerol is then measured following the addition of 10 μg of glycerol kinase.

Typical progress curves with standard glycerol solutions are shown in Fig. 1. The reaction is complete within 1–2 min under these conditions but with plasma extracts 3–5 min is required for completion. Over a range of glycerol concentrations in the assay cuvette of 0·01–0·05 μmole/ml., change of extinction at 340 mμ and glycerol concentration are linearly related and the observed change in extinction is in good agreement with that calculated from the molar extinction of NADH$_2$. The glycerol concentration may be calculated, therefore, from the change in extinction, the molar extinction of NADH$_2$ and relevant dilution factors. The recovery of glycerol added to human blood plasma before deproteinization (amounts 0·025–0·125 μmole of added glycerol per ml. of plasma) gave a mean value of 0·95 ± 0·018 (mean ± *S.E.*). Recovery was thus substantially complete.

Dihydroxyacetone and L-glyceraldehyde are also assayed by this method, but under the conditions used the initial velocity with glycerol as substrate is approximately forty times greater than with similar concentrations of the other substrates.

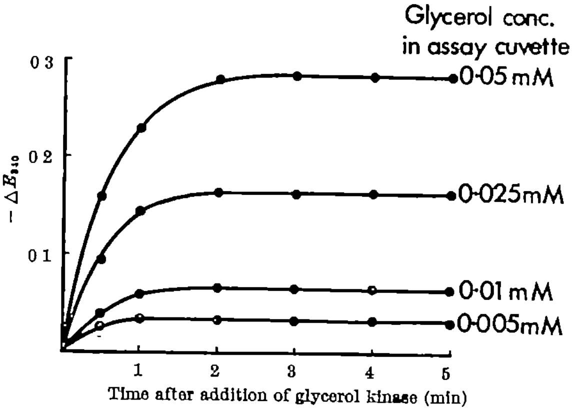

Fig. 1. Time course of reaction

The method can be readily applied to the estimation of glycerol in blood plasma or for measurement of glycerol output by isolated tissues. With this method the mean plasma glycerol concentration in five normal adult males after an overnight fast was 48 ± 4 μmoles/l. and under similar conditions a group of 43 untreated diabetics had a mean plasma glycerol concentration of 90 ± 6 μmoles/l. (mean ± *S.E.*). The outputs of glycerol by perfused heart, isolated diaphragm and isolated epididymal adipose tissue from normal rats were (μmoles/g/h mean ± *S.E.*) 2·7 ± 0·21, 0·66 ± 0·09, 0·87 ± 0·05, and from alloxan diabetic rats 4·6 ± 0·22, 1·15 ± 0·12, 1·26 ± 0·03 (6 tissues in each group).

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Influence of Deoxyribonucleic Acid Content on the Proteolysis of Sputum and Pus

Deoxyribose nucleoprotein (DNP) has been identified as an important constituent of purulent exudates, constituting 30–70 per cent of the total solids[1]. The action of deoxyribonuclease (DNase) in liquefying such exudates has been largely ascribed to the depolymerization of DNA[2–6]. Sherry, Johnson and Tillett[4] observed that acid-soluble nitrogen was progressively liberated into solution following the addition of DNase to the exudate. Similarly, liberation of trichloroacetic acid (TCA) soluble tyrosine was noted during our investigation of DNase action on purulent sputum. It is possible that this liberation of TCA soluble tyrosine resulted from the digestion of protein by an intrinsic proteolytic enzyme which requires depolymerization of interfering DNA. This communication is concerned with the testing of the hypothesis: (*a*) that a nucleoprotein, resistant to proteolysis, exists in sputum and pus; (*b*) that the protein becomes susceptible to a native proteolytic enzyme after the nucleoprotein complex has been split.

Specimens of purulent sputum were obtained from 10 children with cystic fibrosis and 5 patients with bronchiectasis, and 8 samples of pus were obtained from infected wounds, empyema and abscesses. The material was homogenized in distilled water with a

Potter–Elvehjem homogenizer to form 10 per cent homogenates. Aliquots (3·0 ml.) were mixed with 2·0 ml 0·05 M phosphate buffer, *p*H 7·5, and 0·1 ml. DNase solution (5 mg DNase/ml. 0·1 N acetate buffer, *p*H 4·5), and placed in a 37° C water bath. TCA-soluble tyrosine was assayed at 15-min intervals with the Folin–Ciocolteau reagent[7].

DNase caused a progressive (first order) increase of TCA-soluble tyrosine in all the sputum and pus specimens (Fig. 1). Addition of 10 mg soybean trypsin inhibitor (SBTI) to the reactant mixtures resulted in approximately 50 per cent inhibition of proteolysis during a 1-h incubation period. These results suggested that DNase initiated a proteolytic reaction which could be partially inhibited by SBTI, and therefore probably resulted from the action of a proteolytic enzyme in the exudates.

When 0·5-ml. aliquots of the homogenates were added to 5·0 ml. of 3 per cent casein ('Pfanstiehl') in 0·05 M phosphate buffer at *p*H 7·5, a 0-order reaction curve for casein digestion resulted (Fig. 2). The addition of DNase to this mixture did not significantly affect the rate. It was thus apparent that the specimens of purulent sputum and pus contained a proteolytic enzyme capable of digesting casein, but incapable of digesting the intrinsic protein of the exudate unless preceded by DNase action. Further investigation of this protease revealed it to resemble chymotrypsin in that it had specific esterase activity for the aromatic amino-acid ethyl esters (tyrosine, phenylalanine and tryptophan). The protease was present in the sediment of the exudates and was soluble in 2 M sodium chloride.

An experiment designed to demonstrate that the native protease was necessary for the proteolysis induced by DNase was performed as follows: A portion of sputum homogenate was heated in an 80° C water bath for 1 h. This material no longer split acetyl-L-tyrosine ethyl ester substrate, unlike the unheated sputum homogenate. The sediments from this heated specimen and from an unheated specimen were removed by centrifugation at 16,000 r.p.m. at 0° C for 15 min., and resuspended to their original volumes in distilled water. DNase was added to aliquots of each specimen as described here, incubated at 37° C, and periodically assayed for TCA-soluble tyrosine. No proteolytic activity occurred in the heated specimen, whereas proteolysis occurred in the unheated specimen as in Fig. 1. On the other hand, when protease was then added to the heated specimen in the form of 0·5 ml. of unheated sputum homogenate, TCA-soluble tyrosine was released. These experiments demonstrate that the DNase alone is not responsible for proteolysis, but induces proteolysis only by making substrate available to the protease when the latter is present.

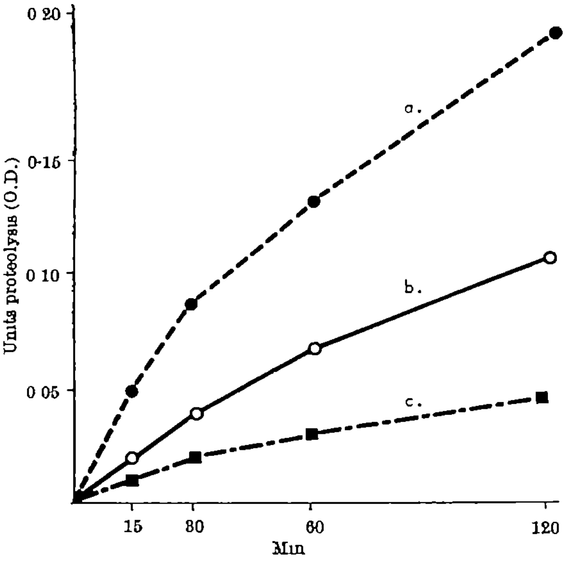

Fig. 1. Effect of DNase on intrinsic proteolysis in purulent respiratory secretions or pus. *a*, Effect of DNase alone; *b*, effect of DNase plus soybean-trypsin inhibitor; *c*, spontaneous proteolysis

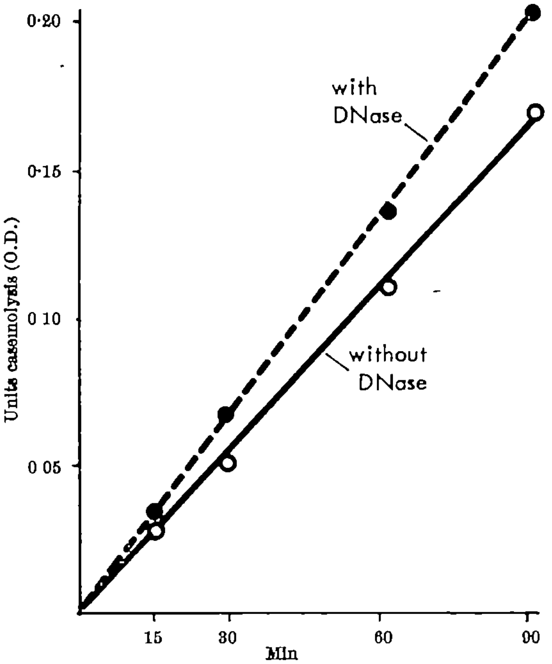

Fig. 2. Caseinolytic activity of protease from purulent sputum and pus; 3 per cent in 0·05 M phosphate buffer, *p*H 7·5

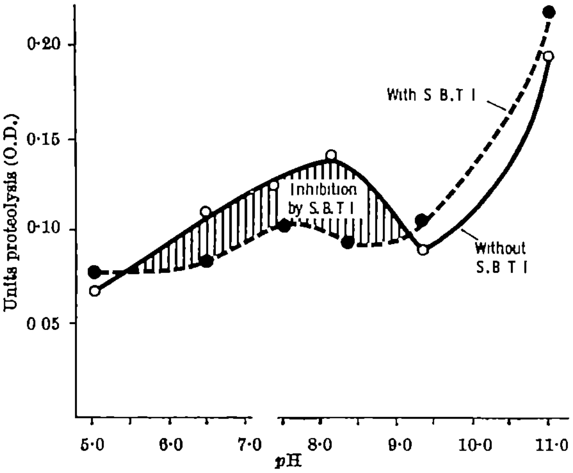

Fig. 3. Optimum *p*H curve for intrinsic proteolysis of purulent sputum resulting from 1·5 M sodium chloride. Shaded area indicates inhibition by soybean-trypsin inhibitor (SBTI)

Further evidence that DNA renders the protein in the complex unavailable to the proteolytic enzyme present in purulent sputum and pus was provided by dissociating the nucleoprotein complex with high concentrations of sodium chloride[8]. Mixing the homogenate with 2 M sodium chloride (1 part homogenate to 2 parts sodium chloride solution) resulted in the same proteolytic activity as occurred with DNase. The effect of *p*H on this phenomenon is shown in Fig. 3. Two peaks of proteolysis are seen: one at *p*H 7·5–8·0, and a second abrupt rise of apparent proteolysis at a more alkaline *p*H around 11·0. The first peak of activity is true enzymatic activity that can be partially inhibited by SBTI as shown in Fig. 3. In addition, this first peak corresponds to the optimum *p*H observed when the protease is tested on a casein substrate. The second peak, however, probably respresents a non-enzymatic hydrolysis.

DNase alone and 2 M sodium chloride alone each produces maximum proteolysis at *p*H 7·5, and there is no additive effect. This suggests that both DNase and concentrated sodium chloride act by dissociating DNA from protein, thereby making protein available for proteolysis.

The chymotrypsin-like enzyme activity of sputum appears to be a function of its purulency; this enzyme was present in all purulent sputa examined, but was absent from non-purulent specimens[9]. A possible bacterial source for the enzyme was ruled out by studying proteolytic activity of bacterial cultures from the specimens. In addition, some of the pus samples were sterile. Mounter and Atiyeh[10] studied the protease activity of buffy coat leucocytes and their description of this activity resembles our observations of the protease in exudates. Although we did not find three peaks of optimum activity at *p*H 3·0, 6·0 and 8·0 as they did, our findings coincide with their description of the enzymatic activity apparent at *p*H 8·0, in so far as its esterase activity and optimum *p*H are concerned. It appears probable that the protease activity of purulent sputum and pus is derived from the leucocytes.

This work shows that the DNA content of purulent sputum and pus acts to prevent enzymatic hydrolysis of protein within the secretions, probably by forming a complex with the potential protein substrate. All purulent specimens examined were found to contain a chymotrypsin-like enzyme that was capable of lysing the exudate protein only following cleavage of the nucleoprotein complex, as by DNase or by 2 M sodium chloride. Thus it appears that the viscosity-lowering action of DNase is at least in part due to the induced enzymatic hydrolysis of protein.

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Thermodynamics of Biological Growth

In a recent communication, Stoward[1] presented data on heat production during the growth of *Aerobacter aerogenes* with the view of contributing information on Prigogine's[2] hypothesis that living organisms tend to a state of minimum entropy production. In view of the fundamental importance of this hypothesis, it seems necessary to examine in some detail the results given by Stoward[1].

These results appear to contain a systematic error, as the thermal data do not follow the form of the growth curve of the bacteria, and no inflection occurs in the heat record at the time at which the stationary phase is reached. This is the behaviour to be expected from a measuring system the time constant of which is too long to follow the rapid changes in rate characteristic of an exponential function. The problem of response time is of particular importance in kinetics; Sirs[3] has shown that, to reproduce the form of an exponential without serious distortion, the time constant of the measuring system must be very much less than the half-time of the exponential. We have found glass containers unsuitable in calorimetric kinetic apparatus[4], and Sturtevant's[5] analysis of the problem shows that even metal vessels may introduce lags in some calorimetric measurements of rate processes. The type of error introduced by thermal lag is shown in Figs. 1 and 2. These compare heat production curves obtained during bacterial growth, using (Fig. 1) glass calorimeter vessels which imposed a serious thermal lag and (Fig. 2) thin metal vessels which enabled this lag to be reduced to a very low level. The data are plotted in the integrated form so that the correlation is obvious. If the determinations of bacterial mass are measurements without time error, but a substantial lag occurs in the thermal estimations, then the functions derived from these measurements will be seriously distorted. We suggest that since Stoward[1] used a glass calorimeter vessel, his data are affected by such a time-lag.

We have recently investigated one aspect of the Prigogine hypothesis, that is, the relative heat production of bacterial cells at different parts of the growth curve[6]. Within the limits of precision of our measure-

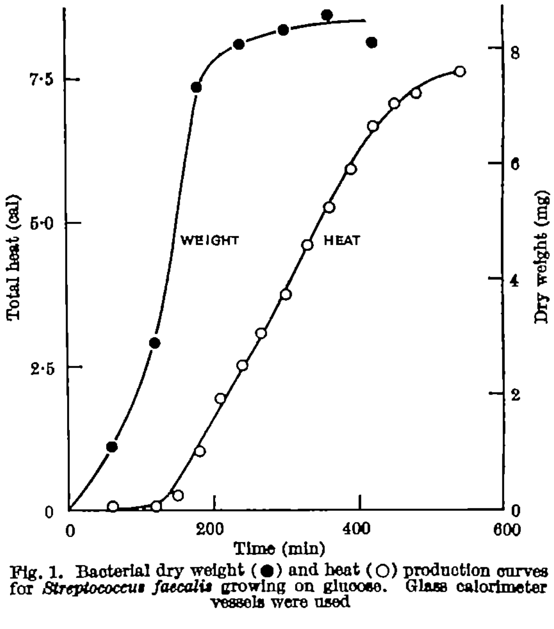

Fig. 1. Bacterial dry weight (●) and heat (○) production curves for *Streptococcus faecalis* growing on glucose. Glass calorimeter vessels were used

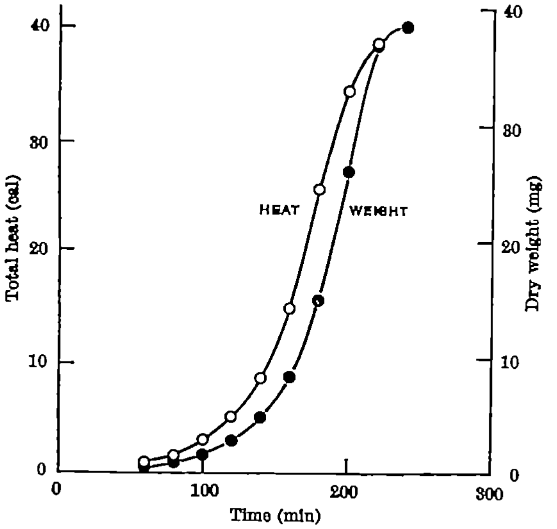

Fig. 2. As in Fig. 1, except that thin aluminium calorimeter vessels were used

ments, it was not possible to demonstrate any significant difference between the heat production of unit bacterial masses at different stages of exponential growth. Heat-changes during the lag phase were too small to allow accurate compilation of data on heat per unit mass, but during the exponential phase the heat evolved per unit mass of bacteria ($\Delta H/M$) against time is constant. On the other hand, the ratio $\Delta H/n$, that is, heat per bacterial cell, shows a tendency to increase during the exponential phase (Fig. 3). This is explicable on the grounds that the average cell size increased during growth. Since, in our experiments, substrate was the factor limiting growth, comparisons with Stoward's[1] data do not extend beyond the exponential part of the growth curve.

The reactions occurring during growth and in the stationary phase are not strictly comparable. During the stationary phase essentially all the substrate present is degraded so that the heat produced can be correlated with thermodynamic data calculated for a simple reaction[6]. However, during growth, synthesis of cell material from the substrate may occur, so that a portion of the substrate is not degraded and ΔH for a simple reaction is no longer applicable.

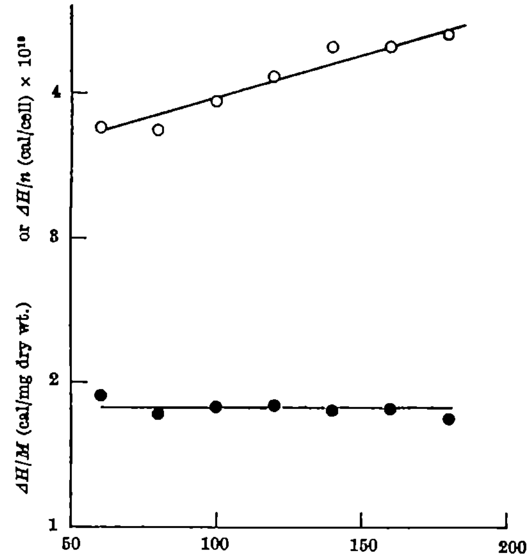

Fig. 3. Heat per cell ($\Delta H/n$) versus time (○) and heat per unit dry weight ($\Delta H/M$) versus time (●). Data used were obtained from the experiment depicted in Fig. 2

We therefore consider that, for the foregoing reasons, the data obtained by Stoward[1] are unsuitable for the determination of the thermodynamic characteristics of living systems.

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The criticisms suggested by Forrest and Walker (preceding communication) that thermokinetic data can be distorted by systematic lags in the measuring apparatus are quite valid in general, but they do not apply to a detailed examination of my apparatus[1].

The principal sources of thermal lag in calorimetric measurements arise from the finite response time of the temperature-recording system, and the failure of the temperature of the calorimeter jacket and head to keep in step with the changing temperatures of the reaction media. The effective response time of my temperature-recording system is less than 2 sec when a sensitive galvanometer is used[1], or less than 10 sec when rises in temperature are displayed on a recording potentiometer[1]. These response times will obviously have little effect on derived temperature-time curves when bacterial growth-rates are measured to the nearest 1–2 min.

On the other hand, errors of thermal lag arising from the comparatively high heat capacities and low thermal conductivities of the calorimeter head and jacket materials can be much more serious[3]. Because of the very low thermal conductivity of silicone rubber, my calorimeter head[1] can be regarded as part of the 'isothermal shield', and therefore any temperature lags must occur in the inner calorimeter vessel itself.

The temperature of the outer wall of the inner (glass) vessel (θ_2) at time t is related to that of the fluid medium (θ_1) by the equation[2]:

$$\theta_2=\theta_1 + (4c_1-2Bc_2)/B^2 - (4c_1/B)t + (2Bc_2-4c_1)/B_2\cdot \exp(-Bt) \quad (1)$$

when θ_1 follows a rate of the form[2]:

$$d\theta_1/dt=c_1t+c_2 \quad (2)$$

where c_1 and c_2 are constants.

The constant $B=4\pi\kappa rl/C_px$, where κ is the thermal conductivity, r the radius, l the length, C_p the heat capacity and x the wall thickness of the inner calorimeter respectively. Putting $\kappa=0{\cdot}002 \times 3{\cdot}6 \times 10^3$ cal. cm^{-1} deg^{-1} h^{-1}, $r=1$ cm, $l=10$ cm, $C_p=4$ cal. deg^{-1}, and $x=0{\cdot}05$ cm, then $B\sim 4\times 10^3$.

Owing to a transcript error the values of $\overline{\Delta H}$ and $\overline{\Delta h}$ in ref. 1 were inadvertently divided by 10. When the correct values of $c_1=0{\cdot}01$ and $c_2=0{\cdot}05$ for *A. aerogenes* growing in a synthetic glucose medium[1] are substituted, the last term in equation (1) is negligibly

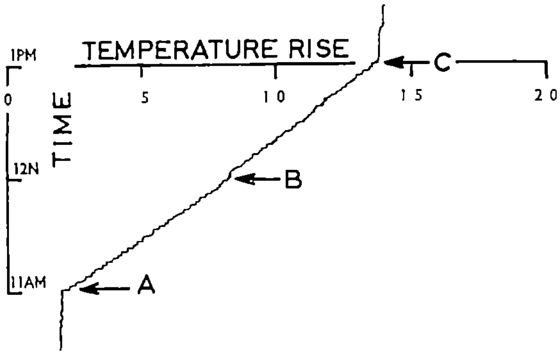

Fig. 1. A typical calibration curve for an adiabatic microcalorimeter. The figure 1·0 on the temperature scale is roughly equivalent to 0·25° C. The constant temperature for the 0·5-h periods before (*A*) and after (*C*) passing the current should be noted

small, while the second and third terms are $\sim -10^{-5}$ after 4 h, which are still very much smaller than the smallest measurable temperature increment (0·005°). Similar numerical results can be obtained if other time functions are substituted for equation (2). This means that $\theta_1 = \theta_2$ within the limits of experimental error.

These theoretical conclusions for the absence of thermal lag have been verified many times in calibration experiments in which a known amount of electrical energy is passed into the calorimeter. No further rise in temperature was observed when the current was switched off in the isothermal calorimeter[1], and a cooling curve can always be plotted immediately. Unfortunately no experimental tracings exist to prove this point, but when the same calorimeter assembly was modified to an adiabatic type[2], calibrations curves such as shown in Fig. 1 are obtained. If thermal lag exists in the calorimeter it will be manifested in further rises in temperature in both the isothermal and adiabatic models when the current is switched off. Fig. 1 shows that the thermal lag is practically zero at all times except possibly for a short period ($\sim$ 2 min) at point *B*, which is probably due to temporary inadequate stirring.

For twin calorimeters as used by Forrest[4], however, it is necessary to construct both vessels from materials of the highest thermal conductivity: this arises from the practical necessity of having two calorimeters of the same thermal leakage. Experiments carried out here confirm Forrest's experience in finding glass vessels unsuitable for this type of calorimeter. Moreover, Forrest and Walker (preceding communication) have not demonstrated unequivocally that glass vessels introduce a thermal lag; a comparison of the numerical results plotted in their Figs. 1 and 2 indicate that their experiments were not carried out under comparable conditions, although their text and the legend of their diagrams intimate that they were.

The most likely interpretation of the different results obtained by Forrest *et al.*[5] and myself lies in the different growth conditions. In my experiments, *A. aerogenes* probably changes its metabolic activity during growth, particularly in the so-called stationary phase. The increasing high growth temperature may also result in the uncoupling of some energy-yielding reactions[6]. The best way of resolving the differences and afterwards testing Prigogine's[7] hypothesis for individual organisms is to carry out simultaneous heat production and Gibbs energy change measurements in synchronously growing cultures under conditions in which the metabolic pathways are strictly controlled.

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Phytylation of Chlorophyllide and the Formation of Lamellæ in Chloroplasts

ONE of the earliest recognizable changes in plastid fine structure after exposure of etiolated leaves to high light intensity is the disappearance of the vesicular centre (prolamellar body) and formation of concentric layers of vesicles[1,2]. A similar structural change was reported for plastids of etiolated leaves after illumination for 24 h with fluorescent light of 400 ft.-candles at 3° C (ref. 3), or after illumination with 2 ft.-candles at 26° C (ref. 4). All these conditions allow photoconversion of those chlorophyll precursors existing in the etiolated leaves, but are unfavourable to chlorophyll accumulation[4]. It has been suggested that the grouping of vesicles into 'ring structures' in plastids is connected with low chlorophyll content[1,4,5].

Etiolated bean leaves contain mainly protochlorophyllide, which is converted to chlorophyllide on exposure of the leaves to light, and is afterwards phytylated to chlorophyll[6]. It has also been proposed that phytylation may be a factor in formation of lamellæ in plastids[7]. Since the organization of vesicles into concentric layers under the influence of light is a very early step in formation of lamellæ, we investigated whether the chlorophyll exists in the phytylated or non-phytylated form in plastids containing these 'ring structures'.

Leaves were detached from 12–14 day old etiolated bean plants and floated on water for 24 h at 26° C under white fluorescent light of 2–5 ft.-candles. This treatment produces plastids with vesicles arranged in concentric layers[4]. Leaves were also floated in complete darkness, under which conditions the vesicular centres remain unchanged in the plastids, and under light of 400 ft.-candles, when a normal lamellar system develops. After immersion of the leaves in boiling water the pigments were extracted quantitatively from leaf samples with acetone, each extract divided into aliquots, from one of which the phytylated pigments were removed by petroleum-ether, as described by Holden[8]. The absorption curves of the extracts of leaves kept in darkness showed the presence of protochlorophyll(ide), while extracts of leaves illuminated with 2 or 400 ft.-candles gave absorption spectra of chlorophyll(ide). In Table 1 the optical density of the petroleum–ether-treated acetone

Table 1. PERCENTAGE OF LEAF PIGMENTS NON-TRASFERABLE FROM 50 PER CENT ACETONE TO PETROLEUM–ETHER

Treatment	100 × optical density ratio*	
Leaves floated in darkness	72 ± 3·6†	(measured at 630 mμ)
Leaves floated under light of 2–5 ft.-c.	73 ± 1·8	(measured at 665 mμ)
Leaves floated under light of 400 ft.-c.	20 ± 3·6	(measured at 665 mμ)

$$* \; 100 \times \frac{\text{Optical density of petroleum–ether-treated acetone extract}}{\text{Optical density of untreated acetone extract}}$$

† *S.E.*

extracts is given as a percentage of optical density of the respective untreated acetone extracts.

Taking these data as an indication of the relative amount of non-phytylated pigments present in the leaves[6,8], it can be noted that in leaves kept in darkness, where the plastids have vesicles arranged in vesicular centres, the main part of the pigments consists of protochlorophyllide. It is also evident that no phytylation occurred after 24-h exposure of the leaves to low light, which thus contained chlorophyllide. Here, as aforementioned, the plastids still show vesicles, although now arranged in concentric layers. Among the variously treated leaves, phytylated chlorophyll occurred only in those which were exposed to 400 ft.-candles, and which have plastids with normal lamellar systems.

These results show that the rearrangement of vesicles from the vesicular centres into concentric layers—which is considered to be a photo-dependent process[1]—may be connected with the photo-conversion of protochlorophyllide to chlorophyllide but not with its change to chlorophyll, and indicate that the inability of the vesicles to fuse to lamellar structures under those conditions may be due to lack of phytylated chlorophyll. The same may be true for the formation of identical ring structures under high light intensity but low temperature[3], since Wolff and Price have shown that phytylation does not occur at 0° C (ref. 6). The results thus tend to confirm the suggestion of Butler[7] that phytylation may be a necessary factor in fusion of vesicles to lamellar structures.

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Carnitine as a Fatty Acid Carrier in Intermediary Metabolism

CARNITINE has been shown to stimulate the oxidation of long chain fatty acids by cell particle preparations[1]. The mechanism of this effect has remained obscure.

Friedman and Fraenkel[2] have reported that carnitine is acetylated by a soluble extract of liver in the presence of coenzyme A, adenosine triphosphate and acetate. They also obtained results indicating that the acetyl transfer from acetyl-coenzyme A to carnitine is reversible.

Recently I published results showing that isolated mitochondria from several tissues of the rat have the ability to deacetylate acetylcarnitine. This deacetylation is paralleled by an oxygen uptake correspondding to a complete oxidation of the acetyl group to carbon dioxide and water. Pyruvate apparently prevented the deacetylation of acetylcarnitine. It was also found that, in the presence of pyruvate, free carnitine was acetylated by mitochondria[3].

On the basis of these results it was suggested that carnitine functions as a carrier of active acetate between compartments within the cell[3].

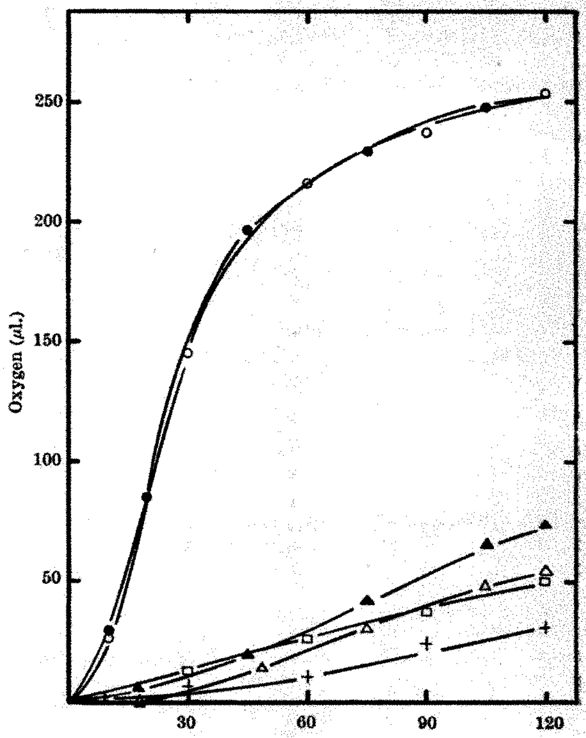

Fig. 1. Effect of DL-carnitine, palmitate and of DL-palmitylcarnitine on the oxygen uptake by rat heart mitochondria. Mitochondria isolated from one rat heart (approximately 1 g), as previously described (ref. 3), were suspended in 6-ml. of 10 per cent sucrose containing 10^{-4} M ethylenediamine tetraacetate. Each Warburg vessel contained in a total volume of 3 ml.: potassium phosphate buffer, *p*H 7·4, 40 µmoles; potassium chloride, 180 µmoles; magnesium chloride, 20 µmoles; AMP, 10 µmoles; succinate, 2 µmoles; crystalline bovine albumin, 30 mg; sucrose suspension of mitochondria, 1 ml. DL-carnitine, palmitate and DL-palmitylcarnitine were added as follows: ○, palmitylcarnitine, 1 µmole; ●, palmitylcarnitine, 1 µmole; +, carnitine, 1 µmole; Δ, palmitate, 1 µmole; ▲, palmitate plus carnitine, 1 µmole of each; □, no addition. The incubation was performed at 30° with air as the atmosphere

As carnitine is most efficient in stimulating the oxidation of long chain fatty acids[1], it may be assumed that carnitine is active as a general acyl carrier in the intracellular transport of fatty acids. To test this hypothesis, I have synthesized palmitylcarnitine and other acyl carnitines and tested these compounds as substrates for isolated mitochondria. Fig. 1 shows that palmitylcarnitine has a strong enhancing effect on the oxygen uptake by heart mitochondria in the presence of catalytic amounts of succinate. Carnitine or palmitate added individually showed no enhancing effect, while the combination palmitate plus carnitine showed a small stimulatory effect in agreement with the observations of Fritz *et al.*[1]. The far more pronounced effect of palmitylcarnitine strongly suggests that this compound is an intermediate in the carnitine stimulated oxidation of palmitate. Similar results were obtained with butyryl—and with octanoylcarnitine.

Previously we have shown that only L-acetyl carnitine is metabolized by mitochondria[3]. The synthetic palmitylcarnitine used in the experiment shown in Fig. 1 is the racemic DL-compound. The net oxygen uptake obtained in the experiment shown corresponds to 40–45 per cent of the uptake expected if all the bound palmitate added had been oxidized. This indicates that only one optical isomer, presumably the L-form, is metabolized.

Thus, palmitylcarnitine seems to represent a water-soluble, activated fatty acid metabolized by mitochondria. The enzymatic synthesis of this compound remains to be demonstrated. If carnitine, as suggested, functions as a carrier of activated fatty acids across the mitochondrial membrane for utilization in the citric acid cycle, an extramitochondrial localization of the carnitine acylating system should be expected.

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Polyphenolic Derivatives in Rhabdomeres of the Compound Eye

THE chemical composition of a homogenized compound eye was reported by Langer[1], but until now nothing was known about the components of rhabdomeres alone, although the presence of photosensitive substances in rhabdomeres has been anticipated by many investigators from a long time ago. During the course of histochemical studies on the compound eye of a house fly, *Musca vicina* Macquart, periodic acid–Schiff-positive substances were found to be distributed in rhabdomeres of a dark-adapted eye. The periodic acid–Schiff reaction of rhabdomeres was always positive both in a dark-adapted eye treated with saliva for 1 h in an incubator and in a light-adapted eye (Fig. 1). In addition to this, Bauer's reaction of rhabdomeres was very weak.

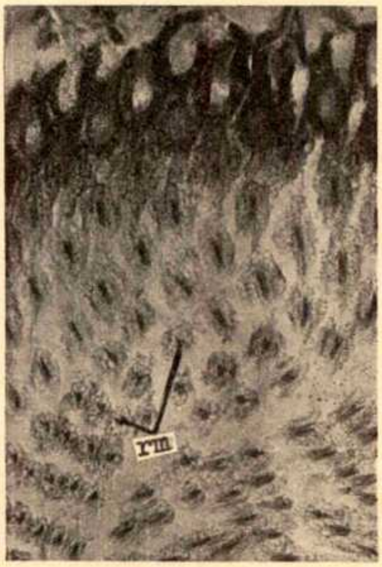

Fig. 1. Cross-section of a light-adapted eye. Periodic acid–Schiff reaction is still evident in the rhabdomeres (*rm*)

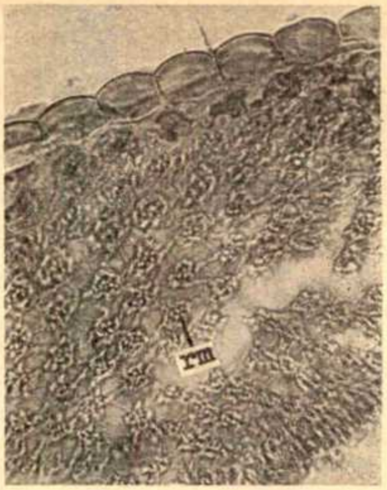

Fig. 2. Chromaffin reaction in a dark-adapted eye (cross-section)

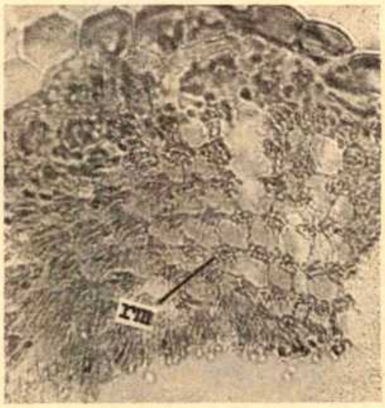

Fig. 3. Chromaffin reaction in a light-adapted eye (cross-section)

From these results, it was considered that most of the substances in the rhabdomeres are not polysaccharides. According to Lison[2], the substances showing a positive periodic acid–Schiff and a negative Bauer's reaction are regarded as proteins or other elements different from polysaccharides. In my investigations, the fly's rhabdomeres were proved to be positive for Millon's reaction, potassium iodate, potassium bichromate and ammoniacal silver, but negative for both the detections of α-amino radical with a ninhydrin and indole radical with a syrupy phospholic acid. In general, the positive Millon's reaction is considered to indicate the existence of tryptophan, tyrosine and other phenolic compounds; but from the negative reaction for both indole and α-amino radicals, it may be suggested that the rhabdomeres of *Musca* do not contain α-amino-acids such as tryptophan and tyrosine. Therefore, in the present circumstances, the positive Millon's reaction of the rhabdomeres may be regarded as proof of the presence of phenolic derivatives. These reactions become more indistinct in a light-adapted eye than in a dark-adapted eye (Figs. 2 and 3).

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Identification of the Origin of Animal Protein by Agar-gel Diffusion

The identification of the origin of various animal proteins proved possible by the agar-gel double diffusion technique[1]. Antisera were prepared in rabbits by multiple injections of alum-precipitated animal sera. Antigens were prepared from animal sera and tissue extracts, tick blood-meals, sun-dried meat or biltong and uncooked sausage meat. They were diffused overnight at 25° C against known antisera simultaneously with the homologous antigen. The agar gels were prepared from 1 per cent agar in borate buffer at pH 8·6 and were about 3 mm thick. A seven-well pattern was used consisting of a 9·5-mm diameter central well set 10 mm from the 7-mm diameter peripheral antigen wells.

Precipitation occurred, and the degree of precipitation appeared to be correlated to the zoological family of the animal of origin. For example, pig and horse sera used as antigens reacted with buffalo antiserum, but the precipitation bands were not as prominent as that which formed with sheep serum used as an antigen (Fig. 1). Within a sub-family, spur formation distinguished different genera (Fig. 2).

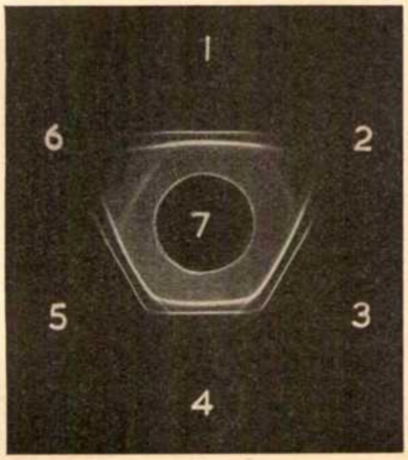

Fig. 1. Wells 1, 3 and 5 contain buffalo antigen; 2, horse antigen; 4, sheep antigen; 6, pig antigen; 7, unabsorbed rabbit anti-buffalo serum

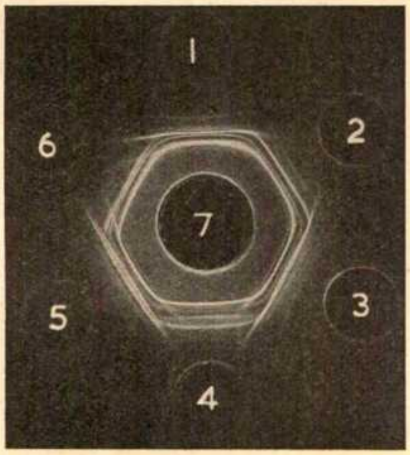

Fig. 2. Wells 1, 3 and 5 contain ox antigen; 2, buffalo antigen; 4, wildebeest antigen; 6, eland antigen; 7, unabsorbed rabbit anti-ox serum

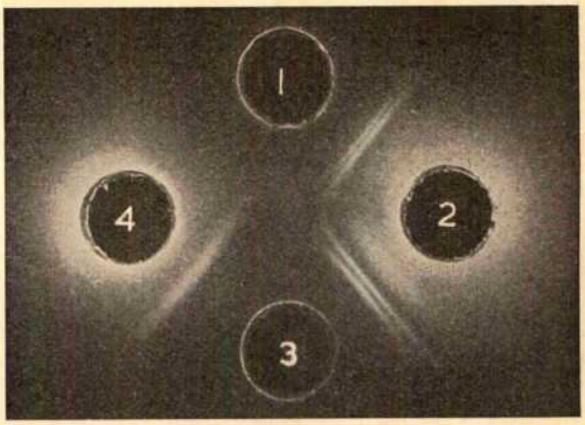

Fig. 3. Well 1 contains horse antigen; 2, unabsorbed rabbit anti-zebra serum; 3, zebra antigen; 4, absorbed rabbit anti-zebra serum

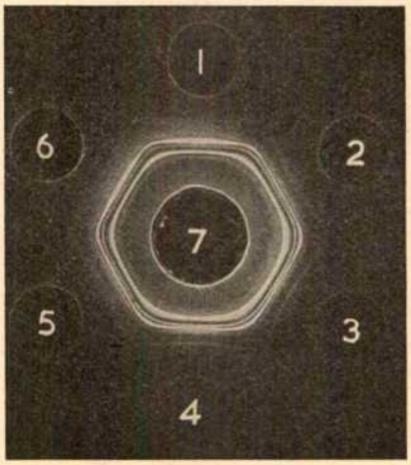

Fig. 4. The peripheral wells contain antigen from six different cattle. The centre well contains rabbit anti-ox serum

The identification of species within a genus was possible when absorbed antiserum was used. For example, an antiserum to zebra antigen reacted with both zebra and horse antigens but when absorbed with horse antigen it reacted only with zebra antigen and not with horse antigen (Fig. 3). Antigens in the form of sera from individual animals of the same species did not form spurs (Fig. 4).

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PHYSIOLOGY

Binding of Calcium Ion to Lecithin Film

The calcium ions in the membrane appear to be involved in some way with the unknown physicochemical reactions which occur in the membrane and which may be responsible for the changes in the membrane permeability or the membrane potential of excitable tissues[1,2]. Inasmuch as the membrane is composed of lipoproteins which possess negative phosphoric sites of phospholipids, it is likely that the calcium ions are bound to these sites. The experiment recorded here, therefore, was projected to determine whether or not calcium ions could actually bind to phospholipids, and if so, whether the binding of calcium ions would be affected by monovalent cations or other substances.

The radiotracer method using a soft β-emitter has proved very successful for the direct measurement of adsorption at the solution-air interface[3]. This technique was applied to study the adsorption of calcium ions (calcium-45) to a lecithin monolayer. The radioactive solution was introduced in a paraffin-coated planchet, and the radiation from the surface was counted by means of a Geiger–Müller counter before and after the spreading of the monolayer film. The radioactive solution (about 20 mc./mM) contained 2·4 sodium bicarbonate (*p*H, 8·2 ± 0·1) except in cases for the study on *p*H. Caution was taken so that counting was done under a constant geometrical condition throughout. L-α-(β,γ-dipalmitoyl)-lecithin (PL) and animal lecithin (AL) were used for the monolayer films. The former was chromatographically homogeneous whereas the latter (shown by Dr. L. G. Abood, of this laboratory) consisted of three kinds of lecithin with a purity of more than 90 per cent. Stearic acid was also used. The spreading solution, prepared by dissolving a material either in petroleum ether or in petroleum ether containing 2 per cent propanol, was carefully spread on the whole surface of the radioactive solution to obtain a uniform surface film.

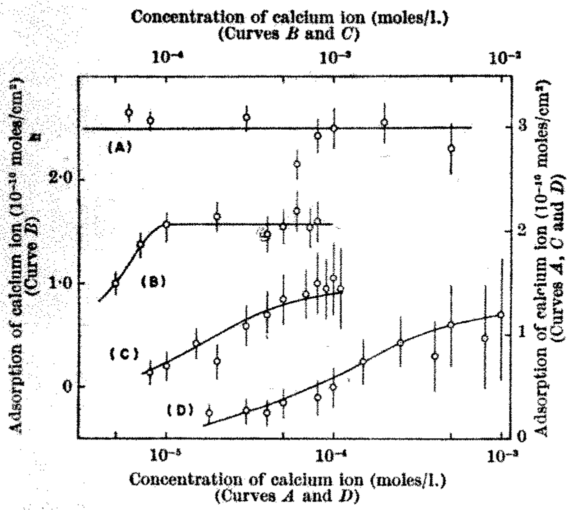

Fig. 1. Adsorption of calcium ions to surface films. Curve *A* represents adsorption isotherm for monolayer of stearic acid (surface concentration: $6{\cdot}0 \times 10^{-10}$ moles/cm²); curve *B* for *AL* film (surface concentration: 24 mg/m²); curve *C* for *PL* film (surface concentration: $24{\cdot}5 \times 10^{-10}$ moles/cm²) and curve *D* for monolayer of *PL* (surface concentration: $2{\cdot}45 \times 10^{-10}$ moles/cm²)

Fig. 1 shows the adsorption isotherms for the given surface concentrations of the lecithin films as well as of the stearic acid monolayer. The adsorption of calcium ions to the stearic acid monolayer is in saturation over the concentrations studied. The saturation value shows the formation of calcium distearate. Similarly, the binding ratio of calcium ion to PL is approximately 1/2. Curve *D* represents the relation calculated from the adsorption isotherm:

$$\frac{\varphi}{(1-\varphi)^2} = KC \qquad (1)$$

where φ denotes a half a fraction of saturation, and *C* and *K* the concentration of calcium ion and the constant, respectively. The value of *K* was $3{\cdot}4_7 \times 10^3$ mole^{-1}. The adsorption isotherm indicates that the surface film is an athermal mixture of lecithin and calcium salt of lecithin, with an occupied area twice as large as the uncombined lecithin.

The adsorption of calcium ions to the multimolecular film (Curve *C*) can be expressed by the isotherm identical with that for the monomolecular film. The saturation value agrees well with that expected for a closely packed lecithin monolayer, provided the occupied area is about 40 Å² per molecule. These findings indicate that only the layer facing the aqueous phase is capable of taking up calcium ions. A similar presumption is applicable to the adsorption of calcium ions to the animal lecithin film.

The effect of foreign substances on the adsorption of calcium ion to animal lecithin was studied with solutions containing 0·1 mM calcium chloride and foreign substances. Results are shown in Table 1. The decrease in the adsorption of calcium ions would be either due to the competition between calcium and monovalent cations, or to the chelating action of the foreign substance.

Table 1. EFFECT OF FOREIGN SUBSTANCES ON THE ADSORPTION OF CALCIUM ION TO ANIMAL LECITHIN*

Foreign substances	Concentration	Fraction of adsorption of calcium ion
No foreign substance	—	1·0
ATP	0·3 mM	0
EDTA	0·4 mM	0
Acetylcholine chloride	5 mM	1·0
KCl	112 mM	0·51
NaCl	112 mM	0·5
H^+	10^{-7} M (*p*H = 7)	0·9
H^+	$10^{-5{\cdot}3}$ M (*p*H = 5·3)	0·5†

* Concentration of calcium ions in solution was 0·1 mM.
† Sodium bicarbonate was not added.

These experiments demonstrate that calcium ions are capable of binding to the monolayer film of lecithin within a physiological range of *p*H. On the basis of this finding, we may conclude that calcium ions are bound to phospholipids present in the membrane of living excitable cells. Results suggest that calcium ions may be binding with two negative oxygen sites and forming a bridge between two radically oriented phospholipids[1]. It is of interest that the binding of calcium ions to the lecithin monolayer is saturated at a relatively small concentration which corresponds to the minimum calcium concentration necessary to maintain the physiological function of living excitable cells. Furthermore, the present investigations indicate that monovalent cations, such as sodium and potassium ions, are in competition with calcium ions. A similar competition probably exists in the case of the living cell membrane[1,2]. The fact that adenosine triphosphate (ATP) hinders the adsorption of calcium ions to the lecithin may provide a key for understanding the actual role of the ATP located in the membrane or its vicinity[4].

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Neuromuscular Fatigue in Dystrophic Muscle

THE effects of the dystrophic process upon muscle function are of continued interest. Dystrophic mouse muscle has been found to exhibit multiple action potentials, spontaneously, as well as in response to a single stimulus to its nerve[1,2]; *d*-tubocurarine or sustained, periodic single shock stimulation shortens the after-discharges[1]. Heightened muscle responsiveness and excitability to dual shocks also develop[1]. In addition, excised dystrophic muscle is less fatiguable and maintains a tetanus for a longer time than the normal[3]. It was felt that an investigation of neuromuscular fatigue might help to further delineate and perhaps clarify some of these phenomena.

Twenty-six experiments were performed on 11 dystrophic mice (strain 129, *dy dy*, Jackson Memorial Laboratory) and 15 normal litter-mate controls. An *in situ*, *in vivo* technique of nerve-muscle preparation was used, with intact circulation to the limb, after general anæsthesia with amobarbital sodium. Isometric muscle tension measurements were made from exposed gastrocnemius muscle stimulated directly or through the sciatic nerve. Fine steel and silver macro-electrodes were used for stimulation and recording. Both tension (through a mechano-electronic transducer tube) and action potentials were displayed on a dual beam oscilloscope and recorded by continuous strip camera.

The results of single shock stimulation through the nerve are shown in Fig. 1*A*. In the control muscle, a single action potential occurs, along with a typical twitch tension response. The response in the dystrophic is characterized by a multitude of asynchronous potentials and a tension record of longer duration than the control, although much less actual tension is developed. This appears to be a brief tetanus response to a single stimulus. Tetanic stimuli, at 100 per second applied through the nerve, also resulted in a more prolonged maintenance of both the tension response and action potential activity in the dystrophic (Fig. 1 *B*).

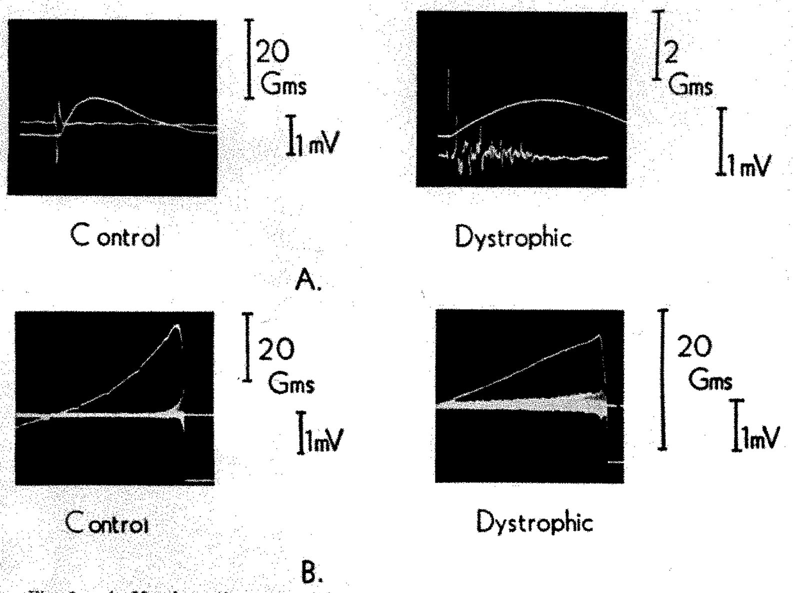

Fig. 1. *A*, Muscle action potential and tension response to single stimulus through nerve; *B*, muscle action potential and tension response to tetanic stimuli through nerve at 100 per sec

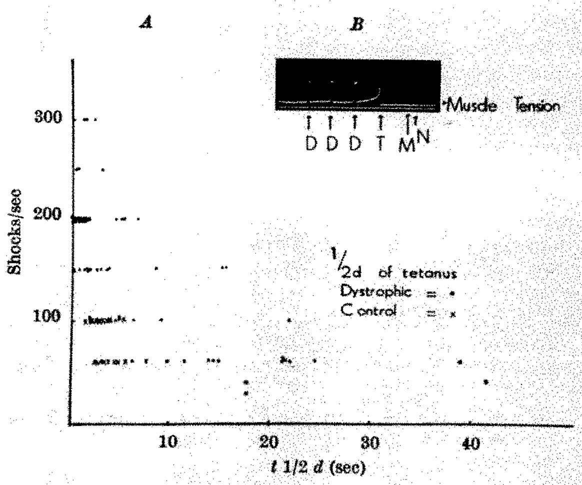

Fig. 2. *A*, One-half time decay of tension developed at varying frequencies of tetanic stimulation through nerve to normal and dystrophic muscles. *B*, Tension recording in single experiment with dystrophic muscle. *N*, single stimulus to nerve; *M*, single stimulus to muscle; *T*, tetanic stimuli through nerve at 100 per sec.; *D*, direct muscle stimulation, at 60 per sec for 1 sec, during neuromuscular tetanic fatigue. Time-base at 5-sec intervals

These results led to a study of the fatiguability of neuromuscular transmission. The method utilized tetanic stimuli, at frequencies varying from 10 to 300 per sec, first applied through the nerve, and then, as fatigue developed, periodically directly to the muscle, usually at a constant frequency of 60 per sec. The tension developed by nerve stimulation declined more rapidly at higher frequencies than at lower, in both the control and dystrophic. Measurements of the one-half time decay of the tension curves indicated that the decrease in tension with frequency was more slowly developed in the dystrophic than in the control (Fig. 2*A*). When this decrease had occurred, for example, at a stimulation-rate of 100 per sec, direct muscle stimulation (at 60 per sec) indicated that the muscle itself was still responsive and capable of maintaining a full tetanus (Fig. 2*B*).

It is considered that the method used in this investigation is a valid test of neuromuscular transmission fatigue[4]. This fatigue occurs in dystrophic muscle at a slower rate than normal, along with maintained muscular responsiveness. The phenomenon has a resemblance to 'myotonia' and may be a general property of dystrophic muscle[1,2], varying in degree in accordance with different types of dystrophic process. The evidence is suggestive of an enhancement of transmission through the neuromuscular junction in the dystrophic as compared to normal. This effect could be related to increased muscle responsiveness or excitability[1,3]. However, since it has been found in the present investigation that direct muscle responsiveness is maintained at a point when neuromuscular responsiveness has declined, the implication exists that an additional factor may be an alteration in neuromuscular transmission independent of any primary muscular dysfunction.

Both electrophysiological and actual morphological evidence is accumulating[1,5-7] of changes at the myoneural junction and terminal innervation in dystrophic muscle.

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Potassium Excretion during Adrenal Insufficiency

IT was first noticed by Harrison and Darrow[1] that in adrenal insufficiency in dogs the renal tubular function of preserving sodium and excreting potassium was disturbed. This was afterwards interpreted generally as the result of insufficiency of the sodium–potassium exchange mechanism, which was advocated by Berliner *et al.*[2] originally for the explanation of the high output of potassium under a special condition of extremely high potassium load. That the abnormality of excretion of potassium in adrenal insufficiency, except in the final stage, was not dependent upon changes in the glomerular filtration rate has been stressed by Harrison and Darrow[1]. However, their experiments were done under special conditions by giving potassium chloride (10 g) to the intact dog or the adrenalectomized dog treated with cortical extract in order to compare their concentration ratio (urine/plasma ratio) of potassium with that of the untreated adrenalectomized dog at the same high serum potassium-level found in the latter. The ratio obtained in the former reached 40–75, whereas that found in the latter was about 10. In our experiences the former values seem to be abnormally high, perhaps due to adaptation to potassium load[2] to which no attention was paid, while the latter lies rather in the normal range. On the other hand, Harrop *et al.*[3] and recently Fukuda and Koyama[4] have noticed that the rise of concentration of potassium in the plasma in adrenal insufficiency paralleled that of urea or non-protein nitrogen and suggested the possibility that the disturbance in excretion of potassium might be the result of reduced glomerular filtration rate. Therefore, experiments were performed to find out whether the clearance of potassium shows direct relation to the glomerular filtration rate in adrenal insufficiency in dogs. The results were compared with those obtained from intact dogs and adrenalectomized dogs maintained with cortisone (1 mg/kg per day) when the glomerular filtration rate was changed by altering the intake of sodium chloride (0–2·0 g/kg per day).

The clearance of potassium and endogenous creatinine[5] was determined on daily urine with the midday blood sample. Together with these the urinary sodium/chlorine ratio was also measured which can be regarded as a delicate index of sodium bicarbonate reabsorption[4]. The results are presented in Fig. 1. In intact dogs the sodium/chlorine ratio tended to decrease below 1·0 when the glomerular filtration rate was reduced beyond a certain limit. The clearance of potassium was found to be almost in a linear relation to the glomerular filtration rate and decreased when the latter was reduced. In adrenalectomized dogs the relation of sodium/chlorine ratio to glomerular filtration rate was quite contrary to that of the intact animal. The sodium/chlorine ratio increased above 1·0 with reduction of the glomerular filtration rate, until actual sodium bicarbonate loss occurred with alkalization of the urine. The same tendency could be observed in cortisone maintained adrenalectomized animals although the reduction of glomerular filtration rate did not proceed so far, as was found in untreated animals. On the other hand, the relation of clearance of potassium to glomerular filtration rate in cortisone-maintained dogs was almost the same with that of the intact animal (Fig. 1, line *A*), while somewhat elevated clearance of potassium in relation to glomerular filtration rate was observed in untreated adrenalectomized dogs (Fig. 1, line *B*). In any event there could be found no default in the potassium-excreting mechanism in the absence of mineral corticoids when the changes of the glomerular filtration rate were taken into consideration. Rather enhanced clearance of potassium in adrenal insufficiency might be due to the adaptation phenomena to the retention of potassium.

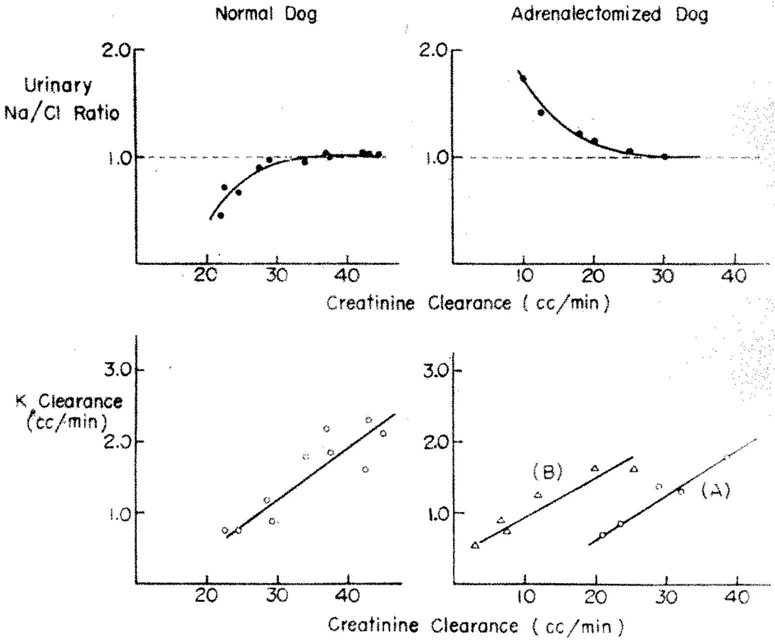

Fig. 1. Relation of the urinary sodium/chlorine ratio and potassium clearance to the glomerular filtration rate. The representative data are taken from three experiments showing similar results. *A*, On adrenalectomized dog maintained with cortisone; *B*, in adrenalectomized dog during the course of adrenal insufficiency

Thus it may be concluded that the main default in the renal handling of electrolytes in adrenal insufficiency in dog was the failure of preserving sodium and especially bicarbonate and that the failure of potassium excretion was merely the result of reduced glomerular filtration rate. That the latter was due to acapnic central deteriorations during the progress of respiratory compensation for the renal acidosis resulting from the excess loss of sodium over chlorine has been fully discussed in a previous report[4]. Glucocorticoids, which can ameliorate this central acapnic deterioration, could prevent the progressive reduction of glomerular filtration rate, as aforementioned (compare the lower limit of glomerular filtration rate in lines *A* and *B* in Fig. 1). Concerning the various kinds of acute experiments showing a short-lived excretion of potassium independent of glomerular filtration rate, care should be taken for leakage of potassium from cells into the tubular urine.

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Peripheral Vascular Effects of Mixtures of Isopropylnoradrenaline and Noradrenaline in Man

IT is well known that the infusion of 0·1–1·0 μg/min adrenaline into the brachial artery in man results in an immediate transient increase in flow of blood in the forearm followed by a fall to below the resting level. Allen, Barcroft and Edholm[1] showed that this biphasic response was not due to dilatation of muscle vessels followed by constriction of skin vessels. Finding that after dibenyline, adrenaline produces only a sustained vasodilatation, Ginsburg and Cobbold[2] suggested that the action of adrenaline when given alone was due to an initial stimulation of inhibitory (β) receptors followed by stronger stimulation of excitatory (α) receptors. The action of adrenaline after dibenyline resembles that of isopropylnoradrenaline. Recently we have found that after dichloroisopropylnoradrenaline, adrenaline causes only sustained vasoconstriction, and under these conditions resembles noradrenaline[3].

If adrenaline stimulates first the inhibitory and then the excitatory receptors, we might expect its action to be matched by a suitable mixture of isopropylnoradrenaline and noradrenaline. We have tested this in a series of experiments, of which Fig. 1 shows a typical example. Forearm blood flow was measured by venous occlusion plethysmography. The typical responses to isopropylnoradrenaline (0·05 μg/min) and to noradrenaline (0·25 μg/min) are shown in the first two panels. When these doses of the two drugs were administered simultaneously (panel 3) the response was an initial vasodilatation followed by a vasoconstriction. This response can be seen to bear a strong resemblance to the response to 0·25 μg/min adrenaline, illustrated in panel 4.

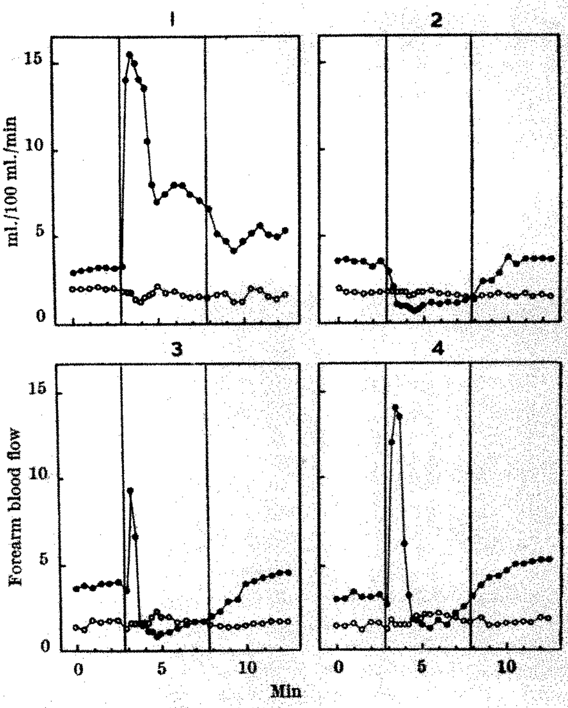

Fig. 1

These results indicate that the response of blood vessels of the forearm to adrenaline can be explained on the basis of the response of the two types of receptor proposed by Ahlquist[4], and thus provide positive evidence for the theory proposed by Ginsburg and Cobbold.

It would be interesting to know if other actions of adrenaline in man and animals can be reproduced by mixtures of isopropylnoradrenaline and noradrenaline, and whether the proportions needed are always the same.

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Receptive Fields of Single Cells in the Rabbit Lateral Geniculate Body

THE activity of single cells in the lateral geniculate body of lightly urethanized rabbits has been recorded with steel micro-electrodes[1]. The retinal receptive fields of these cells have been explored. The light source, a slit lamp bulb, was incorporated in a multibeam photo-stimulator similar to one previously described[2,3] and mounted on a perimeter arc.

In a proportion of cells the receptive field was 'simple' in the sense that the responses evoked by the exploring light spot were the same throughout the receptive field. In other cases in which the receptive fields were larger the response to light varied across the field. The functional organization of such fields is of interest and a typical result is shown in Fig. 1.

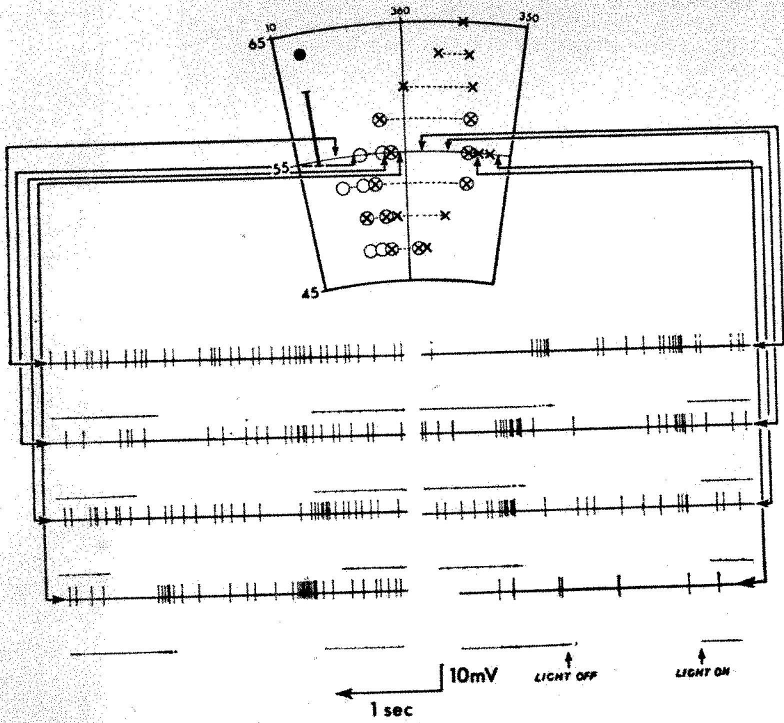

Fig. 1. Receptive field organization of a cell in the rabbit lateral geniculate body. Above, Part of a perimeter chart showing the positions in the rabbit visual field from which responses could be evoked, 360°—vertically above optic axis, 90°—cephalad: the receptive field is therefore in the inferior portion of the rabbit's retina.
×, Off response; ○, on response; ⊗, on–off response. The symbols joined by dotted lines imply that similar responses were evoked in between positions marked. The vertical bar represents a retinal displacement of 1 mm. The small filled circle represents the size of the exploring light spot, stimulus 30′ subtense, 76,000 l./m^2, repetition-rate approximately 2/sec.
Sample records obtained at arrowed points illustrated in lower half of picture right to left. Stimulus single flashes (hand-operated shutter) subtense 45′ intensity 76,000 l./m^2 centred on a 25° constantly illuminated background of 760 l./m^2. Note that when the stimulus lies outside the receptive field light causes neither excitation nor inhibition (that is, slowing of resting discharge). When stimulus lies within a receptive field complex changes in spike-rate occur. The chief changes are that when the left-hand border of the field is stimulated, the geniculate cell gives responses predominantly to the beginning of the light flash and as the stimulus moves across the field the responses change until only the end of the flash is signalled

The upper part of Fig. 1 represents the position of the light source on the perimeter arc, and is in fact part of a perimeter chart. The crosses and circles are placed in that part of the visual field from which responses were obtained. It can be seen that the receptive field is oval and extends in its major axis some 20° of visual angle. The long axis of the field is placed nearly vertical. On-, on-off-, and off-responses were obtained from this field. The specimen records in Fig. 1 show that at the left-hand border of the field the cell responds only to the beginning of the light flash while at the right-hand border the cell responds to the end of the stimulus. In between these points, responses are obtained at both the beginning and ending of the stimuli, the relative preponderance of on- and off-discharges varying across the receptive field. When the stimulus falls outside the field there is no response and no slowing of the spontaneous activity. The area of retina covered by this receptive field was calculated, assuming a posterior nodal distance for the rabbit eye of 8·8 mm, which is a minimal value. The bar in the perimeter chart shows the angle through which the stimulus had to be moved for a retinal displacement of 1 mm.

In the retinæ of all animals investigated[3-6] the receptive fields of retinal ganglion cells are arranged in a different manner to that shown in Fig. 1. The receptive field is circular, and the peripheral portion inhibits the central. The same is true of cells in the cat lateral geniculate body[7]. However, a receptive field organization similar to that of Fig. 1 has been described for cells in the cat visual cortex[8,9]. It appears in the less encephalized rabbit some elements of cortical organization are found in lower nuclei. There are differences between other authors' results[8,9] in cat cortex and the present experiments. The most obvious is that the receptive fields of rabbit geniculate cells are very large—in fact, the example shown in Fig. 1 is one of the smaller fields found. The receptive fields of single retinal ganglion cells are limited by the spread of their dendrites to about 1·5 mm (ref. 10). It is therefore certain that the receptive fields of rabbit lateral geniculate cells are the result of the convergence of several optic nerve fibres which originate in retinal ganglion cells that have overlapping retinal receptive fields. When the intensity of the light stimulus is reduced, receptive fields such as are shown in Fig. 1 become smaller and the responses the same throughout, as though several of the converging afferent fibres had ceased to influence the geniculate cell.

Receptive fields such as shown in Fig. 1 are so large that they can provide little information about the site of a luminous stimulus. On the other hand, it has been found that within the receptive fields there are limited areas which respond when a luminous object moves across them. These areas are frequently located at regions where the type of response changes abruptly, for example, the left-hand border of the receptive field in Fig. 1. The responses to moving objects are in general very much stronger than the responses to stationary flashing lights, and the response only occurs when the object moves in certain directions. The functional organization of the rabbit lateral geniculate body is such that it provides detailed information about the properties of moving objects in spite of the diffuse receptive fields disclosed by stationary flashing lights.

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Arousal Reaction in EEG induced by Blood Pressure

It is supposed by several authors[1-9] that adrenaline-induced EEG-arousal, which originates in the mesencephalic reticular formation and the posterior hypothalamus, is caused by a direct action of adrenaline on these structures. Discharges were recorded from single cells within the posterior hypothalamus[10,11]. The firing-rate of these cells was affected by intravenous injection of adrenaline or by mechanically induced changes of blood pressure. There is no effect of adrenaline if the blood pressure is kept constant artificially. We therefore tested the hypothesis that the adrenaline-induced arousal is a secondary effect of the rising blood pressure.

The following experiments were performed on cats with an *encéphale isolé* preparation. It was observed that the EEG-arousal after intravenous injection of adrenaline occurred about 1 sec after the beginning of the rise in blood pressure. Fig. 1 shows that an arousal is also obtained after an increase of blood pressure, which is induced mechanically by a short, strong injection of Ringer solution into the aorta. The high, slow-voltage pattern simultaneously in all leads changes to a fast low activity, which is typical for an arousal reaction. In this case, the arousal occurs at the end of the injection artefact, when the blood pressure has started to rise. Rothballer[9] found a cortical arousal on injection of adrenaline into the mesencephalic reticular formation without changes in the systemic blood pressure. This seems to support the hypothesis of a local action of adrenaline. In our experiments, a local injection of mammalian Ringer solution into the same area also caused a cortical arousal. Therefore, Rothballer's observation might be explained by a stimulation of the cells induced by the mechanical stimulus of the injection.

It can be concluded from our observations that an increase of blood pressure can excite the ascending reticular activating system. The 'pressosensitive area', described by Baust *et al.*[11], is possibly responsible for these effects. This area can be excited by changes in systemic blood pressure as well as by local pressure stimuli as shown in the experiment mentioned here. The fact that injection of vasopressin leads to EEG-arousal[4] also favours our hypothesis.

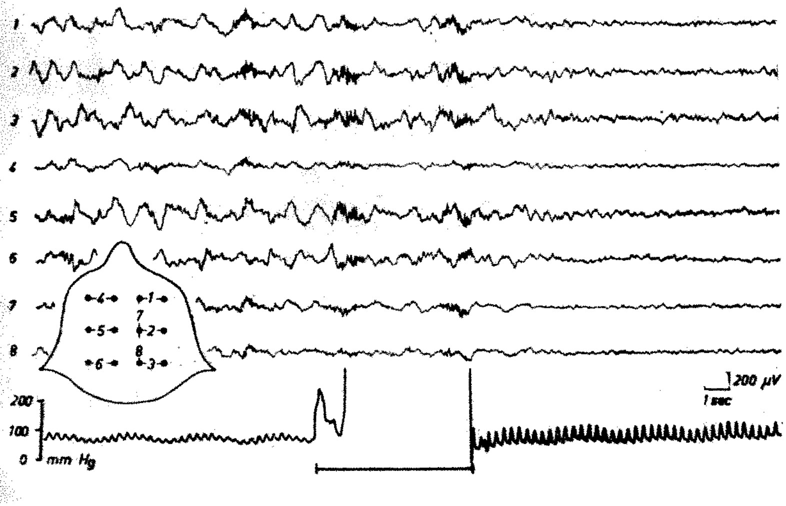

Fig. 1. *Encéphale isolé* preparation of the cat, no narcotics present. Artificial respiration. Lines 1–8, bipolar EEG-records from the cortex; location of electrodes as indicated in the inset. Lowest line, simultaneous blood pressure record in the femoral artery. During the mark (below), 10 ml. of mammalian Ringer solution were injected into the aorta

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Serum Cholesterol in Rats intoxicated with Carbon Tetrachloride

Oral administration of carbon tetrachloride to rats causes an elevation in the amount of β-globulins while reducing the albumin, α_1- and α_2-globulins. Subsequent injury by subcutaneous sponge implantation of the intoxicated rats augments both α_2- and β-regions[1]. Since the α_2- and β-globulin regions of rat serum contain lipoproteins[2], the fatty infiltration of the liver might be a responsible factor in some of the changes in electrophoretic patterns which are observed in injured, intoxicated rats. Carbon tetrachloride poisoning is apparently associated with a reduced rate of lipoprotein synthesis resulting in a lowering of the plasma lipoprotein-level and an accumulation of liver lipids[3]. Preliminary experiments, however, showed no intensification of lipoprotein staining (sudan black) of the paper electropherograms obtained with serum from injured, carbon tetrachloride intoxicated rats.

To test this point further, male white rats (Holtzman) were treated by oral administration of 0·1 ml. of carbon tetrachloride mixed with mineral oil (1·1 mixture) per 100 g of body-weight for a total of six days. Some of the poisoned rats were afterwards injured by the subcutaneous implantation (dorsal) of a 200-mg piece of 'Ivalon' sponge after the fifth dose of carbon tetrachloride; a sixth dose was given 24 h later. Normal control rats, control rats receiving 0·1 ml. of mineral oil in six daily doses and rats injured by sponge implantation only accompanied the experiments with carbon tetrachloride.

24 h after the sixth dose of carbon tetrachloride or mineral oil or 48 h after sponge implantation, the rats were anæsthetized with ether and bled by cardiac puncture. The livers were perfused with physiological saline solution, excised and homogenized with an equal volume of distilled water. The homogenates

Table 1. Serum and Liver Cholesterol-Levels in Injured and Uninjured Rats intoxicated by Carbon Tetrachloride

Tissue	Normal control	Mineral oil treated	Carbon tetra-chloride treated	Injury only	Carbon tetra-chloride treated + injury
Serum (mg%)	73·3 ± 11·1 (20)	64·7 ± 5·8 (6)	44·4 ± 8·8 (13)	78·4 ± 16·7 (14)	51·8 ± 11·1 (16)
Liver (mg/g) dry wt.	14·6 ± 2·2 (12)	13·3 ± 3·1 (6)	25·9 ± 7·5 (6)	16·5 ± 3·0 (8)	23·0 ± 5·0 (6)

No. of rats used for each experiment is given in parentheses. All values are presented as the average ± *S.D.*

were treated with 0·084 per cent solution of ferric chloride precipitating agent[4], heated in a boiling water bath for 20 min and then filtered. Cholesterol-levels were determined in serum and in the filtrate of the liver homogenates by the method of Zak[4].

Table 1 contains the average values and standard deviations for all the rats studied. Injury alone, which increases the proportions of α_2- and β-globulins in the rat, caused no changes in cholesterol-levels. Intoxication with carbon tetrachloride decreased the serum cholesterol-levels and simultaneously elevated the concentration in the liver. This is in accord with its hepatic synthesis[5]. Various hepatoxic agents including carbon tetrachloride lower the level of serum lipids while at the same time causing their hepatic accumulation perhaps by interfering with the synthesis of plasma lipoproteins[3,6,7]. Our results show that injury neither reverses this interference in plasma lipoprotein synthesis nor promotes a release of lipid accumulated in the liver.

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HISTOLOGY

Determination of Refraction Index and Thickness of Histological Sections

In connexion with an investigation into the mechanism of vision of some Arthropoda it was necessary to determine the refractive index, *n*, of the dioptric tissues of an ommatidium. Owing to the small dimension of these tissues, even in such a big insect as the dragon fly, *Aeschna*, it would be very difficult to measure this index with ordinary refractometers because the cells in question cannot be separated without damage. Thus *n* must be determined directly by using histological sections. Due to the fact that cells are never absolutely homogeneous it is possible to determine only an average value of the index. Therefore, the more accurate but circumstantial methods can be replaced by the old and well-known methods of de Chaulnes (1768) if improved in the following way in which only a modern microscope together with a special glass slide device are needed.

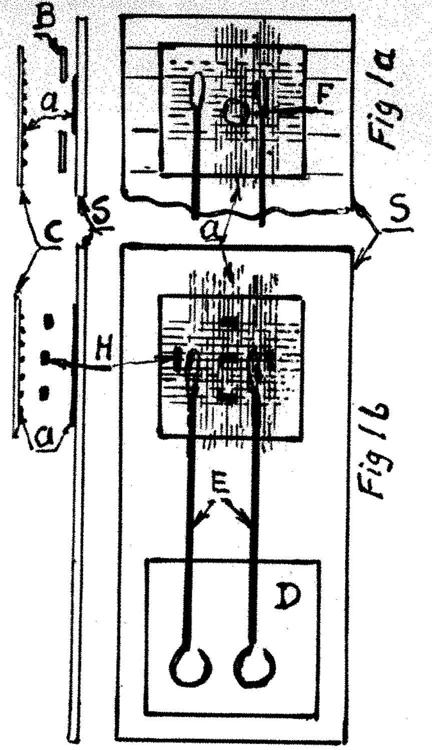

Fig. 1. *a*, Strokes of aluminium; *B*, brackets of cover glass; *C*, cover glass; *D*, metal sheet; *E*, stainless steel springs, fixed to *D*; *F*, drop of liquid or air bubble; *H*, piece of tissue; *S*, glass slide

For the sake of simplicity, I will begin with the refractive index of a liquid (Fig. 1*a*). An ordinary, fairly even glass-slide, *S* (0·8–1·0 mm), is on one side, over an area of about 10 × 10 mm, covered with strokes of metallic aluminium which are easily drawn with a moderately sharpened edge of a piece of aluminium sheet wetted with a drop of water. The aluminium marks, drawn parallel at a distance of 0·2–0·5 mm, will firmly adhere to the glass. The microscope will show that the marks consist of an immense number of very small grains of aluminium. The overposing but not the rest of the marks can and may be removed by a razor blade. In the same way, an ordinary even cover glass, *C* (18 × 18 mm), may also be covered with aluminium marks, *a*. In order not to break this thin glass it should be glued beforehand to a glass slide by wax. Another cover glass is cut in two parts, brackets, *B* (9 × 18 mm), and glued permanently to the glass-slide, *S*, by Canada balsam or resin, one part on each side of the aluminium-marked area. To secure an even upper surface of the two brackets another slide should be pressed on the top of them until the balsam has dried. The aluminium-marked cover glass is now placed on the top of the brackets and with the aluminium strokes downwards and perpendicular to the strokes of the slide. Further, this cover glass is pressed to the brackets by a simple device: on a piece of brass sheet, *D* (about 1 × 20 × 25 mm), are fixed two small wires of hard stainless steel, *E*, of about 0·5 mm diameter. The brass sheet is glued to the slide by a wax–resin mixture in such a way that the flattened ends (about 0·1 mm) of the wire springs may press the cover glass firmly to the brackets in order to prevent the capillary

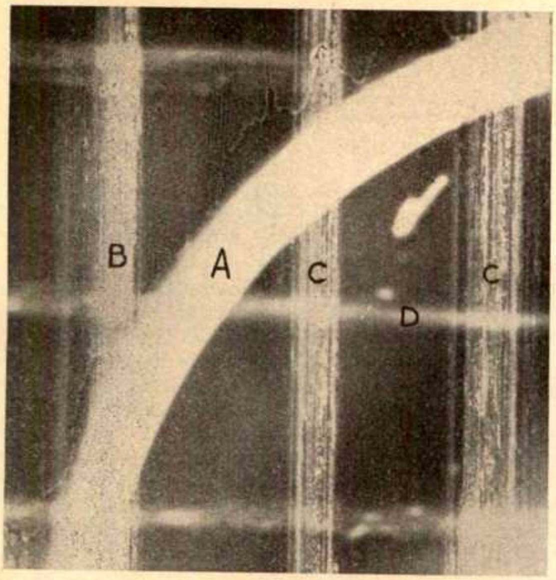

Fig. 2. *A*, Border-line of a droplet of water; *B*, aluminium marks on the glass slide, distinctly seen through air; *C*, aluminium marks on the glass slide, distinctly seen through the water; *D*, aluminium marks on the cover glass, seen very dimly

force of a liquid from lifting the glass. Now we have an interspace between two fairly parallel aluminium-marked glass surfaces, the marks forming a cross net (Fig. 1).

With a microscope magnification of about × 400–800 in light field or more suitably in dark field one is able to focus very sharply on the two marked surfaces. With air in the interspace ($n = 1{\cdot}000$), the microscope-micrometer reading being a for the upper and b for the lower surface, then the thickness of the interspace $d = a - b$. A very small droplet, F, 0·5–1 mm^3, of a liquid of refraction index n_1 may now be put in the midst of the interspace, preferably not into contact with the brackets. Along the borderline of the droplet one will find many points where can be seen, at the same time but with altered focusing, the aluminium marks of the slide through the air on one side and through the liquid on the other side of this borderline, as well as the marks of the cover glass (Fig. 2*b*). If the reading of the slide marks through the liquid is b_1, then $n_1 = \frac{a-b}{a-b_1}$. This simple formula can easily be checked: the interspace = the exact thickness of the brackets can be controlled by a micrometer, and may be d_0. A small correction factor, c, for the difference between d and d_0, that is, $d_0 = d + c$, can, if desirable, be introduced, *inter alia*, due to the fact that the rays through the microscope are not exactly central. Further, the distance $a - b_1$ can be controlled by a liquid of known refraction index, for example, distilled water of $n = 1{\cdot}333$ ($= 4/3$). For the calculation put $b - b_1 = s$. Then we may write the equation $\frac{1}{n_1} = 1 - \frac{s}{d+c}$. If, for example, $d = 127\mu$ and $s = 33\mu$ and $n_1 = 1{\cdot}333$ then c will equal 5. If this value is inserted the equation will be fairly good for other liquids or substances provided the same glass slide device is used.

The determination of refraction index of a biological tissue ought preferably to be made in fresh material or fixed in a medium which will not shrink or swell the tissue appreciably, for example, an isotonic 4 per cent formol solution. The tissue may be cut in a freezing-microtome. The determination of n of the cornea (subst. propria) of a compound eye at right angles to the optical axis of the eye may be used as an example. About 10 sections, 80μ thick, were cut perpendicular to the cornea surface and distributed evenly on the aluminium-marked area of a glass slide. As before, a marked cover glass was placed on top of the sections with tiny springs pressing it to the sections and with, for example, distilled water filling up the interspace and surrounding the sections. In order to prevent any bending of the cover glass a section ought to be placed right under the tip of a spring. Brackets for the cover glass can also be made by small pieces of paraffin cut to the same thickness as the sections. Now one will find many points where, in the same field of view and without moving the slide, one is able to focus on the marks of the cover glass as well as on those of the slide through the water and through the cornea section, the readings being respectively a, b_1 and b_c. Put $a - b_1 = d_1$ and $b_c - b_1 = s$. The index of water or any other reference medium $= n_1$. Then we get for the corneal index $n_c = \frac{a-b}{a-b_2}$, and if this is divided by the previously mentioned equation we get $n_c = n_1 \frac{a-b_1}{a-b_2}$ or $n_c = n_1 \frac{d_1}{d_1 - s}$, and if we invert the equation and insert the correction factor and use water as reference medium: $\frac{1}{n_c} = \frac{3}{4}\left(1 - \frac{s}{d_1 + c}\right)$.

Once c has been evaluated, one has only to measure the three distances a, b_1 and b_c or the difference $b_c - b_1$. For the example given the figures from a statistic series of 25 measurements were found to be: $\frac{1}{n_2} = \frac{3}{4}\left(1 - \frac{4{\cdot}56 \pm 0{\cdot}40}{75{\cdot}20}\right)$. Thus the refractive index will be $1{\cdot}42 \pm 0{\cdot}01$, which seems to be a fairly satisfying value.

This method can also be used to find the approximative thickness and total shrinkage of a tissue, already embedded in, for example, Canada balsam or glycerol–gelatin if the embedding is made between aluminium-marked glasses and the embedding medium is known. If the aluminium marks are not too broad they will scarcely disturb the picture even at great magnifications.

It is obvious that the accuracy of this method is dependent on many factors. Homogeneity, transparency and histological treatment are, of course, important; but this is true for every method. The formula shows, however, that the accuracy of a measuring depends on the exactitude with which the ratio s/d can be determined. An advantage of this simple method compared with the more accurate methods based on total reflexion, for example, refractometers of Pulfrich or Abbe, will, however, be that which obtains the average refractive index of a tissue substance and not only the index of its surface.

The refractive index, n, is important in many ways, not only by itself because it is correlated to other properties, for example, the dielectricity-constant of which n is the square root.

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PATHOLOGY

Association of Cell Surface Antigens with Microsomal Membrane Fractions derived from Ehrlich Ascites Carcinoma Cells

ASCITES tumour cells have been used widely in the study of the localization and mode of action of the cytotoxic antibodies produced by the administration of whole cells to heterologous animals[1-5]. Such investigations have indicated that the cytotoxic antibodies exert their effect on the cell membrane. Recent investigations of Ehrlich ascites carcinoma cells suggest that the surface membrane of these cells form small vesicles on cell rupture, and that these sediment with the microsomal membranes on differential centrifugation[6,7].

The work reported here shows that the microsomal membranes and their various sub-fractions contain potent antigens capable of forming both cytotoxic and agglutinating antibodies to Ehrlich ascites carcinoma cells when injected into rabbits.

Microsomal membranes were prepared from Ehrlich ascites carcinoma cell microsomes by centrifugation in sucrose density gradients as described previously[6,7]. Crude membrane preparations contained all the membrane material floating at a density of 1·19 after centrifugation at 105,000*g* for 18 h in a Spinco *L* ultracentrifuge with a 40 rotor. This preparation contained 0·37 μmole phospholipid phosphorus and 0·02 μmole *N*-acetylneuraminic acid per mole of protein and was free of ribonucleic acid. The microsomes were also further separated into a membrane fraction floating at a density of 1·16 (0·46 μmole lipid phosphorus and 0·028 μmole *N*-acetylneuraminic acid per mg of protein), a second fraction floating at density 1·16 (0·40 μmole lipid phosphorus and 0·017 μmole *N*-acetylneuraminic acid per mg of protein) and material sedimenting into a pellet at density 1·17, containing the ribosomes and 'rough' membranes.

Prior to immunization the various fractions were re-sedimented in 0·25 M sucrose, washed once in 0·15 M sodium chloride and suspended in 0·15 M sodium chloride for immunization.

Adult New Zealand white rabbits weighing about 2 kg were used for immunization purposes. A series of three intravenous injections of antigen was given. Each injection consisted of 0·5 ml. of a saline suspension of membrane material containing 415 μg protein per ml. (66·5 μg protein nitrogen). Immunizations were given on days 0, 2, and 4. Serum samples were collected prior to the first immunization, after the end of the first week (day 7–9) and after the end of the second week (day 14–16) in most instances. Blood samples were allowed to clot at 20° C for 2 h. The serum was removed from the erythrocytes, heated at 56° C for 30 min to inactivate complement, and stored at −40° C until used.

Antibodies capable of agglutinating Ehrlich ascites carcinoma cells were present in the sera collected within 7 days after initiation of immunization with crude membrane material. Sera collected after 12 days contained agglutinating antibodies detectable in dilutions of up to 1 to 2,400 using 4×10^5 cells per ml. in the assay system. No agglutination was observed after 1 h at 4° but agglutination was readily apparent within 15 min at 37°. Neither complement nor divalent cations were necessary for agglutination. Pre-immunization sera did not cause any agglutination reaction. As indicated by an experiment where the antiserum was absorbed with 20 cell equivalents of various cell particulates (Table 1), there is little cross-reaction with nuclei, mitochondria and lysosomes.

Table 1

Anti-membrane serum absorbed with	Agglutinin titre
Microsomes	<1 : 100
Lysosomes	1 : 750
Mitochondria	1 : 2,000
Nuclei	1 : 2,000
Unabsorbed	1 : 2,400

Similar studies using the various microsome subfractions described indicate that the antigen responsible for the production of the agglutinating antibody is associated with the membranes of lowest density.

All sera were examined for their activity in the immune adherence phenomenon[8,9] when reacted with fresh Ehrlich ascites carcinoma cells, guinea pig complement, and human erythrocytes. Antibodies capable of reacting with whole Ehrlich ascites carcinoma cells were found in all serum samples obtained after immunization, and were absent from all the control (pre-immune) sera. Using hæmagglutination patterns as an end-point serum dilutions up to 1 : 16,000 were found to give a significant reaction in the immune adherence system. Examination of the immune adherence mixtures by phase contrast microscopy revealed erythrocytes firmly adherent to the surface of all Ehrlich ascites carcinoma cells (Fig. 1).

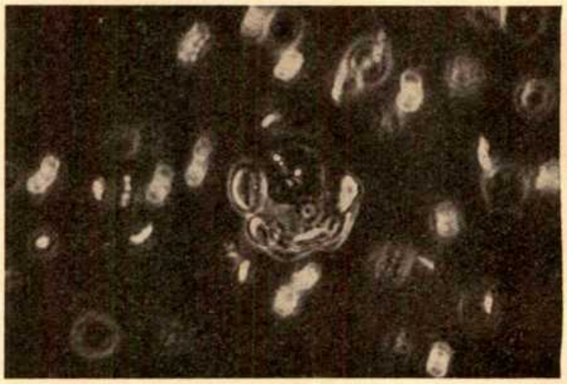

Fig. 1. Immune adherence of human erythrocytes to Ehrlich ascites carcinoma cell in presence of rabbit antiserum against EAT microsomal membrane and complement. The characteristic vacuoles of the EAT cell are visible overlying the nucleus. The non-adherent erythrocytes have been set in motion by tapping on the coverslip. (Phase contrast × *c*. 900)

The action of complement on antibody sensitized cells was accompanied by marked swelling of the cells, presumably due to membrane alterations which were reflected biochemically by: (1) leakage of RNA and soluble nucleotides from the cells; (2) increased protein leakage from the cells; (3) an increase in the ATPase activity of antibody-treated cells which is due to the development of ATP permeability of the cell membrane, thus allowing externally added ATP to reach intracellular loci of ATPase activity such as mitochondria. This alteration of ATPase activity of antibody-sensitized Ehrlich ascites carcinoma cells by complement provides a very sensitive biochemical assay for the presence of cytotoxic antibodies. Pre-immunization sera, with or without complement, showed no cytotoxic effect. Neither the agglutinating nor the cytotoxic antibodies were inhibited by the presence of a 100-fold excess of *N*-acetylneuraminic acid, which is known to be located on the surface of these cells and in the microsomal membranes.

The foregoing results support previous work on the antigenicity of microsomal membranes[10], and provide additional evidence for the localization of the surface membrane of Ehrlich ascites carcinoma cells in the microsomal membranes following cell rupture.

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Dialysable Peptide as the Causative Factor in Experimental 'Allergic' Encephalomyelitis

PREPARATIONS of soluble proteins from brain and spinal cord which were used for the production of 'allergic' encephalomyelitis in experimental animals have been described[1-4]. Roboz *et al.*[5] reported a soluble, collagen-like protein apparently of different nature. Others[6-10] have reported on the association of encephalitogenic properties with proteolipids. Although its chemical pathogenesis is unknown, the disease is almost generally accepted to be an immunological reaction and we therefore prefer to describe chemical fractions obtainable from nervous tissue as 'encephalomyelitis-promoting' (EP) rather than 'encephalitogenic'.

Experiments were undertaken in this Department to determine whether the EP factor, which seemed from experiments on soluble proteins to be a histone, might be present also in proteolipid and other extractives known to be encephalitogenic in combination with Freund's adjuvants. We have found that a histone preparation from bovine cord contained much protein-bound, peptide-rich material which had most, if not all, of the EP activity of the original preparation when separated from the histones by dialysis at *p*H 9. Freed of this material, the basic proteins lost all, or nearly all, EP activity but were shown by zone electrophoresis still to give the same qualitative protein pattern as before removal of the active peptides. The dialysed peptides showed no detectable protein.

Histones with EP activity were also obtained both from the upper aqueous and from the lower chloroform phases yielded in the preparation of proteolipids by the method of Folch and Lees[11], but there was also EP activity in a protein-free fraction from the aqueous phase. Moreover, the paper partition chromatographic pattern of alkaline dialysate of proteolipid (ninhydrin coloration) was found to be identical with that of the histone dialysate from potassium chloride extracts. An alkaline dialysate of an emulsion of the solids of bovine spinal cord soluble in light petroleum ether also contained peptides although its EP activity and that of peptide associated with proteolipid are only now under test.

The soluble proteins were prepared from partially defatted spinal cord by extraction first with 10 per cent potassium chloride and precipitation with fully saturated ammonium sulphate followed by dialysis at neutral *p*H and freeze-drying of the non-dialysable residue. This material had strong EP activity, 50γ injected intradermally in Freund's adjuvants producing paralysis in 8 of 10 guinea pigs. Electrophoretically, 10 protein fractions were present including at least two major histone fractions. The non-histone fractions were confirmed to be inactive but, surprisingly, the purified histones from this material were almost inactive after conventional separations either by ion-exchange celluloses or sucrose density-gradient electrophoresis. Both types of method had involved dialysis of the protein fractions at *p*H 8–9. In several trials on the effects of sucrose and alkali, individually and combined, on soluble protein samples with known EP activity, it was found that mere dialysis of the EP materials in an alkaline medium (*p*H 8·6) was enough to release most of the EP activity into the dialysate, although, qualitatively, the electrophoretic pattern of the non-dialysable residue remained unaltered.

Further support for the foregoing findings derives from the preparation of highly active material obtainable by 10 per cent potassium chloride extraction at *p*H 2 (10 g from 2 kg fresh cord). Electrophoretically, both on cellulose acetate strips and in starch gel it yielded good separation of seven basic proteins and the extract was free of acid proteins. Its EP activity was high (8 of 10 animals were paralysed) at 10-γ dose-levels. On dialysis at *p*H 9, from 1 g of these histones 675 mg of solids passed into the dialysate, about one-fifth being peptide, which could be partially separated from accompanying amino-acids and sugars by passage through 'Sephadex *G*–25'. This dialysate showed high EP activity even at 5-γ doses while the non-dialysable solids (proteins) even at dose-levels of 1 mg were only slightly active.

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Galactose Metabolism : Phenotypic Differences among Tissues of a Patient with Congenital Galactosæmia

It has recently been reported that two patients who had the characteristic syndrome of congenital galactosæmia as infants, and who lack gal-1-P uridyl transferase in their red blood cells, are nevertheless able to oxidize galactose-1-^{14}C to $^{14}CO_2$ *in vivo* in a near-normal fashion[1]. It is therefore apparent that some tissues within these individuals must be capable of metabolizing galactose. Further studies on one of these subjects (T. B.), a 30-year-old Negro who was the first case of galactosæmia reported in the American literature[2], have inquired into possible tissue differences with respect to the presence or absence of the metabolic derangement typical of galactosæmia.

Five different tissues derived from T. B. have been examined *in vitro* for their ability to metabolize galactose. The results obtained with white blood cells[3] and skin cells in tissue culture[4] have been reported previously. These results, together with those obtained for hæmolysates, intestinal mucosa and liver, are presented in Table 1. Of the tissues studied, only liver compares favourably with the normal in its ability to oxidize galactose-1-^{14}C to $^{14}CO_2$. Thus it would appear that this case represents a situation in which marked differences exist among tissues of a galactosæmic subject with respect to their galactose-oxidizing capacity relative to that of their normal counterparts. The implications of these observations in relation to galactose metabolism *in vivo* are not immediately apparent and deserve further study.

The pathway by which the liver of this galactosæmic patient is able to oxidize galactose deserves further consideration. Anderson *et al.*[5] reported that liver obtained by needle biopsy from the same patient had about 5 per cent of the normal ability to incorporate ^{14}C-galactose-1-phosphate into uridine nucleotide in the presence of uridine diphosphoglucose and uridine triphosphate, a finding which indicated a marked reduction of transferase activity as well as of any related alternative pathway. However, the subject was capable of forming glucosiduronic acid from galactose at a normal rate[6], an observation which is consistent with the present results obtained on the patient's liver and the *in vivo* results reported previously[1]. These findings can be explained on the basis that either the low activity detected by Anderson *et al.* is sufficient to account for the results of the *in vivo* and present *in vitro* studies, or that the pathway of galactose metabolism in the liver of this subject differs from that of the normal.

Table 1. OXIDATION OF GALACTOSE-1-^{14}C TO $^{14}CO_2$ BY VARIOUS TISSUES

Tissue	Subjects		B/A
	Subjects Non-galactosæmic (*A*)	Subject T. B. (*B*)	
	mμM $^{14}CO_2$/10^7 cells/h		
White blood cells*	2·0 ± 0·2† (6)	0·09	0·045
Skin‡	1·6 (3) [0·7–2·8]	0	0
	mμM $^{14}CO_2$/ml. packed R.B.C./h		
Hæmolysate§	5·9 (1)	0	0
	mμM $^{14}CO_2$/100 mg tissue/h		
Intestinal mucosa¶	60 (1) 89 (1)	1·7	0·023
Liver‖	1·5 (1) 0·9 (1)	0·9	0·75

Nos. in parentheses refer to the No. of subjects.

* Data calculated from the paper by Weinberg (ref. 3).

† *S.E.*

‡ Data calculated from the report of Krooth and Weinberg (ref. 4).

§ A 20 per cent hæmolysate was prepared by freezing and thawing R.B.C. suspended in Krebs phosphate buffer, *p*H 7·4, after remova of W.B.C. by centrifugation. The incubation medium contained 2 ml. of hæmolysate, 0·1 μc. galactose-1-^{14}C (4·72 μc./mg), 1·1 μM ATP and 0·54 μM TPN. $^{14}CO_2$ was collected as described previously (ref. 7).

¶ Suction biopsy of the small intestine mucosa was performed perorally with the instrument described by Brandborg *et al.* (ref. 8), and comparisons between normal and T. B. were made using tissue from the upper jejunum. 5 mg pieces of tissue were incubated in 3 ml. of Krebs bicarbonate buffer, *p*H 7·4, containing 0·15 μc. of radioactive galactose. Each value represents the average of duplicate determinations.

‖ Liver biopsy of T. B. was performed with a Menghini needle. The 15 mg of tissue were cut into four segments and incubated as described for intestinal mucosa. One piece of tissue was boiled for 2 min before incubation and served as a blank. Liver biopsy of controls was obtained at surgery from two individuals suffering from gall bladder disease. The surgical specimen was cut into 5–10 mg segments for incubation. Incubations were performed in triplicate.

The finding of definite phenotypic differences among several tissues from a single galactosæmic patient when studied for their ability to oxidize galactose *in vitro* indicates that galactosæmia is a more complex disease entity than has been appreciated hitherto.

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Metaplasia of Aortic Connective Tissue to Cartilage and Bone induced by the Intravenous Injection of Papain

The effect of intravenous papain on the cartilage matrix has been reported by L. Thomas[1]. Recently we have been investigating the acute lipæmia induced by the injection of papain solution in adult rabbits[2]. At the end of each experiment, the animals were killed by air embolism and autopsies were performed. Several of these experimental animals showed lesions in the ascending aorta and the arch, and to a lesser extent in the upper portion of the descending aorta. This communication deals with the description of these lesions and of the conditions under which they were developed.

Grossly, the lesions appeared well delineated by a halo of raised tissue with irregular surface and edges

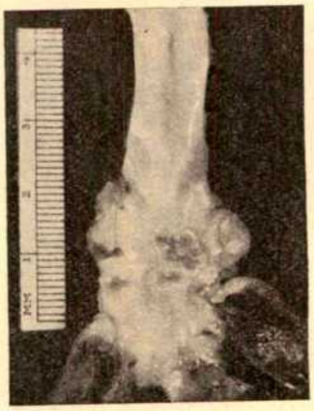


Fig. 1. Aorta of rabbit No. 89, male, 5,670 g, injected once with 15 mg papain/kg body-weight

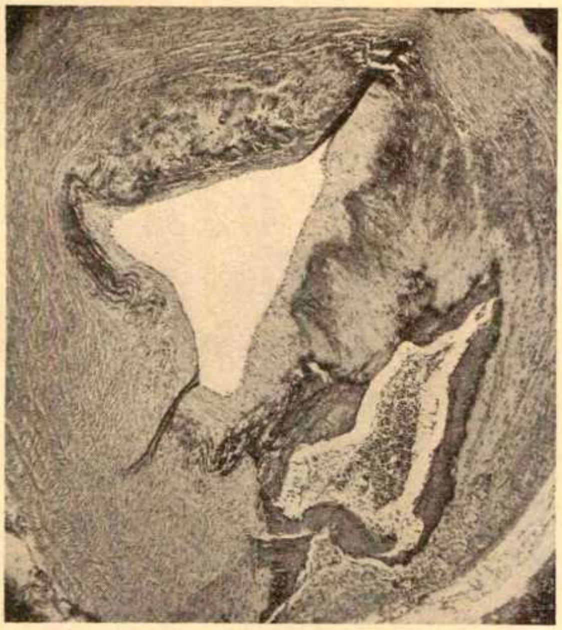
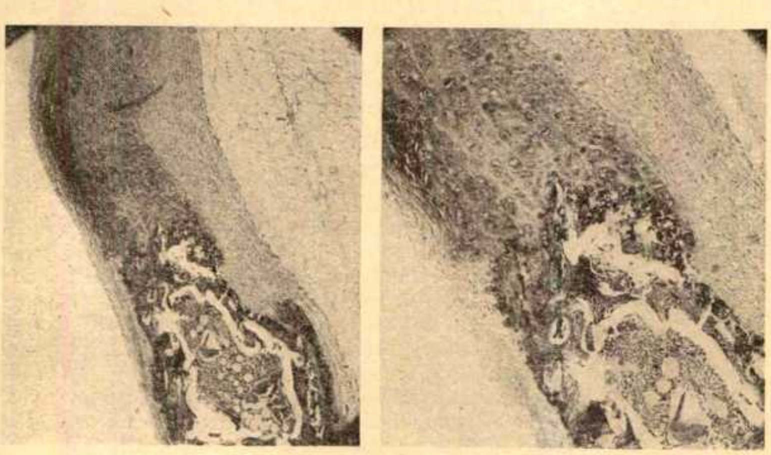


Fig. 2. Photomicrograph of a lesion in the innominate artery of Rabbit No. 137, male, 4,550 g, injected once with 7·5 mg papain/kg body-weight (hæmatoxylin and eosin; × c. 45)



a *b*

Fig. 3. *a*, Photomicrograph of a lesion shown on Fig. 1 (hæmatoxylin and eosin; × *c*. 53); *b*, part of *a* (× *c*. 80)

(Fig. 1). The lesions were hard and presented considerable resistance to the cutting instrument. Their diameters varied from 8 to 12 mm each. No constriction of the lumen was evident, more than that produced by the protruding tissues. In some cases the lesions extended into the major vessels arising from the aortic arch (Fig. 2).

The changes involved primarily the media, but at their maximum point they also involved the entire wall (Fig. 3). Toward the periphery of the lesion, there appeared increased deposition of material, positive to periodic acid–Schiff reaction, presumably polysaccharides in nature. The same material was intensely stained with the usual hæmatoxylin–eosin. In this area, the fibrocytes were quite prominent, and some fibroblastic activity was evident. Many of the fibroblasts were surrounded by a halo of polysaccharides. Further centrally occasional chondrocytes appeared in an environment intensely positive to periodic acid–Schiff. Eventually, the chondrocytes became so numerous as to form true cartilage. In this area calcium deposits appeared which eventually proceeded to form bone with mature bone trabeculæ. Here, the entire thickness of the aorta was replaced by the bone and cartilage and these were covered by a thin layer of connective tissue and endothelial cells. The centre of the ossified area contained active bone marrow. The process thus described is slowly reversed on the other side of the lesion until normal aortic tissue becomes again evident.

All animals in these experiments weighed 2,500–5,000 g. They were maintained on laboratory rabbit purina chow *ad lib*. One single injection of purified (but not crystalline) papain was given intravenously. The papain was prepared as recommended by L. Thomas[1]. The dose varied from 7 to 15 mg/kg body-weight. Usually solutions of 2–3 per cent were used for injection. The animals were killed 3–6 weeks after the injection of papain. The lesions described here appeared in animals killed at least 3 weeks after the injection of papain. Smaller lesions without cartilage and bone formation and without bone marrow were quite regularly seen as early as one week after receiving one injection of 7–10 mg/papain/kg body-weight.

The mechanism of metaplasia of connective tissue elements in adult life towards cartilage and bone has been speculated on by pathologists and histologists, but without concrete evidence. As a likely explanation for this metaplasia I would like to refer to the effect of papain on the cartilage matrix. I have shown[3] that the injected papain induces hydrolysis of the cartilage matrix and that the products of such hydrolysis, namely, sulphated polysaccharides, circulate in the blood in high concentrations and become deposited in various organs[4].

It would seem reasonable that one such site would be the aorta, where polysaccharides are evident in excess in a variety of disorders. How the metaplasia arises from this deposition is only a matter of speculation. That papain as such has a direct action on the aorta seems rather unlikely in view of the conclusive evidence that the intravenously injected papain is rapidly inactivated and is completely destroyed within 10–15 min after it is injected[1]. Direct trauma to or of the aorta could be of significance for the development of these lesions even though there was no evidence of trauma in these experimental animals.

Finally, these lesions offer a possible tool for the study of connective tissue metaplasia either in experimental animals or in cases of delayed callus formation in fractures.

This work was supported by United States Public Health Service grants *H*-5647 and *H*-4613.

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BIOLOGY

Inhibition of Fruit-Bud Formation in Apple with Gibberellic Acid

Bradley and Crane[1] have reported inhibition of fruit bud formation by gibberellin in apricot, almond, cherry and plum; Hull and Lewis[2] in peach and Griggs and Iwakiri[3] in pears. Gibberellic acid also inhibits flower formation in *Fragaria* spp.[4] and *Kalanchoe blossfeldiana*[5]. No report of inhibition of fruit buds in apples has been found, although Hull and Lewis[2] obtained no effect on flowering or vegetative growth from a single application of a spray of 100 p.p.m. of gibberellin.

An experiment at Mylnefield has shown that gibberellic acid can cause inhibition of fruit-bud formation in apples without affecting bursting of buds in the following spring. In the summer of 1960 branches of six varieties of apples were selected, and treatments were allocated at random so that each treatment was applied to two branches on each variety. Drenching sprays of gibberellic acid (*YF* liquid formulation supplied by Plant Protection, Ltd.) at either 10 or 50 p.p.m. were applied to the leaves and stems of the branches at approximately weekly intervals from May 31 until August 29, making 14 applications in all. Control branches were not sprayed. Counts of buds were made in spring 1961 and the records are presented in Table 1. Some branches were broken in winter and this accounts for the missing data. Taking the six varieties and both levels of treatment together, the percentage of spurs bearing blossom clusters was reduced from 40 per cent on unsprayed branches to 14·7 per cent on sprayed branches. There was also a pronounced inhibition of fruit bud formation on the new wood of the variety Laxton's Superb.

Table 1. EFFECT OF GIBBERELLIC ACID (GA) ON FRUIT-BUD FORMATION IN APPLE

Variety	No GA		10 p.p.m. GA		50 p.p.m. GA	
	No. of fruiting spurs or buds	No. of vegetative spurs or buds	No. of fruiting spurs or buds	No. of vegetative spurs or buds	No. of fruiting spurs or buds	No. of vegetative spurs or buds
Benediction (spurs)	12	38	1	27	0	27
Delicious (spurs)	9	27	—	—	1	22
Madresfield Court (spurs)	13	8	7	8	0	11
McIntosh Red (spurs)	4	9	—	—	1	16
Lord Derby (spurs)	11	12	0	31	0	20
Laxton's Superb (spurs)	15	2	5	2	14	18
Laxton's Superb (buds on new wood)	42	1	4	57	0	47
Total (spurs and buds)	106	97	17	125	16	161
Per cent fruiting	52·2		12·0		9·8	

The same rates of application on plums caused either death (50 p.p.m.) or a severe delay in bud break (10 p.p.m.) as has been reported before[1].

Microscopic examination of buds of *Prunus* by Bradley and Crane[1] showed that growth of both fruit and vegetative buds was retarded by gibberellin. However, because growth of fruit buds was retarded at lower levels of gibberellin than were required to retard vegetative buds they suggested that gibberellins may be specifically blocking floral initiation as well as generally retarding cell division at higher concentrations.

Apple buds were not examined microscopically at Mylnefield, but the fact that there was no delay in bud break in spring suggests that any check to bud growth that there may have been during treatment was not severe. Nevertheless an indirect morphological effect rather than specific inhibition of flower induction remains possible, especially as Fulford[6] found that small differences in numbers of nodes in apple buds appeared to determine the presence or absence of flowers.

It is possible that sprays of gibberellic acid could be used to restrict flower bud formation in the non-fruiting year of biennial bearing apple trees.

The assistance of Mrs. R. N. Smith is gratefully acknowledged.

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A Species of *Tetrahymena* from the British Garden Slug *Milax budapestensis*

HISTOPHAGOUS species of the ubiquitous holotrich genus *Tetrahymena* (Ciliata: Hymenostomatida) have been reported from the body spaces and tissues of a variety of vertebrate and invertebrate organisms[1]. Slugs and snails have been named as hosts in a dozen widely scattered and usually very brief reports published during the past 30 years, the molluscs having been collected in South Africa, the Congo, Poland, Nova Scotia, and from sites in California, Oregon, Virginia, and Illinois in the U.S.A. In work by one of us (A. C. S.) on osmotic relations in gastropods, a small (about 40μ long) ciliated protozoon was detected in a blood sample withdrawn from one of the common British garden slugs, *Milax budapestensis* (Hazay, 1881), collected in the University of Exeter greenhouse. Subsequent examination of additional specimens of this species of keeled slug revealed similar infections; the ciliates were apparently present throughout the alimentary tract and in the liver, although not occurring in great numbers. Study of the protozoon by silver impregnation[2] and other techniques made possible its identification as a strain of *Tetrahymena limacis* (Warren, 1932) Kozloff, 1946, in agreement with the careful redescriptions of this species published by Kozloff[3-5].

The finding of *T. limacis* in British slugs extends its geographical host range, which one may now suspect is truly world-wide. Of more significance, however, is the relationship of this species of *Tetrahymena* to its host, especially in comparison with other tetrahymenid ciliates which also have been said to exhibit facultative parasitism. Of the 13 species of *Tetrahymena* described so far, 8 have been implicated in some degree of parasitic or commensalistic association with vertebrate or invertebrate hosts; only 5 have been found solely in free-living (fresh-water) habitats. In the total range, from a completely non-parasitic existence or merely occasional or accidental parasitism to incipient or possibly true obligate parasitism, *T. limacis* appears to fall near the latter extreme. The whole series has very recently been considered of possible evolutionary significance in the origin of the endoparasitic habit from a free-living ancestry[6].

T. limacis has no encysted stage in its life-cycle and is seldom found outside its host, although it can be cultured with facility in simple media in the laboratory. It has a mouth and an active contractile vacuole, but this also holds for numerous species of ciliates long known to be obligate endoparasites. Experimental work[4,5] suggests that the organism enters a fresh host in nature via fæcal contamination in the host's edaphic environment. Infection is not fatal and the number of ciliates per host is never high: these points particularly stand in contrast with the situation observed in such species as *T. chironomi*, *T. corlissi*, and *T. stegomyiae*[1].

All the species of *Tetrahymena* involved in any degree of parasitism *sensu lato* are histophagous, tissue-eating, ciliates. One species, *T. rostrata* is, in addition, an edaphic form when found free of its hosts, which, like *T. limacis*, include snails and slugs; but it is not difficult to make a taxonomic distinction between these two species on both life-cycle and morphological grounds. Whether *T. limacis*, unlike most others in the series alluded to here, may more properly be considered facultatively free-living, thus being an obligate rather than facultative parasite of its host, is an important and intriguing question which both demands and warrants further investigation.

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Furunculosis in Salmon Kelts

DURING the spawning season of 1960–61 investigations were initiated in Ireland into the occurrence of furunculosis among spawning and spawned salmon. In that season 159 kelts were examined from four rivers; only one fish gave a positive reaction[1]. These investigations were continued during the spawning season of 1961–62 when 49 kelts were examined. All fish were dead when taken from the water. The distribution and results are given in Table 1.

Table 1

River	No. of kelts examined		Hatchery or naturally spawned	Result of test	Period
	Male	Female			
River Owenea (Co. Donegal)	25	11	All hatchery stripped	All negative	January 9–February 7, 1962
River Erne (Co. Donegal)	4	2	All naturally spawned	All negative	March 15–May 29, 1962
River Blackwater (Co. Waterford)	—	1	Hatchery stripped	Positive	December 6, 1961
River Boyne (Co. Meath)	1	—	Naturally spawned	Positive	December 3, 1961
River Lee (Co. Cork)	2	—	Hatchery stripped	Negative	January 23, 1962
River Moy (Co. Mayo)	3	—	Naturally spawned	1 positive	March 1–3, 1962
Total	35	14		46 negative 3 positive	

As can be seen, three fish (6·1 per cent) gave a positive reaction.

I thank Mr. E. Murphy, of the Veterinary College of Ireland, Ballsbridge, Dublin, who undertook the bacteriological examination of these fish.

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Development of a DDT-Tolerant Strain of Laboratory Mice

A COLONY of mice selected by breeding the survivors of DDT treatments showed a definite tolerance to the insecticide by the ninth generation. The level of this tolerance was assessed by comparing the LD_{50} values with those of a control colony started from the same parent colony and reared under similar conditions.

In the summer of 1959, a colony of white laboratory mice, *Mus musculus* L., was divided into two parts: one part was maintained as a control; in the other, each generation was subjected to treatment with DDT. DDT dissolved in sesame oil was administered by intraperitoneal injection at the rate of 0·01 c.c. solution/g mouse weight and expressed in mg of DDT/kg mouse weight. All treatments to select tolerant mice were made at the age of four weeks. In order to reduce variation in weight between individuals in different litters, all litters were culled back to nine individuals per doe when the young were a week old. The doe was removed from the litter at approximately 3½ weeks. After treatment, mice were kept until eleven weeks old, at which time they were mated.

The original LD_{50} value for mice from the control colony and parent colony at four weeks of age was estimated to be approximately 500 mg/kg. At generation four, the LD_{50} value for the control colony males was estimated at 500 mg/kg and for the females at 550 mg/kg, whereas the colony under DDT pressure had an estimated LD_{50} of 650 mg/kg for males and a proportionately higher value for females. These approximate LD_{50} values were based on selected arbitrary dosages decided on after numerous preliminary tests.

At the ninth generation, using the method of moving averages and tables published by Weil[1], the LD_{50} value and confidence interval for the control colony males was estimated as 495–541–590 mg/kg and for females as 526–567–612 mg/kg. The value for the colony under DDT pressure was 905–944–983 mg/kg.

Observations were also made on the reaction of individuals to the treatment. Members from the colony under DDT pressure reacted much more slowly to the effects of the treatment, and the symptoms caused by the insecticide were much less noticeable until death was imminent.

The foregoing observations indicate that a change is occurring in the colony of mice in which each generation is subjected to DDT pressure. Whether this change may be classified as a true resistance to the insecticide comparable with that found in many insect species is uncertain. With insects, the change to resistance is frequently slight for the first few generations and then increases rapidly. The tolerance-range of the mouse to DDT is very narrow. The dosage to produce an LD_{50} for the tolerant (selected) colony is, at the ninth generation, only 1·7 times higher than that of the LD_{50} value for the control colony. This increase of the dose by 1·7 times is, however, sufficient to kill all the control colony animals. This small difference is therefore a very significant one.

We think that with the development of this DDT-tolerant colony of mice, a useful biological tool has been produced for use in further study of the complex subject of chemical tolerance.

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Spurious Negative Correlation between Age and Weight per Day of Age in Beef Calves

Weight per day of age is commonly used to assess growth in beef calves. It can be shown that there is a very high correlation between weight per day of age and gain per day (Fig. 1), and since the former is a simpler calculation, and does not require a birth weight, it appears on the surface to be a practical substitute for the latter.

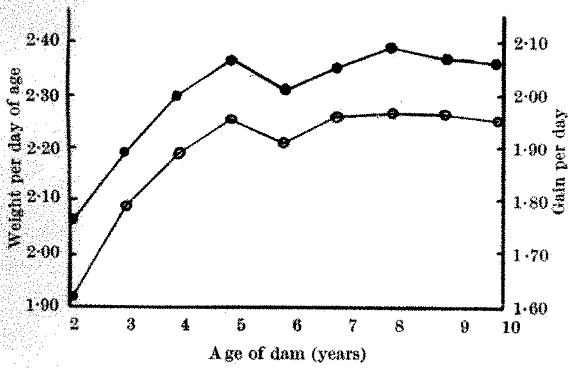

Fig. 1. Correlated change in weight per day of age and gain per day with increasing age of dam in pre-weaning growth period of beef calves. ●, Weight per day of age (lb.); ○, gain per day (lb.)

In 1958 a Canadian technical committee, considering procedures for collecting and summarizing data on beef cattle performance, actually recommended that birth weight be considered as zero in the calculation of growth-rates. This recommendation in effect would substitute weight per day of age for gain per day. Later, in an investigation to determine the magnitude of age of dam effects on pre-weaning growth of beef calves in Canada, the spurious nature of the relationship between weight per day of age and age of calf became clear.

Let us assume that two calves each have a birth weight of 70 lb. One calf is weighed at 120 days of age and is 310 lb., the other is weighed at 250 days of age and weighs 570 lb.

$$\text{Calf 1}\begin{cases}\text{gain per day} = \dfrac{310-70}{120} = 2\cdot00 \text{ lb.}\\[2ex] \text{weight per day of age} = \dfrac{310}{120} = 2\cdot58 \text{ lb.}\end{cases}$$

$$\text{Calf 2}\begin{cases}\text{gain per day} = \dfrac{570-70}{250} = 2\cdot00 \text{ lb.}\\[2ex] \text{weight per day of age} = \dfrac{570}{250} = 2\cdot28 \text{ lb.}\end{cases}$$

These calves are exactly equal in their rates of gain; but calf 1 weighs 0·30 lb. per day more than calf 2. This difference is simply because the birth weight of 70 lb. is spread over only 120 days in calf 1 and therefore contributes 0·58 lb. per day, while in calf 2 it is spread over 250 days and contributes only 0·28 lb. per day. The effect of the 70 lb. of birth weight follows a curve of diminishing effect as the age increases until at 700 days of age it contributes only one-tenth of a pound per day of age and is of no particular practical significance. Even in the post-weaning period, however, it is clear that weight per day of age is not a valid measure of growth.

The spurious negative correlation between weight per day of age and age of calf is clearly evident in data gathered on Hereford calves in the Canadian record of performance programme and is illustrated by Fig. 2.

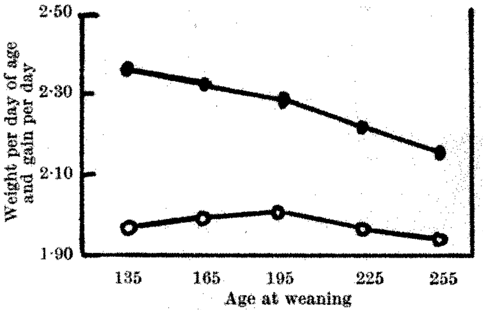

Fig. 2. Relationship between two methods of expressing pre-weaning growth in beef calves as a function of age at weaning. ●, Weight per day of age; ○, gain per day

The lines in Fig. 2 represent the two ways of expressing average growth of the same calves weaned at each age. A total of 5,373 calves are represented. Each sex has been given equal weight in determining each point in Fig. 2. Weight per day of age shows a fairly consistent drop as weaning ages increase while gain per day remains relatively constant.

The negative correlation is masked in Fig. 1 since, within each age of dam class, the average weaning age of the calves is the same within the sampling error and except for any association there may be between weaning age and age of dam.

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ENTOMOLOGY

Control of the Ventral Diaphragm in an Insect

The control and origin of heart muscle activity present an unresolved problem in many groups of animals. The hearts of vertebrates and many invertebrates contract myogenically, but rate of beat is controlled by nerve fibres associated with the central nervous system. In the arthropod groups neurogenic hearts have been described in the crustacea and *Limulus*[1], and less completely in some insects[2]; but as pointed out by Beard[3] no general pattern of control and origin of heart-beat has emerged in insects.

Many insects possess in addition to the dorsal heart pumping blood anteriorly a large ventral accessory organ driving the hæmolymph posteriorly and dorsally. In the locust *Schistocerca* this ventral diaphragm extends from the metathorax to near the

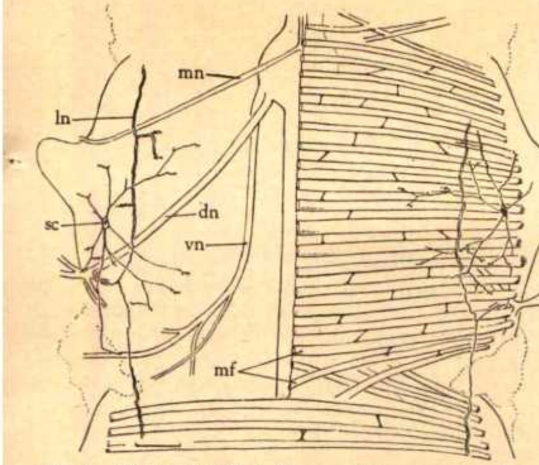

Fig. 1. Greatly simplified diagram of the sternal region of an abdominal segment as seen from above. The ventral diaphragm has been removed on the left side. *dn*, Dorsal nerve; *ln*, lateral nerve; *mf*, muscle fibres; *mn*, median nerve; *sc*, sense cell; *vn*, ventral nerve. Scale, 300μ

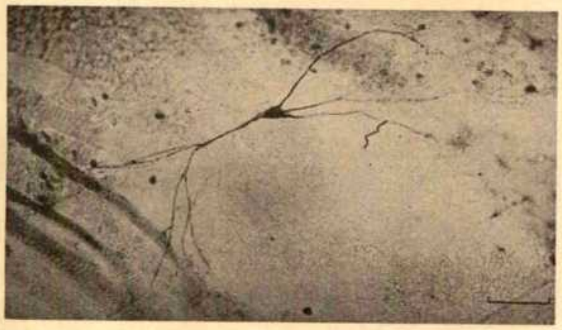

Fig. 2. Sense cell showing processes passing to muscle fibres. Axon process is the uppermost of the left-hand group of processes. Scale, 100μ. *Photograph by D. Warhurst*

extremity of the abdomen, and consists of transverse muscle fibres lying on a connective tissue membrane, stretched across the sternal region of the abdomen. The transverse muscle fibres (Fig. 1, *mf*) are connected by fine anastomoses, and are grouped in fields of 30–40 fibres corresponding to the skeletal segments. At the boundary between these segmental muscle fields oblique muscles overlap the two fields. Each abdominal muscle field contains a pair of sense cells (Fig. 1, *sc*, and Fig. 2) with dendritic endings on the muscle fibres, of a generally similar morphological type to the stretch receptors described by Orlov[4], and Lowenstein and Finlayson[5]. The sense cell terminals end on 15–20 muscle fibres, and there are usually at least three endings on adjacent nerve trunks. The dendrites have flattened leaflet endings (varicose or muff type figured by Orlov). The axon, which is fine and difficult to trace, runs some way posteriorly to join the segmental ventral nerve (Fig. 1, *vn*). The motor fibres have their origin from the segmental dorsal nerve (Fig. 1, *dn*) at the intersegmental level, forming a lateral nerve (Fig. 1, *ln*); this is similar to the paramedian nerve of Schmitt[6]. At least two fibres can be seen in the trunk at most levels, and many muscle fibres can be seen to have endplates contributed to by two fibres. Some at least of these fibres are anterior or posterior rami of single fibres in the dorsal segmental nerves.

The median nerve (Fig. 1, *mn*), which has a spiracular function[7,8], has a connective tissue junction with the lateral nerve, but there appears to be no exchange of fibres.

In the active diaphragm waves of contraction pass posteriorly at the rate of 8–12 waves/min at 18° C; this is normally between a quarter and a fifth of the frequency of beat of the ventilatory muscles in the same preparation. Reversal of beat, a characteristic of invertebrate hearts, sometimes occurs. If the nerve cord is removed, or all the segmental nerves are severed, two changes usually occur in the active diaphragm: first, the rate of beat is about doubled to 20–25/min; secondly, while the segmental muscle fields still contract in series their co-ordination is less regular.

Furthermore, in the intact preparation, light mechanical stimulation of the outer surface of a segment causes rapid twitching of the diaphragmal muscle field of this segment; this is abolished by section of the segmental nerves.

In rather quiescent intact preparations that have been lightly stained the sense cells can be picked out with forceps, and slightly reduced activity is then observable, but this may be due to muscle damage. On the other hand, section of the ventral nerves alone may lead to increased activity and loss of co-ordination.

Division of the active denervated diaphragm across segmental boundaries destroys co-ordination between adjoining fields, which may depend on tension in the oblique muscle fibres. The denervated diaphragm is responsive to direct mechanical stimulation, the fibres going into a state of contracture which may take 10 sec to die away; and to electrical stimulation. Bursts of 25 c/s sawtooth pulses at 0·5-sec intervals will initiate fast rhythmical activity of a similar frequency; much lower or higher frequencies are not effective.

The pattern of afferent and efferent nerve impulses in the segmental nerves is dominated by the rhythm of tracheal ventilation as noted by Hughes[9]. In the motor pathways of the dorsal nerves no very obvious activity is correlated with the slow rhythm of the diaphragm; however, a recording was made from the very delicate lateral nerve (Fig. 1, *ln*) in a rather quiescent preparation (Fig. 3) and this showed a regular discharge in one or perhaps two fibres at about 1/5th-sec intervals; the small value of the spikes, − 0·2 mV, may be partly due to poor contact with the preparation. Records from the ventral nerve cut at the ganglion do show a small group of impulses occurring at intervals of 4 or 5 sec, not present after section of the preparation in the region of the sense cell axon, but it is hoped to make records from the axon itself. One of the main difficulties in making recordings from these preparations is presented by the liveliness of the active preparation.

The evidence summarized suggests that muscular activity in the diaphragm is myogenic in origin and propagated largely by tension. Inhibitory fibres from the ganglia slow the rate of beat, and there are also fast fibres associated with a twitch response. Evidence for a general accelerator fibre has not been

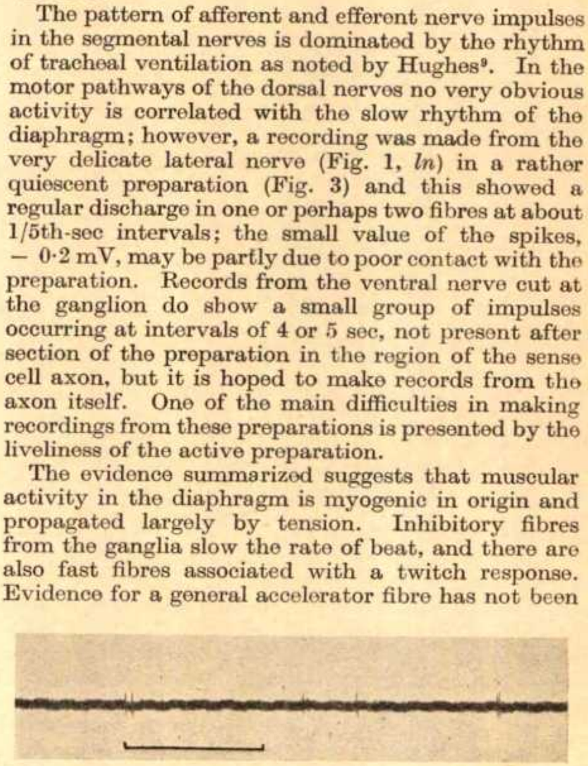

Fig. 3. Recording from the lateral nerve at high amplification. Scale, 0·2 sec

found so far. The function of the sense cell is probably in association with inhibition.

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A Case of Dominant Dieldrin Resistance in *Anopheles gambiae* Giles

In the course of field investigations by one of us (J.H.) in the village of Gaoulou near Sassandra, Ivory Coast, a population of *Anopheles gambiae* Giles was found which showed similar low mortalities when exposed to 0·4 and 4·0 per cent dieldrin in 'Risella' oil for 1 h, and high but incomplete mortality when exposed to 4·0 per cent DDT in 'Risella' oil for 1 and 2 h. Detailed results of these exposures as well as results of exposures to the organophosphorus insecticides, malathion and 'Baytex', are given in Table 1.

Table 1. Insecticide Susceptibility of the Gaoulou-Sassandra Population of *Anopheles gambiae*. (Tests made in Africa)

Insecticide (%)	No. of females tested	Exposure period (h)	No. of dead females	Mortality (%)
DDT	Wild caught gravid and freshly fed females			
0	120	1 and 4	5	4
0·5	131	1	13	10
1	156	1	58	37
2	184	1	128	70
4	162	1	147	91
4	108	2	104	96
4	116	4	116	100
Dieldrin	Wild caught gravid and freshly fed females			
0	21	1	1	4
0·4	112	1	13	12
4	116	1	10	9
Malathion	Laboratory bred females, freshly fed			
0	100	1	3	3
0·4	81	1	2	2
0·8	101	1	18	18
1·6	99	1	86	87
3·2	79	1	79	100
Baytex	Laboratory bred females, freshly fed			
0	Same controls used as for malathion			
0·2	38	1	1	3
0·4	99	1	21	21
0·8	118	1	97	82
1·6	80	1	80	100

In May 1962 eggs from this population were dispatched to the Ross Institute in London, where a colony was successfully established. The results of testing the susceptibility of adults reared from these eggs and of adults of the first laboratory generation on DDT and dieldrin are given in Table 2. Similar low and almost identical mortalities are shown on 0·4 and 4·0 per cent dieldrin for 1 h, and these are very similar to the mortalities found in the field. On 4·0 per cent DDT for 1 h, however, no survivors were found in London. It is concluded that the increased tolerance to this insecticide in the field is the result of a slight 'vigour tolerance' and not to the presence of individuals specifically resistant to DDT.

Table 2. Insecticide Susceptibility of the Gaoulou-Sassandra Population of *Anopheles gambiae*. (Tests made in London on Laboratory-reared, Unfed Females and Males, 1–4 days old)

Insecticide (%)	No. of mosquitoes tested	Exposure period (h)	No. of dead mosquitoes	Mortality (%)
0	35	1	1	3
0	39	5	0	0
DDT				
4·0	289	1	289	100
Dieldrin				
0·4	97	1	13	13
4·0	104	1	11	11
4·0	137	5	54	39

Females of the Sassandra colony have been crossed with males of an insecticide-susceptible strain of *A. gambiae* from Diggi, Western Sokoto, Northern Nigeria, and with a homozygous dieldrin-resistant strain from Kano, Northern Nigeria. These belong to the two partially incompatible fresh-water forms of *A. gambiae* described by Davidson and Jackson[1]—Diggi to the group *A* and Kano to the group *B*. F_1 males of the Kano × Sassandra cross were sterile while those from the Diggi × Sassandra cross were normal. The Sassandra strain thus belongs to group *A*.

The results of testing the F_1 and F_2 generations of the Diggi × Sassandra cross on 0·4 and 4·0 per cent dieldrin for 1 h are given in Table 3. The F_1 adults show no mortality on either dosage, while the F_2 adults show a similar mortality on both dosages, and this mortality is very close to 25 per cent. It is concluded, therefore, that the Sassandra females used on this cross were homozygous for dieldrin resistance, and that resistance in this strain is dependent on a single dominant genetic factor.

Table 3. Mortalities on Dieldrin of F_1 and F_2 Generations of Cross, Diggi ♂ × Gaoulou-Sassandra ♀. (Tests made in London on Laboratory-reared, Unfed Females and Males, 1–4 days old)

Dieldrin (%)	No. of mosquitoes tested	Exposure period (h)	No. of dead mosquitoes	Mortality (%)
		F_1 generation		
0·4	7	1	0	0
4·0	51	1	0	0
		F_2 generation		
0	39	1	0	0
0·4	530	1	118	22
4·0	545	1	145	27

In respect of its dominance this type of resistance differs from those previously recorded for three other strains of *A. gambiae* from West Africa and for other anopheline species[2]. Here dieldrin resistance was found to be monofactorial and semi-dominant, 0·4 and 4·0 per cent dieldrin for 1 h being the dosages discriminating between susceptible, heterozygotes and resistant individuals.

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MICROBIOLOGY

Production of a Gibberellin-like Substance by *Arthrobacter globiformis*

The ability to synthesize plant growth substances such as indolyl-3-acetic acid (IAA) is widely distributed among micro-organisms. But the production of gibberellins appears to be much more restricted, fungi of the genus *Gibberella* being the chief microbial source. However, in recent work on IAA production by species of *Arthrobacter*[1] we detected another growth-promoting zone on chromatograms of culture filtrates by means of a modified *Avena* coleoptile section test. These observations along with a report of gibberellic acid production by *Azotobacter*[2] prompted further work on the synthesis of gibberellin-like substances by *Arthrobacter*.

Arthrobacter globiformis (415), the type species of this genus, was grown in a synthetic medium containing per litre distilled water, sucrose, 10 g; KNO_3, 0·5 g; biotin, 10 μg; salt solutions *A* and *B* (ref. 3), 5 ml. each; *p*H 6·8–7·0. The culture was grown at 26° C on a rotary shaker for varying periods of time and the cells removed by centrifugation. The cell-free culture medium was stirred with 50 g/l. of cellulose and carbon (4 : 1) for 2 h and the mixture filtered. The aqueous fraction was discarded. The residue was washed with 95 per cent acetone, using 1/3 of the volume of the original culture medium, the eluate evaporated under reduced pressure, taken up in water and, after *p*H adjustment to 3·0, extracted with ether and/or ethyl acetate. The extract was concentrated under reduced pressure, chromatographed and segments of the chromatogram or eluates of these assayed at different time intervals for growth-promoting activity by means of a modified *Avena* coleoptile section test.

Three solvent systems were used for chromatography: (*a*) isopropanol/ammonia/water (8 : 1 : 1); (*b*) *n*-butanol/acetic acid/water (4 : 1 : 1); (*c*) ethanol/ammonia/water (18 : 1 : 1). Extracts enriched with authentic IAA and gibberellic acid at levels sufficient for colour detection were chromatographed in addition to the extracts alone. In one-way chromatography with the three solvents and in two-way chromatography with solvents (*a*) and (*b*) a growth-promoting substance which co-chromatographed with gibberellic acid was detected consistently. In all instances, unlike IAA, and like gibberellic acid, the substance did not produce curvature of coleoptile segments; furthermore, depending on the degree of purification, the time-activity curve was similar to that of gibberellic acid. Since the chromatographic and biological behaviour of the substance suggested gibberellic-like activity other tests accepted as more or less specific for gibberellic acid were performed with eluates of the gibberellic acid zones of the chromatograms: the lettuce seed germination test of Cathey *et al.*[4], the lettuce hypocotyl test of Frankland and Wareing[5] and the dwarf pea test of Brian and Hemming[6]. Typical results are shown in Table 1; whereas the substance has no significant activity in the pea test, the increase in per cent germination and the elongation of the hypocotyls as well as the activity detected by means of the *Avena* coleoptile test all suggest that it can be considered a gibberellin. The characterization of this substance is in progress.

Unlike *Azotobacter*, members of the genus *Arthrobacter* occur in large numbers in most soils. Rouatt and Katznelson[7] have reported that 45 per cent of the isolates from soil extract agar plates of root-free soil and 34 per cent of those from wheat rhizosphere soil representing respectively 54 × 10^6 and 240 × 10^6 organisms per gram soil were of this genus. In consequence, significant amounts of biologically active substances may be produced with corresponding effects on seed germination, root development and possibly on mineral nutrition of plants[8]. These effects may be even more striking under conditions favouring the production of precursors of these substances as in the case of tryptophan for IAA synthesis[1,9].

Table 1. PRODUCTION OF A GIBBERELLIN-LIKE SUBSTANCE BY *Arthrobacter globiformis*

Test used	Gibberellin acid μg/ml. buffer*				Extract ml. equiv. original culture/ml. buffer*				Control
	0·01	0·1	0·5	5·0	1·0	5·0	10·0	20·0	
Lettuce seed germination (per cent)		30	35	45	30	45		30	20
Lettuce hypocotyl elongation (mm)	13	19				14	13		9
Dwarf pea internode elongation (mm)	15	24			9	9	9	10	8

* Concentration for pea test should read /plant. Figures represent average of ten plants.

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PSYCHOLOGY

Detection of Cosmic Ray μ-Mesons by the Human Eye

THE detection of ultra-violet light and X-rays in the human eye, through a fluorescence mechanism, is well known[1], and it would be expected that a high flux of ionizing particles would be similarly detectable. There is apparently no record in the literature of the detection of single minimum ionizing particles, though there are several mechanisms by which a relativistic particle may release a detectable amount of energy in traversing the eye. These include the emission of Čerenkov radiation[2] in the vitreous humour. An ultra-relativistic singly-charged particle, in median passage through a normal eye, from lens to retina, generates approximately 600 photons in the visible, which will be collected over a large part of the retinal area. The visual signal would be equivalent to that from an illuminated disk, with intensity increasing from about 0·5 photon/mm^2 at the periphery to about 10 photons/mm^2 at the centre. This light is emitted essentially instantaneously (within 10^{-10} sec) and has an intensity comparable with that at the threshold of vision[3]. In addition to Čerenkov radiation, light may be released by scintillation in the crystalline lens or vitreous humour, or direct excitation of the retina may occur.

Preliminary tests have been carried out with a number of observers, to verify this possibility. The observer lay on a couch in a dark room, and a small Geiger telescope was attached to a pair of darkened goggles worn on the head, so that cosmic ray μ-mesons, passing through the right eye within 15° of the vertical, were selected. The telescope operated a mechanical register which was audible to the observer,

Table 1

	Genuine counts				Spurious counts				
Observer	Total	Yes	No	Yes/total	Total	Yes	No	Yes/total	P
1	18	6	12	0·33	112	19	93	0·17	0·10
2	23	3	20	0·13	233	20	213	0·09	0·35
3	59	12	47	0·20	190	28	162	0·15	0·17
4	34	11	23	0·32	213	40	173	0·19	0·06
5	33	15	18	0·46	157	47	110	0·30	0·08
6	26	3	23	0·12	152	21	131	0·14	0·70
7	20	2	18	0·10	179	8	171	0·04	0·23
8	30	3	27	0·10	196	15	181	0·08	0·40
9	31	10	21	0·32	181	49	132	0·27	0·35
All observers	274	65	209	0·237 ± 0·029	1,613	247	1,366	0·153 ± 0·010	

and a scaler. Mesons were counted at a rate of approximately 1/min. A somewhat larger Geiger telescope placed some distance away, operating at about 5 counts/min, was connected to the same mechanical register, but to a different scaler. Thus identical audible signals were given to the observer at random intervals, and only 20 per cent of these corresponded to the passage of a genuine μ-meson through the eye. The scaler readings were recorded by an operator in communication with the observer. The observer answered 'yes' or 'no' to each signal, and the results were analysed, to look for a higher proportion of affirmative responses in the case of the genuine events. Nine observers were tested. A period of dark adaptation was allowed before commencing observations. Operation was possible for a period of about 30–45 min, after which fatigue and discomfort due to the goggles became serious. The results are summarized in Table 1.

P is the Poisson probability for each observer that the observed or a greater number of counts should be found in the genuine channel, when the expectation number is derived from the spurious channel. Eight of the nine observers recorded a higher proportion of affirmative responses for genuine signals than for spurious, but none is individually significant. The total results summed for all observers do, however, appear to indicate a significant effect, at about the 0·4 per cent level, assuming the ratios to be distributed normally. The effect of summation is to reduce the significance-level of the results, but since the numbers of genuine events were small for individual observers, the use of χ^2 was not considered legitimate. The signal detected may not in fact be due to Čerenkov radiation; observers described the visual sensation associated with the genuine signals as a bright point of light, rather than the diffuse disk expected, and it is possible that the detection is through a direct excitation of the retina. The energy deposited by ionization in traversing a rod or cone is about 500 eV.

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Immediate Detection of X-rays by the Rat

Recently we postulated that mammals perceive radiation and have commenced an investigation of its perceptual aspects and its underlying physiological receptor mechanism. Small doses of radiation (10–20 r.) have been used as a noxious stimulus to establish avoidance reactions in blind and normal laboratory animals under a wide variety of experimental conditions[1–4]. While these experiments indicate that animals perceive radiation, they afford no measure of the reaction time of the animal to the onset of the radiation exposure. We are now measuring this important parameter in rats by using X-rays as a warning stimulus to signal the occurrence of a subsequent electric shock to the animal's paws.

The animals are tested and trained in a standard instrumental conditioning apparatus which consists of a small acoustically treated compartment equipped as follows: A lever, operated by the rat, actuates a mechanism adjusted to deliver a pellet of food for every twelve responses. The response-rate is recorded by a cumulative response recorder. The compartment floor is an electric shocking grid and a speaker provides a continuous wide-band noise to mask extraneous sounds. Behaviour can be observed visually through a double pane window.

The compartment is situated directly under an X-ray therapy machine with factors of 250 kV, 0·5 mm copper filter, 1·7 copper, half-value layer. Output is measured by a 'Victoreen' chamber lying in the animal's compartment, hence includes back scatter. A plate of 3/16 in. lead suspended between the X-ray tube and the animal's compartment can be moved inaudibly to expose the animal while the machine remains in continuous operation. As a precaution, sham exposures are alternated with actual exposures under identical procedures except that the copper filter in the X-ray machine is replaced by a lead plate which effectively blocks the X-ray at its source.

Training is a variation of the Estes–Skinner conditioned-suppression technique[5]. As a preliminary procedure the lever is removed from the compartment while the animal is conditioned with radiation and electric shock. In a typical case this training consisted of a 10-sec X-ray with intermittent shock during the last 8 sec of exposure. Ten exposures were administered at irregular intervals (1–3 min). This was repeated every 2 or 3 days until 40 conditioning exposures were administered.

Testing is conducted while the animal is actively pressing the lever for food. The suspended shield is moved and the animal is exposed without the subsequent electric shock. In many cases this brief exposure causes an immediate arrest in the animal's lever pressing behaviour. Fig. 1 is a sample of the cumulative response records of two animals trained and tested with a 10-sec exposure at 1·0 r./sec (rat A) and a 15-sec exposure at 0·2 r./sec (rat B). The horizontal step in the slope of the record indicates that the rats ceased to press at the onset of the shock-free exposure and resumed activity immediately after the exposure was terminated. Since sham exposures did not suppress the response it is apparent that the X-ray is the critical stimulus.

Other animals did not exhibit conditioned suppression in the first tests and were subjected to the following procedure. If a rat pressed the lever during exposure, the response was considered an error and he was given an electric shock. Lever pressing between exposures were rewarded as in the previous training. Each animal was trained until he performed five consecutive errorless, hence shock-free, trials, or received a maximum of twenty trials. The mean performance of four rats exposed to 10-sec X-ray at 1·0 r./sec is given in Fig. 2. Errors, that is, lever pressing during exposure, decline with increased training much as one would expect with discrimination training with stimuli such as visible light or sound stimuli. During the sham exposures the animals continued to press the lever as indicated by the dotted line (Fig. 2). All the animals (12) trained in this manner exhibited repeated conditioned suppressions during shock-free exposures within 10 trials.

While this procedure is often referred to as discrimination training, it is apparent that the animal can perceive X-rays before the training begins. During our efforts to habituate animals to the X-ray room we observed that animals tended to go to sleep during sham exposures and to remain awake during actual exposures. Furthermore, after a few treatments with X-ray and electric shock during preliminary training many animals attempted to escape from the compartment at the onset of X-ray, indicating that defensive responses to shock had quickly been conditioned to radiation. Subsequent training to inhibit the lever pressing response during exposure serves to provide objective evidence of the animal's ability to detect the presence of X-rays.

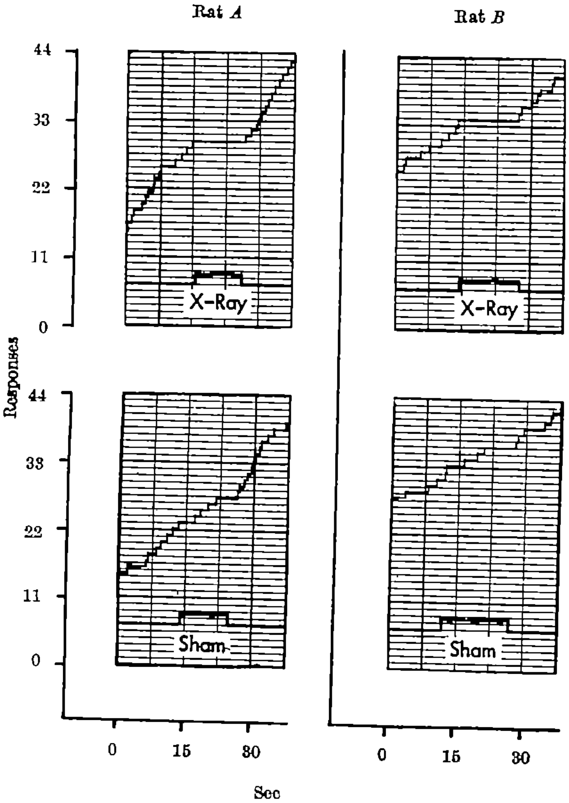

Fig. 1. Conditioned suppression of lever pressing response in rats with X-ray. Rat *A* was tested with 10-sec exposure at 1·0 r./sec and rat *B* with 15 sec at 0·2 r./sec. In prior conditioning X-ray had served as a warning stimulus for electric shock. No shock was used during these tests

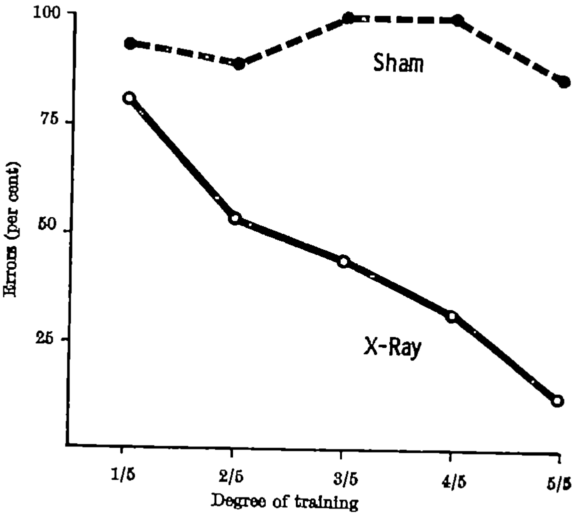

Fig. 2. Mean performance of four rats during discrimination training with 10-sec X-ray exposure (1·0 r./sec). Errors, that is, responses during exposure, were followed by shock. Responses during sham exposure led to food. Training is plotted in successive fifths of total trials received by each animal. Criteria was five consecutive errorless (shock-free) trials with maximum of 20 trials

Preliminary experiments indicate the ophthalmectomized animals can also detect X-rays, thus phosphenes or other visual effects cannot account for the phenomena. Our present evidence indicates a receptor system of extremely fast reaction time and great sensitivity. Animals can detect the very onset of a dose-rate of 0·2 r./sec. Experiments with lower doses are in progress. (*Note added in proof.* Experiments conducted since submission of this report indicate dose-rates of 0·05 r./sec or less are effective.)

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COSMOLOGY

Transfinite Numbers and Cosmology

A COMPLETE analysis of Dr. Schlegel's communication on this subject[1] would take too long: but some correction should be made.

In Dr. Schlegel's equation:

$$N = N_0 e^{kt} \qquad (1)$$

t is finite. The equation is only an idealized approximation: it would be better enunciated for the large but finite number N_0 of atoms in a large but finite initial volume V_0: for all that, the equation is not objectionable. But the substitution of an infinity for t is objectionable. The infinity to which t tends is not $\aleph_0$. In Dr. Schlegel's formula :

$$N \geqslant e^{\aleph_0} \qquad (2)$$

the right-hand side is meaningless: and the argument does not yield:

$$N \geqslant 2^{\aleph_0}$$

as Dr. Schlegel claims. It yields only that $N \geqslant K$ for any finite integer K, that is:

$$N \geqslant \aleph_0 \qquad (A)$$

The hypotheses involved here are that the universe is expanding, and that it looks the same everywhere at all times.

To obtain the opposite inequality:

$$N \leqslant \aleph_0 \qquad (B)$$

one is much less committed. Irrelevant are all questions of isometric embedding: relevant only is the question whether the universe can be exhausted by a countable set of small neighbourhoods: this is in essence the question whether the universe is 'separable'. There exist (in the mathematical sense) topological manifolds which are not separable, but that sort of thing is excluded by the assumption of a global metric: it is a familiar fact that any Riemannian manifold is separable. Even if we abandon the hypothesis of a Riemannian metric in some quantized theory, it can scarcely be doubted that (B) would still follow from the steady-state hypothesis, in any event if this included some hypothesis of isotropy.

On the usual interpretation of the red shift we can have no future physical connexion with atoms beyond a certain finite distance. This leads to a difficulty about the derivation of (B) which may trouble some. Do such atoms 'exist'? How can they be 'counted'? Such questions invite confusion between conceptual existence (on one of the steady-state-expanding-universe concepts) and physical-existence-for-us: and doubt may easily result. But the universe consists of those atoms, and parts of space, which are connected with us not necessarily by one such physical connexion but by a finite chain of such physical connexions as are envisaged (reception and transmission of signals). This gives a closed system: to conceive the 'existence' of any further atoms is to indulge in idle fantasy: this closed system is the universe: and it is separable.

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THE application of transfinite numbers to physical theory is admittedly novel, and should be open for critical discussion; none the less, I see no force in the arguments which Mr. Ursell presents.

In my communication I implicitly made use of the accepted relation $2^{\aleph_0} = n^{\aleph_0} = C$, where n is any integer greater than 1 and C is the power of the continuum, and on the basis of this relation I set $e^{\aleph_0} = C$. It is true that $e^{\aleph_0}$ has not been defined in transfinite-number theory; we may, however, readily come to the non-denumerability of atom-spaces, in steady-state theory, without making use of this quantity. The growth equation $N = N_0 e^{kt}$ requires $N/N_0 = 2$ for a time-interval $t_2 = (1/k)\log 2$. It follows, then, that any chosen finite set of atom-spaces will be doubled in time t_2. Indeed, over-looking fluctuations in the presumed creation process, we can say that in time t_2 each atom-space becomes two atom-spaces. Hence, each atom-space in a given set becomes two atom-spaces in an interval t_2. Since, by hypothesis, the universe has existed for a time $\aleph_0$, and $\aleph_0 \cdot t_2 = \aleph_0$ (we may choose time units so as to make t_2 an integer), we have a set of $\aleph_0$ factors of 2. Hence, a set of atom-spaces with cardinal number $2^{\aleph_0} = C$ must have been formed in the universe. We could equally well have worked with a t_n, defined for an n-fold increase of any finite group of atoms, with the same result: there is a set of atom-spaces with cardinal number $n^{\aleph_0} = C$.

It is a basic tenet of steady-state theory that the universe has always existed with its present large-scale features. I take this to mean that the universe has existed for an achieved infinity of years: a set of years with the cardinal number $\aleph_0$. The alternative assumption is that the life-time of the universe is t years, where t 'tends to infinity', that is, is indefinitely large, but is still a finite number. This assumption would not give the universe the steady-state property of having existed for ever.

Mr. Ursell proposes that cosmological considerations must be limited to those parts of the universe with which physical interaction, as through signal chains, is possible. Such interaction is a factor of doubtful relevance in the consideration of the consistency properties of cosmological models (as opposed, of course, to matters of empirical confirmation). In any event, he presents no argument that would allow a set of C atom-spaces to be fitted into a universe which everywhere has the spatial properties (in accordance with the cosmological principle used in steady-state theory) that we find in our observed universe.

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THE force of my arguments must, it seems, be a matter of opinion. Dr. Schlegel "may readily come" to various conclusions, but mostly without me.

Dr. Schlegel's central fallacy, on my evaluation, lies in the easy substitution of an infinity for a finite number in a formula. This is how he leaves the slippery slope of ambiguities for the abyss: this is where I do not follow. In the foregoing communication the error is implicit in his phrase, "set of $\aleph_0$ factors", explicit in the next sentence. I concede a factor of 2^n between the year n B.C. and now: I do not concede his factor of $2^{\aleph_0}$ between, presumably, the cold bleak world of $\aleph_0$ B.C. and now.

Dr. Schlegel's objection to my final paragraph is disheartening. I am there defending, against some quite possible criticism, the statement (B) above which he himself made (ref. 1, p. 666, line 12). Even when I agree with him, he disagrees with me.

H. D. URSELL

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, December 10

Institution of Mechanical Engineers, Manipulative and Mechanical Handling Machinery Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Universal Excavators *versus* the Front End Loader".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Sir John Hunt, C.B.E., D.S.O.: "A British–Russian Expedition to the Pamirs".

Monday, December 10—Tuesday, December 11

Powder Metallurgy Joint Group of the Iron and Steel Institute and the Institute of Metals (at Church House, Great Smith Street, London, S.W.1), at 11.15 a.m. on Monday and 10 a.m. on Tuesday—Winter Meeting.

Tuesday, December 11

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

Institution of Electrical Engineers, Science and General Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. G. Heaton: "Thermoelectrical Engineering".

Institution of the Rubber Industry (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. C. Musgrave: "Colouring of Rubber". 7 p.m.—Panel Discussion on "Rubber *versus* Plastic in Footwear".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. Richter. "Brain Biochemistry in Relation to Psychiatric Disorders"*. (Seventeenth of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers, Automobile Division (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. D. K. MacKenzie, Mr. T. P. Newcomb and Mr. B. D. Watton: "Car Brake Usage Test Schedules".

Wednesday, December 12

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. H. Harris: "Inborn Errors of Metabolism".*

Colour Group (Great Britain) (in Lecture Theatre No. 2 of the New Physics Department, Imperial College, London, S.W.7), at 3 p.m.—Dr. F. J. J. Clarke: "Further Studies of Extra-Foveal Colour Metrics". 4 p.m.—Dr. W. A. H. Rushton: "Visual Adaptation".

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. A. H. Strude: "Current-swept Sea-floors near the Southern Half of Great Britain"; Dr. F. Howitt: "Stratigraphy and Structure of the Purbeck Inliers of Sussex".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. C. D. Foster and Dr. M. E. Beesley: "Estimating the Social Benefit and Loss from Investing in the Victoria Line".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. A. Saxton: "Radio Meteorology".

British Institution of Radio Engineers, Education Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Discussion on "Teaching Mathematics to Engineers".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. F. H. S. Brown: "Duty and Development of Modern Power Station Plant" (Parsons Memorial Lecture).

Royal Institute of Chemistry, London Section (at Shell-Mex House, Strand, London, W.C.2), at 6 p.m.—Annual General Meeting, followed by Mr. George Dring, "Qualifications in Chemistry".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Brian Parkyn: "Glass Fibre Reinforced Plastics".

Society of Environmental Engineers (in the Mechanical Engineering Department, Imperial College, London, S.W.7), at 6 p.m.—Mr. A. P. Longmore: "Simulation of a Space Environment".

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, London, W.C.1), at 6.15 p.m.—Dr. A. R. Meetham: "Mechanical Indexing".*

Society of Chemical Industry, Food Group (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. J. A. O'Keefe and Dr. Magnus Pyke: "Nutrition and the Law".

Society for Analytical Chemistry, Atomic Absorption Spectroscopy Discussion Panel (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Inaugural Meeting—Mr. D. J. David: "Aspects on Atomic Absorption Analysis".

Society of Instrument Technology, Control Technology Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. D. H. A. Harvey and Mr. G. S. Kardoosh: "Hot Strip Rolling Mill Looper Control"; Mr. A. F. Watters: "Position Control of a 150-Ton Forging Crane".

Wednesday, December 12—Thursday, December 13

Association for the Study of Animal Behaviour (at Birkbeck College, Malet Street, London, W.C.1), at 11 a.m. on Wednesday and 10 a.m. on Thursday—Symposium on "Gregarious Behaviour".

Thursday, December 13

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. F. Brailsford and Dr. V. R. Mazza: "An Experimental Investigation into the Alternating Magnetic Flux Distribution in Right-Angled Corners of Transformer Laminations".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. N. P. Davies. "Cancer in the Tropics, with special reference to Neoplasms in Childhood".* (Last of eighteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Chemical Society (at Burlington House, Piccadilly, London, W.1), at 6 p.m.—Scientific Papers.

Prestressed Concrete Development Group (at the Royal Institute of British Architects, 66 Portland Place, London, W.1), at 6 p.m.—Mr. Felix Adler. "Prestressed Concrete in Building".

Association of Supervising Electrical Engineers (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 6.15 p.m.—Mr. L. Landon Goodman: "The Electrical Engineer's Contribution to Productivity".

Society for Analytical Chemistry, Biological Methods Group (in the "Feathers Restaurant", Tudor Street, London, E.C.4), at 6.30 p.m.—Eighteenth Annual General Meeting, followed by Dr. A. J. Amos: "The Assessment of Vitamins in Feeding Stuffs".

Institute of Metals (joint meeting with the London Branch of the Institute of British Foundrymen, at the Institute of Metals, 17 Belgrave Square, London, S.W.1), at 7 p.m.—Mr. R. E. Berry: "Trends on Non-Ferrous Melting Practice".

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, London, W.C.2), at 7 p.m.—Mr. R. A. Turrell and Mr. B. J. Rogers: "Colour Television Services".

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Symposium on "Medical Problems Presented by the Tristan da Cunha Community". Speakers: Dr. H. E. Lewis, Dr. C. K. Thacker, Dr. D. Taylor-Robinson, Dr. D. A. J. Tyrrell and Prof. A. Sorsby.

Friday, December 14

Biochemical Society (in the Department of Chemistry, Westfield College, Hampstead, London, N.W.3), at 9.30 a.m.—Colloquium on "The Organic Chemist's Approach to Enzymology" and original communications.

Society of Non-Destructive Examination (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1), at 3.30 p.m.—Dr. R. Jones. "The Uses of Ultrasonics".

Institute of Navigation (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 5.30 p.m.—Capitaine de Frégate L. Oudet: "Routing in the Dover Strait".

Society for Analytical Chemistry, Microchemistry Group (joint meeting with the Midlands Section, at the University, Edgbaston, Birmingham), at 6.30 p.m.—Dr. A. M. G. Macdonald: "The Determination of Carbon and Hydrogen in Organic Materials".

Friday, December 14—Saturday, December 15

Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom (in the William Beveridge Hall, University of London Senate House, London, W.C.1), at 2.30 p.m. on Friday and 10.30 a.m. on Saturday—Home Universities' Conference.

Monday, December 17

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. R. H. Hughes: "Hong Kong—Far Eastern Meeting Point".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Prof. H. E. M. Barlow, F.R.S.: "A Proposed New Method of Measuring Microwave Power and Impedance using Hall Effect in a Semiconductor"; "High-Frequency Radiation Pressure and Hall Effect in Semiconductors"; "A Microwave Electrostatic Wattmeter".

Institute of Metal Finishing (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. J. Carder: "Satin Nickel + Chromium Plating".

Monday, December 17—Tuesday, December 18

Association of Applied Biologists (in the Physics Department, Imperial College of Science and Technology, London, S.W.7), at 2.30 p.m. on Monday, and 10.30 a.m. on Tuesday—General Meeting.

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Lecturer or Senior Lecturer (qualified to supervise post-graduate studies and research in functional analysis) in Pure Mathematics—The Registrar and Secretary King's College, (University of Durham), Newcastle upon Tyne (December 15).

Research Assistant (with good qualifications in chemistry or physiology), to work on biochemical effects of radiation—The Personnel Officer, St. Bartholomew's Hospital, London, E.C.1 (December 15).

Plant Physiologists, Microbiologists and Biochemists, for work involving fundamental research (with ample scope for original work and its publication)—The Director, Brewing Industry Research Foundation, Nutfield, Surrey (December 17).

Assistant Lecturer in Pure Mathematics—The Secretary, The University, Northcote House, The Queen's Drive, Exeter, Devon (December 31).

Chair of Applied Chemistry in the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

Senior Lecturer in Mathematics at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

Senior Lecturer or Lecturer (with special qualifications in plant physiology and preferably the ability to conduct an elementary course in plant genetics) in the Department of Botany, University of the Witwatersrand, Johannesburg—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, December 31).

Chair of Clinical Ophthalmology at the Institute of Ophthalmology—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 2).

Sembal Chair of Experimental Ophthalmology at the Institute of Ophthalmology—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 2).

Lecturers (2) (qualifications in analysis will be relevant for one post) in Mathematics—The Secretary, The University, Aberdeen (January 8).

Chair of Philosophy—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (January 5).

Lecturer (man or woman) in Botany—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (January 7).

Senior Lecturer in Geophysics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 7).

Senior Lecturer or Lecturer (with experience in the fields of inorganic and/or physical chemistry) in Chemistry at the Australian National University—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 7).

Lecturer (especially interested and qualified in the field of structural geology) in Geology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 10).

Senior Fellow, Fellow, or Senior Research Fellow in a Unit of Cell Biology and Immunology within the Department of Experimental Pathology, The John Curtin School of Medical Research, Australian National University, to engage in studies on acquired immunity and on mechanisms involved in the responses and interactions of cells—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London. S.W.1 (Australia and London, January 11).

Jubilee Research Fellow (woman graduate of any nationality), for research in any subject within the Faculties of Arts or Science—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (January 14).

Lecturers/Senior Lecturers (2) in Biochemistry at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

Assistant Lecturer (with qualifications (1) in any branch of pure mathematics, (2) in numerical analysis or statistics) in the Department of Mathematics—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff.

Junior Scientific Assistant (with training in either genetics or microbiology and preferably with some knowledge of both) for mutation research on *Neurospora*—Dr. Charlotte Auerbach, F.R.S., Institute of Animal Genetics, West Mains Road, Edinburgh.

Postdoctoral Research Assistant, for work on nuclear magnetic double resonance techniques—Dr. D. W. Turner, Chemistry Department, Imperial College of Science and Technology, London, S.W.7.

Senior Research Assistant, to join a team doing research in high pressure chemistry, with some applications to geochemistry——Dr. R. S. Bradley, Department of Inorganic and Structural Chemistry, The University, Leeds 2.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 721, Vol. 245, (4 October 1962): Biology and Physics of Locust Flight. 5: Strength and Elasticity of Locust Cuticle. By Martin Jensen and T. Weis-Fogh. Pp. 137–169+plate 55. 12*s*. No. 722, Vol. 245, (4 October 1962): Studies on the Ontogeny of *Tritonia hombergi* Cuvier (Gastropoda Opisthobranchia). By Dr. T. E. Thompson. Pp 171–218. 15*s* 6*d*. (London: The Royal Society, 1962.) [910

Royal Observatory Bulletins. No. 51: Space-Velocity Vectors for 3483 Stars with accurately determined Proper Motion and Radial Velocity. By Olin J. Eggen. Pp. E79–E398. 40*s*. net. No. 57: Geomagnetic Secular Variation for the Epoch 1955.0. By B. R. Leaton. Pp. D245–D270. 4*s*. net. No. 58: Studies in the Magellanic Clouds. 4: Photometry of Cepheids in Variable Field I, LMC. By R. v.d.R. Woolley, A. R. Sandage, O. J. Eggen, J. B. Alexander, Linda Mather, Elizabeth Epps and Susan Jones. Pp. E31-E87+4 plates. 9*s*. net (London: H.M. Stationery Office, 1962.) [910

Ciba (A.R.L.), Ltd. Technical Notes, No. 237, (September, 1962): Aerolite Powder for Chipboard. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1962.) [910

Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 63: 50-Point Traffic Census—the First 5 Years. By J. C. Tanner and J. R. Scott. Pp. vi+38. (London: H.M. Stationery Office, 1962.) 3*s*. net. [1610

National Association for Mental Health. Annual Report, 1961–1962. Pp. 55. (London: National Association for Mental Health, 1962.) [1610

Bulletin of the British Museum (Natural History). Historical Series. Vol. 3, No. 2: Supplementary Letters of Sir Joseph Banks. Edited by Warren R. Dawson. Pp. 41–70. 8*s*. Zoology. Vol. 9, No. 1: Land Birds of Guadalcanal and the San Cristobal Group, Eastern Solomon Islands. By Ian C. Galbraith and Ebba H. Galbraith. Pp. 1–86. 26*s*. (London: British Museum (Natural History), 1962.) 26*s*. [1610

The British Cast Iron Research Association. Forty-first Annual Report, 1961–1962. Pp. 74. (Alvechurch, Birmingham: The British Cast Iron Research Association, 1962.) [1610

Other Countries

Canada: Department of Northern Affairs and National Resources. National Museum of Canada. Bulletin No. 180: Contribution to Anthropology, 1960, Part 1. Pp. vi+190 (29 plates). 2 dollars. Bulletin No. 181, (Biological Series No. 67): Annotated List and Bibliography of the Abyssal Marine Molluscs of the World. By Arthur H. Clarke, Jr. Pp. vi+114. 1.50 dollars. Natural History Papers. No. 17, (August 27, 1962): Fish Remains from Ontario Indian Sites 700 to 2500 Years Old. By D. E. McAllister. Pp. 6. Anthropology Papers. No. 2: Museum Ethnology and the Algonkian Project. By Tom F. S. McFeat. Pp. 80. (Ottawa: Queen's Printer, 1962.) [910

United States Department of Health, Education and Welfare. Public Health Service. Publication No. 935: Activities of the National Institute of Health in the Field of Gerontology—Extramural Research and Training Projects Active on January 31, 1962, and Intramural Research Projects carried on during Calendar Year 1961. Pp. viii+47. 35 cents. Publication No. 921: Research Highlights in Aging: a Review of a selected number of scientific papers on research in aging carried out or supported by the National Institutes of Health, 1961. Pp. v+29. 20 cents. Publication No. 989: Research Programs in Aging: Studies Conducted or Supported by the National Institutes of Health during 1961. Pp. v+17. 15 cents. (Washington, D.C.: Government Printing Office, 1962.) [910

New Zealand Forest Service: Forest Research Institute. Forestry Research Notes, No. 31: The Effect of Some Water-Borne Wood Preservatives on the Immediate Bond Strength of Glued Joints in Radiata Pine Sapwood. By A. G. Stanger. Pp. 27. Report of the Director-General of Forests for the year ended 31 March 1962. Pp. 62. (Wellington. Government Printer, 1962.) [910

European Organization for Nuclear Research. Annual Report 1961. Pp. 153. (Geneva: European Organization for Nuclear Research—CERN, 1962.) [910

Commonwealth Scientific and Industrial Research Organization, Australia. Annual Report of the Division of Plant Industry, 1961–62. Pp. xlviii+184. Fourteenth Annual Report of the Division of Coal Research, 1961–62. Pp. iii+25. Annual Report of the Engineering Section for year ending June 30, 1962. Pp. 27. Annual Report of the Division of Forest Products, 1961–62. Pp. iii+44. (Canberra: Chatswood; Melbourne: Commonwealth Scientific and Industrial Research Organization, 1962.) [910

Canada. Review of the National Research Council, 1962. Pp. 383. (Ottawa: National Research Council, 1962.) [910

Institute of Technology, Nihon University. Report No. 4: Internal Cavity Reflex Klystron Amplifiers. By Koryu Ishii. Pp. ii+65. (Tokyo: Institute of Technology, Nihon University, 1962.) [910

Parliament of New South Wales. Report of the Department of Agriculture, New South Wales, for the year ended 30th June, 1961 Pp. 137. (Sydney: Government Printer, 1962.) [910

Cornell University Agricultural Experiment Station: New York State College of Agriculture. Memoir No. 379: Growth, Nutrition, and Metabolism of *Mentha piperita L.*, Parts 1–7. Pp. 144. Ithaca, New York: Cornell University Agricultural Experiment Station, New York State College of Agriculture, 1962.) [1510

Radiochimica Acta, Band 1, Heft 1, (September 1962). Pp. 1–48. Price per volume (4 issues) 72 D.M.; 18 dollars (Frankfurt a M.: Akademische Verlagsgesellschaft, New York and London: Academic Press, Inc, 1962.) [1510

Australia. Commonwealth Scientific and Industrial Research Organization. Fourteenth Annual Report, 1961–62. Pp. iv+153+10 plates. Annual Report of the Division of Meteorological Physics for the year ending June 30, 1962. Pp. iii+17. Annual Report of the Fodder Conservation Section, 1961–62. Pp. 10. Annual Report of the Division of Physics for the year ending June 30, 1962. Pp. v+26. Second Annual Report of the Wheat Research Unit for the year ending June 30, 1962. Pp. i+10. (Melbourne and Sydney: Commonwealth Scientific and Industrial Research Organization, 1962.) [1610

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THE NATIONAL LENDING LIBRARY FOR SCIENCE AND TECHNOLOGY

THE official opening of the new National Lending Library for Science and Technology on November 5 (see also p. 1034 of this issue) was properly an occasion for the Department of Scientific and Industrial Research, and it was appropriate that the opening ceremony should be performed by Lord Hailsham, both as Minister for Science and Lord President of the Council, to whom the Council for Scientific and Industrial Research is responsible. Nevertheless, while both the Chairman and the Secretary of that Council were present, the platform party included no representative of the Advisory Council on Scientific Policy, although, as Lord Hailsham indicated, it was by that body that the creation of a new national science lending library was recommended some ten years ago. The Advisory Council had earlier, in 1951, recommended the establishment of the National Reference Library of Science and Invention and has steadfastly advocated both projects since after almost a decade of wavering and indecision the Government finally rejected the imaginative conception of a Science Centre in London. It is true that, as Sir Harold Roxbee Cox remarked at the opening ceremony, the Council for Scientific and Industrial Research is now charged with some specific responsibility for the dissemination of scientific literature, and that the by return of post lending service, the rapid copying service, the translation service and the training schemes at which the new Library aims, go far beyond anything contemplated in the Science Centre, but nevertheless it is fitting that the part played by the Advisory Council on Scientific Policy and the wide responsibilities of the Council in this field should be recognized at the inauguration of this important project.

The location of the new Lending Library and some features of its planning and staffing have naturally not escaped criticism, but this is no occasion for other than congratulations to the director of the Library, Dr. D. J. Urquhart, and to all concerned. Dr. Urquhart was able on this opening day to show an operating establishment which in planning and equipment makes full use of the remarkable advances in scientific technique in this field during the past two decades. He has had the courage to break imaginatively with many current library techniques and practices, and although it is as reasonable to wait for experience to attest the soundness of his judgment at all points as it would be unreasonable to expect a new venture to be entirely free from teething troubles, it was clear to all that the claim was fully justified that here was something unique in Great Britain. Moreover, what may fairly be described as the most outstanding development in libraries in Britain during the past decade or more and a valuable contribution to more effective communication between science and industry is already staffed by those whose keenness and enthusiasm was as obvious as it was refreshing to the visitor.

Here there is already an achievement which reflects the highest credit on all responsible and for which the scientist and technologist has every reason to be grateful. That gratitude should, however, as Dr. Urquhart has already indicated, and Lord Hailsham repeated, be expressed, at least in part, in co-operation and in support. Important as it is, the new National Lending Library for Science and Technology is, as its name implies, only one element in a national library system. Its full possibilities and efficiency will only be realized in the context of a national system, and its inauguration should give further impetus to the establishment of the National Reference Library for Science and Invention, still at the planning stage.

It may well be true that the implementation of the conception of a Science Centre in London on the lines which Sir Robert Robinson so untiringly advocated offered the maximum economy of national resources, but it is none the less open to doubt whether in such a setting a National Lending Library could have possessed anything like the spaciousness that has given Dr. Urquhart his opportunity for such imaginative design and break with traditional practice. Here, however, new techniques and developments have been fully utilized, but the benefits will be reaped largely as local libraries respond to Dr. Urquhart's appeal for co-operation, for example, in regard to improving their holdings of abstract publications. Other libraries, too, must take a forward look and endeavour to meet the needs of the future by adopting new techniques and discarding, where possible, techniques that are obsolete as well as traditional.

Nor is this all. No institution like the new Library is likely to flourish without constructive criticism, but such criticism must be inspired by clear ideas of what functions the institution is intended to perform. It is also essential that the institution should be assured of the resources in staff as well as equipment and finance needed to enable it to keep pace with the steadily increasing demands resulting from the exponential trends in scientific and technical literature to-day. The parsimony and short-sightedness of successive Governments in the past do not warrant excessive optimism as to the absence of any check to the development of the new Library in the future, and the Ministry for Science and the Advisory Council on Scientific Policy may well need active and sustained interest and support both from industry and from scientists and technologists if progress is to be maintained.

Beyond this, there must be an end to the dilatoriness that hindered the development of the scheme for the new National Reference Library for Science and Invention and has inhibited any action on the Roberts Report towards the evolution of a real national library system. There is still a long way to go, and it is only as part of a national system in which the public libraries, the university libraries and the libraries of the colleges of technology, the professional institutions, a host of special libraries are all linked up with these two new national libraries and the National Central Library that the vision and courage and technique that have gone to the establishment of the new Library opened on November 5 can have their full effect. Those who were present had indeed a glimpse of the possibilities. It now lies with them to ensure that their colleagues in industry, in the universities, the colleges of technology, the Library Association and other professional bodies are made fully aware of those opportunities and of their responsibilities to see, so far as possible, that no lack of vision or false economy limits the project.

Dr. Urquhart has been fully justified in the emphasis he has laid on the economic aspects of library services and functions and in his determination to eliminate unnecessary routine and recording. Only so can the resources available hope to keep commensurate with the expansion visualized over the next century, even when the utmost advantage is taken of mechanization. Impressive, however, as is the mechanization of the new Library, probably the most encouraging feature for the future is the keenness and enthusiasm of its staff. It remains as true to-day as when it was written in these columns more than thirty years ago that the efficiency of the technical library for research purposes is determined as much by the personality and enthusiasm of the librarian as by the mechanical perfection of its indexing system. Growing mechanism, in fact, makes even larger demands on the library staff if the real needs of users are to be met. Their understanding of the problems encountered, their enthusiasm and initiative can go far to offset inherent drawbacks in mechanization. Moreover, if they can scarcely hope, for example, to provide a real substitute for the invaluable browsing which is impossible in a library planned on such lines as the new National Lending Library, such qualities could do much to provide stimulus in other ways, whether in personal contact or initiating new means for exchanging ideas and information. Contrariwise, mechanization of outlook and touch on the part of the library staff could wreck the co-operation and understanding on the part of users of all kinds which, as Lord Hailsham reiterated, is a vital element if this new venture is to realize its full potentialities.

THE SCIENCE OF METEOROLOGY

The Challenge of the Atmosphere

The Science of Meteorology. By Sir O. G. Sutton. (Hutchinson Science Library.) Pp. xiii + 227. (London: Hutchinson and Co. (Publishers), Ltd., 1962.) 21*s*.

THERE is always a risk that the writings of the head of the department in which the majority of meteorologists in the country are employed shall be regarded as being *ex cathedra*. One hopes that his sober statements on the negligible effects of hydrogen bombs on the weather and the slender possibilities for rain-making will be widely studied; but it would miss the point of the book to accept them on account of his authority. Every opinion must be carefully argued, and this is the essence of the "Challenge of the Atmosphere". The basic physics and dynamics of the air have been understood for a long time, but the situations are so complicated that the end-product—the weather—is infinitely varied. By comparison the game of chess, the rules of which are also well known, is trivial.

Meteorology is the most difficult of the physical sciences because the ground is already chosen: it cannot be fabricated in the laboratory to suit the state of our knowledge. Sir Graham takes us through close but delightful argument from a complex physical situation to a statement of the challenge it poses to the intellect, and then extracts the essence and states it in terms of the concepts of classical mechanics and physics. He shows us how one has to think about complicated problems.

If he had dealt only with aspects of the atmosphere in which this kind of approach has been particularly successful, the book would have been biased, and rather dull for the less-philosophical reader. But when he begins to write descriptively, and at second hand, much of the sparkle vanishes, and one can find many better accounts by less-eminent authors. This, however, is a personal point of view, and a newcomer to meteorology might well find the descriptive chapters most interesting at first reading.

The challenge and the philosophy retain their interest even when the theories in terms of which they are stated have been superseded. Thus it is difficult at the distance of three or four decades to believe that anyone really hoped that the atmosphere would turn out to have a constant eddy transfer coefficient. Yet apparently many did. It is obvious nowadays that the magnitude of eddy transfer must depend on the thermal stratification of the air. But how many people who use Sutton's formula for the dispersion of smoke appreciate the niceties of the philosophy by which it was realized that it must also depend on distance from the source ? The chapter on this topic is the most exciting because it is really part of the author's memoirs. However, he does not give us the latest instalment of the story—the realization that mixing length theory is only an aspect of dimensional analysis.

In looking ahead one can readily share his optimism and commend his enthusiasm for the future development of the subject without accepting all the details of his philosophy. In particular, one may question the view that the motion of the atmosphere can be divided into a mean motion with a superstructure turbulence, for he does not adequately face the problem of defining the mean. Because of the non-isotrophy and wave properties of the atmosphere one can also dispute his hope that the ideas of Kolmogoroff will be applicable to it. One could quibble over "All predictions of forthcoming weather are statements of the probability of an event"; while it may be all right for insurance companies dealing with many cases, 'probability' has doubtful meaning in the context of a single occasion and continuously variable possibilities rather than discrete alternatives.

One cannot be quite so happy about his treatment of thunderstorms. The ideas behind C. T. R. Wilson's theory of charge separation are good fun, but they have never been enthusiastically accepted. Also the descriptive account of the life of a thundercloud given by the 1946 *Thunderstorm Project* is interesting though unsatisfactory because it has no sound mechanical basis: and so it is a pity that Sir Graham ignores the insight which has been gained in the past eight years into both the mechanics of showers and the role of the freezing of supercoded droplets in producing temperature differences which cause the charging of ice crystals.

To other minds the atmosphere poses other challenges not discussed in this book, but a personal point of view is always the most stimulating. It is to be hoped that the challenge will be accepted by more of our brighter young scientists in the future.

Finally, the British reader should be warned that the book was obviously written for the American market.

R. S. Scorer

LANGUAGE AND MAKERS OF CHEMISTRY

Historical Studies in the Language of Chemistry

By Maurice P. Crosland. Pp. xvii + 406. (London: Heinemann Educational Books, Ltd., 1962.) 50*s*.

Great Chemists

Edited by Eduard Farber. Pp. xxvi + 1642. (New York and London: Interscience Publishers, Inc., 1961.) 222*s*.

THERE are divers ways of tracing the progress of chemistry through the ages, among which a study of the changing modes of expressing observations and ideas, as adopted in Crosland's book, is not the least interesting. From the dawn of alchemy to modern chemistry the written word has always been intimately associated with symbolic representation. Both these forms of expression as used by the alchemists were marked by a picturesque obscurity: this was largely involuntary, seeing that, in the words of Liebig, the alchemists "propounded in an unintelligible language that which, in their own minds, was only the faint dawn of an idea". Sometimes, however, the obscurantism is found to be deliberate on the part of esoteric adepts "whose noble practise doth hem teach to vaile their secrets with mistie speach".

During the alchemical millenium and the later era of iatrochemistry much knowledge was accumulated concerning chemical operations and materials, so that more precise forms of literary expression and an extended symbolism gradually came into vogue. The ancient planetary symbols for the metals were now supplemented by symbols for individual substances, processes, forms of apparatus, duration of operations, and so forth. But a static terminology could not keep up with a dynamic science; for example, with a growing knowledge of the nature of specific substances, such names as *sal polychrestum Glaseri*, *salt of Mars* and *Mercury precipitate* became outmoded. Later, the clumsy nomenclature of the era of phlogiston gave way to the French system drawn up at Paris in 1787, of which Black complained that by using the "latinized French words" of the French chemists it would in future "scarcely be possible to think on chemical subjects in a way different from their theories". In another field of linguistics a similar plaint may be found in an old folksong of the West Frisians: "We will not let our language go, for without the Frisian tongue there can be no Frisians more".

From the time of Dalton and Berzelius, modern chemical expression, nomenclature and symbolism have become increasingly precise and meaningful; so that an amazing amount of information, both qualitative and quantitative, may be conveyed by a chemical equation or a structural formula.

Crosland has rendered a considerable service to chemistry and chemists by producing an integrated account of a scattered and complex subject: it is well expressed, easy to read, and excellently documented and indexed. The five plates include an interesting reproduction of a photograph of the members of the historic Geneva Congress held in 1892 to consider the nomenclature of organic compounds. There are also eight text-figures of symbols.

In 1871 Clerk-Maxwell wrote: "In Science, it is when we take some interest in the great discoverers and their lives that it becomes endurable, and only when we begin to trace the development of ideas that it becomes fascinating". The massive book edited by Dr. Farber enables the reader to follow both these lines of approach in chemistry. A work of this kind cannot take the place of a history of chemistry; but it can become a valuable aid and incentive to all chemists who are possessed, as they ought to be, of a vivid interest in the origins and progress of their science.

The present list of more than a hundred biographical studies is arranged in chronological sequence, with the interspersion at suitable points of four short 'interludes', acting as a kind of connective tissue. These, as also some of the biographies, are written by the editor under the following titles: "Philosophers and Practitioners, from about 800 B.C. to A.D. 800"; "Philosophical Alchemists and Practical Metallurgists, Thirteenth to Sixteenth Centuries"; "The Chemists' Language"; and "Scientists and Industrialists".

Nearly all the 114 numbered sections of the book are devoted to individual chemists, but there are a few collective sections dealing, for example, with Babylonian and Arabic chemists. Chronological pride of place is given to a certain *Tapputi-Belatekallim*, 'perfumeress' of a Babylonian household, whose name, to adopt an alchemical phrase, has been "brought out of the black darkness" of centuries through the medium of a cuneiform tablet of ancient Mesopotamia. The individual biographies vary a great deal in treatment, a feature which breaks the possible monotony of a work of this kind. Thus, Lord Brougham's charming personal reminiscences of Joseph Black could not carry an evaluation of his researches and views such as we find in Prof. Partington's detailed and very fully documented account and assessment of van Helmont. Many of the biographers are themselves eminent chemists, and the articles are often reprints or translations of earlier notices. The sequence extends to modern times, but no living chemist is included.

Portraits of the subjects form a welcome addition, but a few of them have suffered in reproduction: also it would have been of interest to know the ultimate provenance of each. Naturally, a selection of a limited number of great chemists must always be somewhat arbitrary; but it is certainly surprising to miss the junior W. H. Perkin from this list. Indeed, Ruzicka refers to him in this very volume as "the complement of Wallach in the history of the chemistry of the terpenes, as well as of organic chemistry in general . . . the great synthesist, William Henry Perkin, founder and unrivalled master of alicyclic synthesis"—and, of course, of much else in organic chemistry.

This age-long cavalcade of chemistry, charged with human interest, variety and colour, fully deserves the motto of Nicolas Flamel, the Parisian alchemist of the early fifteenth century: *Moult plaist a Dieu procession, S'elle est faicte en devotion.*

John Read

SOCIETY: A PIECEMEAL VIEW

Society

Problems and Methods of Study. Edited by A. T. Welford, Michael Argyle, D. V. Glass and J. N. Morris. Pp. vi+586. (London: Routledge and Kegan Paul, Ltd., 1962.) 50*s*. net.

THIS work is introductory, "intended both for those setting out upon a systematic study of social processes, and also for those whose main interests lie elsewhere but who wish to orientate themselves in particular parts of the field". It consists of thirty-one different contributions, each by a different author or authors, and is divided into two parts, the first emphasizing various approaches and methods and the second examining a number of areas of study.

Inevitably, the particular contributions vary in quality, and in the extent to which they meet the objectives of the book. Some, such as those on the census, on the measurement of abilities, attitudes and personality traits, and on experimental studies of small social groups, place too much emphasis on technical details at the expense of the general significance and limitations of the work discussed. Others are too introductory and descriptive—for example, the contributions on personnel selection and vocational guidance, on urban sociology and on rural sociology. Not many of the articles are really outstanding, although honourable mention should be made of those on social medicine, on social factors in the major functional psychoses, on social mobility and on the sociology of education.

As the editors allow, selection is always a problem in a work of this kind. Nevertheless, some of the decisions made are curious. Contributions are included on road accidents, on operational research and on operational research in industry which have virtually no 'social' content at all, although the questions discussed might well have been approached with this emphasis. On the other hand, a number of important questions in the field of social administration, which are very relevant to social policy, such as poverty, are ignored. These omissions cannot be excused on the grounds either that it was desired to limit contributions as far as possible to those from sociology, or that applied research was to be excluded. The particular examples already quoted suffice to emphasize that the book covers studies of many miscellaneous aspects of social processes, contributed by a variety of disciplines, and ranging from the basic to the most applied approach.

The serious weakness of the book lies, however, in the absence of any overall view. The four editors assure us in the briefest of introductions that "the editorial hand has been kept light"; it certainly has, and in respect of the absence of a satisfactory introduction the editors appear to have underestimated their responsibilities. Particularly in an introductory text, it is essential that the reader be given a clear and concise outline of the wood before he is plunged among the trees. Moreover, as several of the individual authors stress, the boundaries between the different areas they discuss are often no more than convenient conventions, and theoretical considerations seem to suggest increasingly that less attention should be paid to them. An introduction on the main social sciences, emphasizing the distinctive approach and hence limitations of each, would have added greatly to the value of an introductory work of this kind, which is wide-ranging yet very specific in each of its many parts. This would have served not only to highlight the complementariness of the approaches of various social sciences for the study of certain problems; it would also, by providing an overview, have made it easier to appreciate both the relevance of the particular techniques discussed and the adequacy of the research into specific problems which is described.

W. H. Scott

ANCIENT GLASSES

Analyses of Ancient Glasses, 1790–1957

A Comprehensive and Critical Survey. By Prof. Earle R. Caley. (Corning Museum of Glass Monographs, Vol. 1.) Pp. 118. (Corning, New York: Corning Glass Center, 1962.) n.p.

ALTHOUGH the town of Corning, the headquarters of the famous glass-making concern of that name, is in up-state New York, many miles from large centres of population, the Glass Center there was visited by hundreds of thousands of people in the first few months after opening. In the Glass Center the Museum of Glass and the Stuben glass house, where the extremely high-quality decorative pieces are made, are open to the public; in addition there is a fine library of books on glass and facilities are provided for scholars studying the history of the art and technology. As another means of fostering such studies the museum is to publish monographs, from time to time, of which the present volume is the first.

This is a very handsome quarto volume in which the results of analyses of ancient glass are collected, beginning with the work of Klaproth (1743–1817) and covering publication up to 1957. Prof. E. R. Caley, who has for many years been applying his chemistry to archæological subjects, was obviously well chosen for the present task. The first chapter describes in detail Klaproth's analyses, published in 1801, of some coloured Roman glasses, and they were surprisingly good; the remainder of the chapter discusses analyses, mainly of Roman and Egyptian glasses, carried out by various people up to about 1880. The critical commentary directs attention to analyses that would preferably be repeated using modern methods and facilities and, unfortunately, often notes with regret the lack of careful description of the place in which the specimen was found and its probable date.

The long second chapter reports investigations of the past fifty years, many of which are concerned with ancient coloured glasses. Copper and manganese and the ever-present iron are the main sources of colour; some Egyptian yellow and brown glasses are shown as containing up to 1 per cent of sulphur, but Prof. Caley does not appear to suggest that these might be what are now described as 'iron–sulphur' ambers. Several deep blue glasses are mentioned in this section, but cobalt is shown to occur only in a few of these until the work of Farnsworth is reached; he apparently settled a controversy about the presence of cobalt in blue Egyptian glasses and showed that many, but not all, of the deep blue glasses did contain cobalt.

Chapter 3 deals specifically with Egyptian glasses and includes a discussion on the sources of raw material.

The next two chapters consider glass from the Near and Far East and Roman glass, and there is a final chapter on altered or decomposed ancient glass. Altogether it is a most interesting and scholarly book which has set a high standard for the series of monographs of which it is the first. R. W. Douglas

RARE EARTH METALS AND THEIR ALLOYS

Rare Earth Alloys

A Critical Review of the Alloy Systems of the Rare Earth, Scandium and Yttrium Metals. By Dr. Karl A. Gschneidner, Jr. (Prepared under the auspices of the Office of Technical Information, United States Atomic Energy Commission.) Pp. xiii+449. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1961.) 96s.

This compilation of physical properties of the rare earth metals and their alloys is primarily for those engaged in metallurgical research, although workers in the field of applied research and solid-state physics should also find portions of the book useful. Although yttrium and scandium are not members of the lanthanide series, because of their similarity and association with the rare earths, they are included as part of the same group.

The book is divided into four main sections. In the first section the author deals with the physical properties of the pure rare-earth metals. In the chapter devoted to the electronic structure of the metals, Dr. Gschneidner gives the outer configuration as $(5d)^1$ $(6S)^2$ except for europium $(4f)^7$ $(5d)^0$ $(6S)^2$ and ytterbium $(4f)^{14}$ $(5d)^0$ $(6S)^2$. While the majority are predominantly trivalent in their chemistry and Yb and Eu are divalent in the metal, the recognized configuration, at present, of the outer shells is $(5d)^0$ $(6S)^2$ except for gadolinium $(4f)^7$ $(5d)^1$ $(6S)^2$ and Lu $(4f)^{14}$ $(6d)^1$ $(6S)^2$. This should be borne in mind when the author is discussing the validity of the hypothesis put forward by the Russians on the work function of some of the rare earth hexaborides.

Section 2 is concerned with the alloy systems involving rare earth metals as one or more of the components. Each system is followed by information on its crystallographic, physical, thermodynamic and mechanical data. In the introduction to the general alloying theory the author makes use of a modified form of the Darken and Gurry plots for selecting elements likely to form extensive solid solutions. This type of prediction should only be used with care since several examples are known of non-rare earth systems, for example, copper–gold, silver–copper, where extensive solid solutions are formed with the solvent which would be predicted otherwise when based on the given Darken–Gurry plots.

In the presentation of the phase diagrams the author has taken into account the data given by the experimenters reviewed, such as purity and melting points of the elements used, techniques, etc. The resultant diagrams are constructed from the most reliable data, in the author's opinion, and many are composites made up of two or more sources.

Section 3 includes crystallographic details of atomic positions and parameters, and has been arranged according to the Strukturberich notation. In Section 4 there is a list of more than 650 references and an excellent cross-referenced index of the alloy systems.

Dr. Gschneidner has reviewed the data of some sixty-years work and has presented it in a concise and well-written volume. One of the most valuable aspects of this book lies in the comprehensive bibliography of work published up to 1960, and it is as a reference book for those engaged in, or who are about to be initiated into, the metallurgy of the rare earth metals and their alloys that this book will be most appreciated. J. G. Day

POLAROGRAPHY

Progress in Polarography

Edited by P. Zuman, with the collaboration of I. M. Kolthoff. Vol. 1: Pp. xiv+1–356. Vol. 2: Pp. x+357–807. (New York and London: Interscience Publishers, 1962.) 85s. each volume.

These two volumes present a comprehensive international coverage of recent trends and advances in both polarographic research and instrumental development. The individual sections are written by research investigators of acknowledged status in the electrometric field, and although the present publication, for this reason, will possess a considerable appeal to the expert, there is much detail lucidly presented which will prove of greatest value to the tyro polarographer.

Marie Heyrovska, in the first chapter, gives an interesting historical record of the growth of polarographic science, and lists those text-books, journals, reviews and research papers which have been published in the period 1931–60.

In recent years, as a producer of polarographic research papers, Czechoslovakia easily heads the list of those twenty-three countries which have made published contributions in this field. When one considers that this science originated with Prof. Heyrovsky at Charles University, and that Prague has a Scientific Institute uniquely devoted to polarographic studies, the result of this census of related papers is not surprising.

Volume 1 has a distinctly theoretical bias which is largely balanced by the more practical tone of Volume 2. It is impossible in a review of this nature to mention all the eminent contributors by name; but in order to illustrate the general nature of the work it may be noted that Volume 1 has theoretical papers on polarization and electrode kinetics (Randles); double-layer structure (Delahay); kinetic currents (Brdicka *et al.*); adsorption (Reilly), etc. More practical approaches are made by Reynolds (inorganic polarography) and Zuman (organic substances). Fujinaga describes the infrequently practised technique of constant current polarography.

Volume 2 has chapters on modern instrumentation in the United States, Japan and Europe written by Meites, Tanaka and Serak respectively. Square wave and pulse polarography are concisely described by Barker. Zagorski describes recent refinements in polarographic cells; Kolthoff and Okinaka present a valuable paper on newer electrode systems; and Kemula outlines his now well-known chromato-polarographic technique. Milner's succinct description of sample treatment should prove of greatest value to practising analysts.

The second volume concludes with several readable chapters by various specialist authors on the applications of polarography to metallurgical, pharmaceutical, biochemical and other related forms of analysis.

There can be little doubt that as a collection of up-to-date reviews of scientific advances in this ubiquitous field, this publication will win much acclaim from those serious disciples of the Heyrovsky method. D. T. LEWIS

AN INTRODUCTION TO MAMMALS

Introduction to Mammalogy

By Prof. E. Lendell Cockrum. Pp. viii+455. (New York: The Ronald Press Company, 1962.) 9 dollars.

Laboratory and Field Manual for Introduction to Mammalogy

By Prof. E. Lendell Cockrum. Second edition. Pp. v+116. (New York: The Ronald Press Company, 1962.) 4 dollars.

THE production of a book designed primarily for college students and entitled *Introduction to Mammalogy* would seem to be an ideal opportunity to correct the impression frequently given by an orthodox, elementary, vertebrate course that the class Mammalia consists of rabbits and rats (with dogs' skulls). The book is divided into two sections, the first comprising six chapters on the "Biology of Mammals", and the second a systematic treatment of the class with an account of each family. Both parts leave much to be desired. The first section is indeed a useful and comprehensive account of general mammalogy, but it seems a pity that the opportunity was not taken to omit much that can be found in any vertebrate text-book, and include more information on more strictly mammalian characteristics. Much space is occupied by trivialities, for example, the same space (one paragraph) is taken to describe the fluorescence of some pelages under ultra-violet light as to describe the scent-glands, which play a dominant part in the life of most species of mammals. Inaccuracies abound; for example, in the labelled illustration of a skull (coyote) the zygomatic arch is called the rostrum and the mandible the maxilla.

The systematic section would be an invaluable compilation if it were accurate. In fact errors occur on page after page. Each family is accompanied by a map of its world distribution, most of which show gross inaccuracies. For example, the Dasypodidae (armadillos) are shown to be confined to the north-eastern half of South America, when in fact they reach Patagonia; apes are shown on the East African coast; the Indian river dolphin is correctly described in the text as being confined to the Rivers Ganges, Brahmaputra and Indus, but although these rivers are clearly indicated on the map they are left blank, while southern peninsular India is shaded: rhinoceroses are indicated as absent from both India and West Africa, which fortunately is not yet the case; and squirrels are shown to be absent from West Africa, a situation that would save many a taxonomic headache if it were true! The text is scarcely more reliable. The statement that the gaur (the Indian wild ox) is a typical African species is enough to dispel any confidence in any of the great mass of potentially valuable information contained in this volume.

The accompanying laboratory and field manual contains keys to the genera of North American mammals and many useful suggestions for practical work. But the exercise of filling in blanks with observations is scarcely likely to develop an original, inquiring approach to any subject.

G. B. CORBET

PATHOLOGY OF TREES AND SHRUBS IN BRITAIN

Pathology of Trees and Shrubs

With special reference to Britain. By T. R. Peace. Pp. xx+723+16 plates. (Oxford: Clarendon Press, 1962.) 90*s*.

THIS is no mere description of diseases of trees and shrubs. It is far more than that, and it is so compiled and written that it will be of great value to those interested in the pathology of trees and shrubs, be they forester, student or research worker.

More than one-third of the book is devoted to an exposition of the various agencies which may cause damage to trees such as climate, unsuitable edaphic conditions, man, animals, insects and plants. This section includes well-written summaries of diseases of general importance in the nursery and on stems, shoots and leaves. Then follow chapters on the diseases of forest trees grown in Britain and a chapter on the diseases of ornamental trees and shrubs. Control, pruning, tree surgery and quarantine measures are adequately dealt with and there is a valuable list of host species. All this information is due to the author's own experience in Britain, in Europe and in North America, and to his having consulted a vast amount of world literature. The listed bibliography amounts to nearly 2,500 entries and this section alone will be of great worth to research workers and others requiring more detailed information about a particular subject.

Although one agrees to a certain extent with the author's contention about changes in botanical nomenclature, it is a pity that he has held out so strongly against such names as *Picea abies* and *Quercus petraea*, which even most diehards have accepted, except perhaps some French foresters.

The author is quite emphatic about not attempting to provide keys for identification purposes. The book is concerned with the symptoms and diagnosis of tree diseases and therefore details of spore-sizes and microscopic anatomy of fruit-bodies have no useful place in it and would add considerably to the book's already large size if they had been included.

In the preface Peace feels that he may have been over-ambitious in attempting to cater for a wide range of needs. No apology is required, for a work of this kind has long been wanted. It has succeeded by the material provided and by the way it has been presented and illustrated. Tom Peace was chief research officer of the Forestry Commission. This book is a worthy memorial to his work. His untimely death last September more or less coincided with the date of publication. C. J. TAYLOR

THE ROYAL SOCIETY

ANNIVERSARY ADDRESS*

By Sir HOWARD FLOREY, F.R.S.

AWARD OF MEDALS, 1962

Copley Medal : Sir Cyril Hinshelwood, O.M., F.R.S.

THE contributions of Sir Cyril Hinshelwood to chemical research have been recognized repeatedly, and universally. Most of his scientific work has been concerned with the mechanism of a wide variety of chemical reactions, homogeneous and heterogeneous, and in all states of aggregation. These have included processes of fundamental importance in both chemistry and biology. His work has been characterized by a flair for the elucidation of complex processes by the ingenious planning of relatively simple experiments, together with a remarkable insight into the implication of the results. He has influenced the whole pattern of research in chemical kinetics during the past forty years.

His early investigations of the mechanism of homogeneous gas reactions clarified theories about molecular activation through collisions, and also led to new ideas about chain propagation in thermal systems. He developed the theory of branched chain explosions, and by a skilful use of both catalyst and inhibitors he has disentangled many complex reactions involving free radicals. In the course of this work he has made important advances in our understanding of combustion and also of some reactions of hydrocarbons which are of considerable practical significance. From measurements on reactions in the liquid phase he has been able to discover essential differences from the corresponding gaseous processes.

He has applied the principles of chemical kinetics to study the growth of bacteria, and in particular their adaptation when subject to special influences during growth. He has analysed the stages of the growth process and the effect on them of different growth media, of inhibitors, and of specific elements. In this way he has been able to discover much about the mechanism of drug resistance and to provide a new approach to many problems of bacteriology.

Hinshelwood's ability as a brilliant expositor is well known. He is truly a natural philosopher in the classical tradition, versatile in the arts and the sciences, and no one has been more faithful to the aims of the Royal Society for the Improvement of Natural Knowledge.

Rumford Medal : Prof. D. M. Newitt, F.R.S.

D. M. Newitt has been an outstanding investigator for many years in the field of chemical engineering, and is particularly well known for his work at high pressures. Starting in 1923 from work on combustion in gaseous systems at high pressure, he moved on through studies of slow oxidations to reactions in the liquid phase at pressures ranging up to 20,000 atmospheres. He made fundamental observations in physics, thermodynamics and chemistry, and the work involved the solution of major engineering problems in the design of appropriate plant-pressure vessels, compressors and measuring apparatus. The field is complex in its basic science, highly important in its technological application and hazardous in its exploration. Newitt is an acknowledged master in it.

He has also made important contributions to our knowledge of gaseous combustion, ignition, explosion and pyrolysis. In the field of chemical engineering his researches have included important studies of the drying of solids, and of the theory and practice of mining, agitation and granulation.

He has for forty years been associated with the development of chemical engineering as a distinct discipline both professionally and educationally, and he has a distinguished record of public service, both in the two World Wars and in peace.

A Royal Medal : Prof. S. Chandrasekhar, F.R.S.

Prof. Subrahmanyan Chandrasekhar has for many years conducted intensive and highly successful mathematical investigations into the difficult subject of the stability of motion of real (viscous) fluids. It is well known that many of the peculiar difficulties of hydrodynamics arise from the fact that although relatively simple patterns of flow, amenable to mathematical analysis, can occur with certain geometrical shapes of the boundaries, all such motions are inherently unstable in that the patterns are liable to break down if their characteristic parameters exceed certain limits. It is the task of the mathematician to elucidate the mechanism of transition from one state to the other and thus help to bridge the gap between the dynamics of ideal and real fluids.

Such a task calls for the most painstaking and intricate mathematical analysis coupled with strong physical insight. These are the qualities that Chandrasekhar has brought to bear. The problem attracted the attention of mathematicians and physicists since it was first noticed by James Thomson in 1882; but it is to Chandrasekhar that we owe the most thorough-going and complete analysis.

Convection is of fundamental importance to meteorology, geophysics and certain aspects of astrophysics, and Chandrasekhar has extended the original investigation, notably by discussing quantitatively the effects of rotation and of an externally impressed magnetic field. He has also made significant contributions to the problem of the onset of thermal instability in fluid spheres and spherical shells with internal heat sources, essential topics in the examination of patterns of convective motions which might occur in the fluid core of the Earth and in relation to theories of the origin and nature of the Earth's magnetic field. Among other problems that he has studied in the same general field, the stability of Couette motion, of flow between coaxial cylinders and of stratified fluids, must be mentioned. All these have profound implications for the environmental sciences.

Chandrasekhar's superbly efficient mathematical technique has enabled him to replace much of the empiricism which formerly characterized this study by mathematical rigour, and has thus provided the environmental physicist with the tools necessary for the development of the subject on sound lines.

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* Delivered at the Royal Society on November 30.

A Royal Medal : Sir John Eccles, F.R.S.

Anyone who attempts to review the research of Sir John Eccles is faced with a formidable task. Since his early work with Sherrington in the late 1920's, Eccles has produced a steady stream of scientific papers and books. The main theme of his work has been the investigation of the mammalian spinal cord. At first the inquiry was along Sherringtonian lines, and reflexes were described in terms of central excitatory or inhibitory states. Then followed a period in which Eccles tried to frame an electrical theory of central nervous action. Later, he became convinced of the essential correctness of the chemical theory of synaptic transmission, and during the past ten years he has been one of the most enthusiastic exponents of the role of chemical transmitters at synapses. Perhaps his most important contribution is the demonstration that there are clear-cut electrical differences between the central effects of excitatory and inhibitory impulses. Using microelectrodes to measure the membrane potential of motoneurones, Eccles and his colleagues showed that excitatory impulses caused a transient breakdown of the electrical potential difference across the surface, while inhibitory impulses stabilized the potential difference at or near the resting level. More recently, he has directed his attention to the demonstration and analysis of a phenomenon newly observed in the central nervous system, that of presynaptic inhibition.

Besides his massive studies of the central nervous system, Eccles has made important contributions to our knowledge of smooth muscle, of neuro-muscular transmission and of the differences between various kinds of skeletal muscle. Red muscles such as the soleus give longer twitches than white muscles such as the gastrocnemius. The difference resides in the muscles and cannot be attributed to the type of message which travels down the nerves, yet Eccles and his colleagues have shown that if the nerves which normally supply fast and slow muscles are crossed, the fast muscle becomes slow and the slow one becomes fast. The reason for the change in behaviour is unknown, but the phenomenon is clearly of great interest, both for its own sake and for the light which it may throw on the differentiation of tissues during development.

Sir John Eccles has established a flourishing school of neurophysiology at the Australian National University, Canberra.

Davy Medal : Prof. H. J. Emeléus, C.B.E., F.R.S.

Prof. Emeléus has been recognized for many years as an outstanding inorganic chemist, and it is almost entirely due to his researches and his influence that there has been a revival in Great Britain of interest in this field, leading to the discovery of new compounds of unexpected value and importance.

His first interests were the oxidation reactions of phosphorus, sulphur and arsenic, and similar chemiluminescent processes, and he sought to clarify these processes by isolating intermediate species such as sulphur monoxide. His attempt to extend the ideas of reaction chains to the supposedly spontaneous oxidation of volatile hydrides led him into the chemistry of silicon hydrides and chlorosilanes, of germanes and related compounds, fields of inorganic chemistry where little was known. He continued work on the pyrolysis of silanes and germanes, but the emphasis shifted from the physicochemical aspects to the inorganic chemistry of compounds which were, or could be, formed. He studied the chemistry of the higher silanes, synthesized and examined derivatives of the silyl radical, and devised new reactions by which a variety of halogenated silanes are formed.

Some twenty-five years ago he took up the long-neglected subject of fluorine chemistry, and became involved in the separation of uranium isotopes by thermal diffusion using uranium hexafluoride. This work led him to new developments with fluorides of non-metallic elements such as sulphur, arsenic or antimony, and with interhalogen compounds. He studied the reactions of bromine trifluoride and iodine pentafluoride, discovered and interpreted the electrical conductivity and reactions of solutions in these media, and also the formation of new kinds of interhalogen anionic complexes based on the auto-ionization of bromine trifluoride and iodine pentafluoride. He took up intensively the organometallic chemistry of perfluoroalkyl compounds, and this is still yielding a wealth of valuable knowledge.

In the course of all this work, Emeléus has developed new experimental techniques for handling the volatile and reactive metallic fluorides and for studying their reactions. Throughout, he has displayed skill and refinement in the methods he has chosen for isolating and characterizing materials, placing emphasis on experimental and analytical techniques and where relevant on the use of physical methods.

Darwin Medal: Prof. G. G. Simpson, For.Mem.R.S.

Prof. G. G. Simpson, former curator of fossil mammals and birds in the American Museum of Natural History, may be regarded as the leading authority on mammalian palæontology and as one of the most important writers of to-day on the general theory of evolution.

He first established his reputation by two outstanding monographs on Mesozoic Mammalia of America and in the British Museum (Natural History). Since then he has produced a continuous succession of important papers dealing with fossil mammals—in particular those from Palæocene and Eocene levels. Also noteworthy are his studies of cretaceous Mongolian insectivores, of the Oligocene tupaiid *Anagale*, and of Palæocene and Eocene primates.

His fine book on *The Principles of Classification and a Classification of Mammals* was published in 1935. This extensive study is now regarded as the standard work of reference on the classification of mammals. His book on *Tempo and Mode in Evolution*, published in 1945, is perhaps the most serious attempt yet made to apply the data of palæontology to the quantitative study of rates and determinants of evolution, and the elucidation of evolutionary factors such as the role, intensity and direction of selection, the nature of adaptation, and the mechanism of speciation. His most recent book, *The Principles of Animal Taxonomy*, is likely to be for many years the standard work on one of the most important branches of biological thought.

Buchanan Medal : Sir Landsborough Thomson, C.B., O.B.E.

Over a period of nearly forty years, Sir Landsborough Thomson has given outstanding service as an administrator of research in the medical and biological fields. He joined the headquarters staff of the Medical Research Council in 1919 when it was still a small organization, and remained with it as deputy to the Secretary until his retirement in 1957. During that period the Council's activities and responsibilities expanded until they were of national scope, and it was Thomson who was primarily responsible for its administrative policy and method of working. By his consistent good sense and informed understanding he maintained within the Council's organization that administrative flexibility which is essential to any organization which aims at forwarding the progress of research.

Thomson's approach to each problem as it arose showed his high quality and the breadth of his wisdom, so that his advice was widely sought not only by his administrative colleagues but also by research workers both within and without the Council's employ and on problems which concerned other organizations besides his own. His combination of administrative skill and scientific understanding made his administration a model which had influence beyond his immediate field.

In this achievement Thomson was substantially helped by his own scientific interests in biology, interests which led him ultimately to election as president of the Zoological Society of London.

Hughes Medal : Prof. B. Bleaney, F.R.S.

Prof. Bleaney is distinguished for his experimental work on magnetic and electrical phenomena and the interpretation of the results in terms of atomic and molecular properties.

During the Second World War he was engaged on the design of the first klystron oscillators for the production of 3-cm and 1-cm waves, and constructed prototypes for the models which were later produced commercially. He used these new sources of long wave-length radiation to make the first analysis of the spectrum of ammonia by what is now generally called microwave technique. He also made measurements on the broadening of these inversion lines both by ammonia itself and by added gases, and obtained estimates of the collision diameters.

Most of his other work has been connected with paramagnetic resonance. He first made measurements at low temperatures and showed that transitions occur in the chrome alums. Later, he discovered and analysed hyperfine structure with manganese and cobalt salts. He has determined the nuclear spin moments, magnetic moments and electrical quadrupole moments of a large number of nuclei, including some radioactive species. His paramagnetic measurements have also been applied in studying the structure of a number of inorganic crystals. His early work was the basis of theories on the paramagnetism of the rare earths and of neptunyl and plutonyl compounds.

He suggested the first successful method of nuclear alignment, which has since been widely applied.

ACTIVITIES OF THE ROYAL SOCIETY

This year is the tercentenary of the granting of our first Charter by Charles II, and the occasion was celebrated by a soirée at which we were honoured by the presence of one of our Royal Fellows, Her Majesty Queen Elizabeth, The Queen Mother. This important milestone in our history has been marked by some evidence that the activities of the Society are still growing, for our capacity to foster research has recently been substantially increased. The Government has promised us funds to establish five research professorships, to which are attached resources to finance at least part of the work associated with them. Our gratitude to the Government is great for what is, in effect, a very large new endowment. This recognition of the part we can play in helping investigation is greatly to be welcomed, both for itself and because it directs attention to a way in which we can keep vigorous the Founding Fathers' idea that the Society exists for "improving natural knowledge". In the rapidly changing times in which we live some of the functions that the Society used to have may disappear, and during the coming years interest in promoting research may have to be exerted in ways undreamed of by the original Fellows. Let us hope that the Government's initiative will prove fruitful in our hands, and that in the future the number of such professorships will be increased still further.

We are also indebted to the Leverhulme Trust for asking us to administer a new scheme for appointing visiting professors to India. Such a scheme is perhaps an example of how we may all profit from the changing conception of Commonwealth ties.

The fact that we have been supported by both the Government and the Leverhulme Trust in this way is a clear indication that confidence still persists in our capacity to assist scientific research. But the present complexities of the financing of science by the Government has made unnecessarily difficult certain activities which the Society used to administer with relatively little trouble. I refer to such matters as organizing expeditions promoting investigation of the Sun, fostering foreign relations and so on. We can, therefore, heartily welcome the setting up of a Government body, commonly known as the Trend Committee, to review the methods of financing civil science. We hope that, among other things, some of the Society's difficulties will be lessened as a result of its deliberations. As Fellows know, the Society is doing its best to produce for this Committee the considered views of its fellowship.

I should like to mention two other matters which appear to be of more than usual importance. I said last year that the possibility of improving the accommodation of the Society, which has been deficient for so many years, was being explored, and I had hoped that by this anniversary meeting I should have been able to tell the Society in some detail what has been going on. All I can say at present, however, is that negotiations for improved accommodation have proceeded so far that unless unexpected and insoluble difficulties arise I shall look forward to giving a cheerful presidential address next year on the history of the accommodation of the Society, thus bringing up to date what was said by Sir Henry Dale in 1943.

The other matter which has occupied a good deal of attention of the Council and Fellows is whether we should increase the number of Fellows elected annually. It appears that there is a fairly even division among the Fellowship as to the wisdom of increasing the numbers at this time. As a preliminary to taking a decision in this matter, a Committee of Council has overhauled the methods of nomination and selection and has made suggestions which we hope will be an improvement on previous practice. In due course new draft statutes will be presented to Fellows.

During the often interesting discussions which took place on these matters, I fancied that I detected some signs that the members of most sections thought that they could do with a few more places, but that their colleagues in other disciplines were not quite so well placed: and further, I believe I saw signs that those on the 'A' side were a little worried that those of us on the 'B' side were not quite up to standard. There is little doubt that one of the difficulties of the times is for scientists trained and oriented in one or two disciplines to understand what colleagues in other disciplines are doing, and indeed how they set about solving their problems.

SCIENCE AND HUMAN PATHOLOGY

It occurred to me that as those who attend the anniversary meeting have not been forced to listen to a pathologist before, I might be permitted to spend a little time in letting those Fellows who are not professional pathologists or medically qualified have a glimpse of some of the methods that are used to try to elucidate the causes of disease, and to show that the solution of many pathological problems demands the employment of diverse techniques.

There is perhaps at the present time a tendency to disparage too many scientific observations which have no experimental content, and indeed I was somewhat startled this year to know that a certain body had turned down some proposals carefully thought out by a Committee of the Society on the ground that the intended work was merely the collecting of data. A great deal of pathology, at least in the past, has been data-collecting, and it would be a bold man who would proclaim that this had been unfruitful. Perhaps I can illustrate my point adequately if I select one disease which is now a household word. I refer to coronary thrombosis, a condition which is responsible for much disability and many deaths, and of which we hear a great deal too much in connexion with our friends. Much of our knowledge of the underlying changes in this condition has been acquired by the classical methods of pathology, namely, observation of the diseased parts after death and the correlation of the findings with the symptoms that the patient had while alive.

The disease of the arteries underlying coronary thrombosis is now commonly known as atherosclerosis, and that it is not exclusively a by-product of modern civilization is demonstrated by the fact that fragments of the aorta from Egyptian mummies dating from about 1500 B.C. to A.D. 525 show typical lesions. It is perhaps not inappropriate that one of the earliest descriptions of the disease is that of the aorta of a famous anatomist, J. J. Wepfer, who died in 1695 at the age of seventy-five. The picture of his aorta has a somewhat macabre association for it was published in a posthumous edition of Wepfer's own anatomical observations.

So long ago as 1770, Edward Jenner, famous for his introduction of vaccination, and who, may I remind you, was a Fellow of the Society, first noticed the connexion between certain pains in the chest and narrowing of the arteries supplying the heart. In 1850, Cruveilhier connected disease of the arteries of the heart with alterations in the blood which led to obliteration of the arterial lumen. The activities of the morbid anatomists and morbid histologists, which have been intensified in recent times owing to the apparent great increase in the evil consequences of arterial disease, have now furnished a picture of what may happen in the arterial wall.

The wall of a normal artery, such as one of the coronary vessels which supply the heart, consists of an inner lining of flattened cells, called endothelium, which adhere to a tissue consisting of mucopolysaccharides in which are interwoven fibres of collagen and elastic tissue. This internal layer, known as the intima, lines the main pressure-resisting layer, known as the media, which consists of elastic tissue, collagen fibres, and smooth muscle cells. External to this there is an adventitia which consists of small blood vessels and fibrous tissue. The changes leading to well-developed atherosclerosis occur in the intima just subjacent to the endothelial lining. Precisely what the initial changes are still escapes detection for even the electron microscope has so far not yielded anything of a striking nature.

Features of the fully developed disease are, however, easy to recognize. The obvious lesion of atherosclerosis is thickening of the arterial intima due to the formation of new collagen fibres and elastic tissue, an increase in the number of smooth muscle cells, and the deposition of lipid substances. Not only does this thickening tend to constrict the lumen of the vessel, but also its presence may encourage the formation by the blood of a thrombus at the site, leading to the all too familiar disease of coronary thrombosis. The thickened intima, besides containing deposits of lipid, often in the later stages accumulates considerable amounts of calcium salts, with consequent hardening of the vessel wall in that part.

The prominent lipid material, which is composed of cholesterol and cholesterol esters as well as phospholipids and glycerides, has attracted attention for more than a century, and the theory has been propounded that the disease is produced by abnormalities in the blood lipids, so that these are deposited in the intima in excessive quantities after being released from combination in the lipoproteins of the blood. When not combined in the lipoproteins they are relatively insoluble, so they may remain in the intima for an indefinite time.

To test these views, an enormous body of data has been collected on the level of cholesterol, the phospholipid/cholesterol ratio and lipoprotein concentrations in human blood, in populations living in a wide variety of nutritional and environmental circumstances. These statistical studies tend to show that populations that have a low level of blood cholesterol have relatively little coronary thrombosis. Whether this type of research will provide crucial clues about the cause of the disease remains to be seen, but up to the present no proposition arising from these investigations has received universal acceptance.

Other methods of investigation depend on the work of biochemists and physical chemists, who are investigating in great detail the composition of lipids met with in the body and in arterial lesions. Considerable interest has been aroused in the discussion of whether the kinds of fatty acid which are met with in lesions of atherosclerosis are of importance. One school of thought maintains that the polyunsaturated fatty acids are all-important and that a relative lack of these in the diet is the cause of the disease, while others on what appears to be good evidence deny that this is so. So far a mass of statistical, biochemical and biophysical observations has not produced any satisfactory or agreed solution about the cause of the disease.

It may well be asked whether a crucial experiment could not be performed to demonstrate whether cholesterol really is of importance in the development of arterial disease. Experiments, which at a first glance would seem to have returned an unequivocal answer to this question, were performed by Anitchkov more than forty years ago. He fed very substantial amounts of cholesterol to rabbits, which normally have a low level of cholesterol in the blood and a low intake of cholesterol from the foodstuff. The rabbits developed after some weeks or months lesions of the arterial wall which bore some resemblance to those of the human disease; but these and similar experiments in other species suggest that the lesions produced are not identical with those seen in man. Nor after many investigations is it even clear precisely how the cholesterol is deposited in the arterial wall.

At the present time the possibilities of comparative pathology are being explored by investigating whether the natural arterial diseases which occur in domestic and wild animals have any similarity to those of man. If a species could be found in which the lesions that occur spontaneously are so far as can be ascertained identical with those in man, the way would be open to a more penetrating experimental study. Some observations of great interest have been made during recent years on pigeons. It has been found that in a breed known as White Carneau, gross lesions occur in the aorta of all birds more than three years old, and that these lesions bear a striking histological resemblance to those of man. But of further interest is the fact that while White Carneau and Silver King pigeons develop lesions of this nature, other strains known as Shoe Racer and Racing Homer do not, even when fed on identical diets of grain and kept under the same conditions of housing and exercise. The occurrence of these susceptible and non-susceptible strains has enabled their discoverers to make a comparative study of the various factors which might be concerned in the development of lesions. Their different susceptibilities cannot be attributed to differences in the cholesterol phospholipid or β-lipoprotein levels, for both susceptible and non-susceptible strains have what would be considered in mammals a high blood cholesterol, of the order of 300 or more milligrammes per cent. There is certainly a genetic element here, and at the present time a detailed examination for possible inherited biochemical abnormalities is being conducted on this species.

Recent investigations of the domestic pig have shown that it suffers from a disease which appears to be indistinguishable from the early stages of the disease in man. In my opinion the way is open for the performance in this species of some decisive experiments on the role of diet in the genesis and development of atherosclerosis. The pig resembles man in its omnivorous habits, and surpasses man in its ability to take diets of peculiar composition without complaint. It is indeed an excellent experimental animal for the exploration of dietetic factors. Such experiments might well resolve some of the unsolved problems which have been thrown up by the collection of data in man.

While much attention has been concentrated on the behaviour of lipids, both in the arteries and in the body in general, it must not be forgotten that other components of the tissues are altered in arterial disease. There may well be changes in the mucopolysaccharides of the matrix in which the fibres of the intima are set, but methods for investigating these substances are very imperfect and so far no clear indication has been obtained that the origin of the disease is to be found in aberrations of the mucopolysaccharides.

Changes also occur in the elastic tissue of the vessel wall very early in the development of atherosclerosis. It is not very many years since atherosclerosis was generally considered to be a natural part of the ageing process in the human body, and indeed it is well known that the disease is more frequent in the ageing than in the young. There is probably a good deal of substance in this idea, for it is difficult to believe that the elastic tissue of the arteries does not go through much the same changes as the elastic tissue of the skin. We have only to look at the faces of our colleagues to see the signs of ageing, from the fact that their skin becomes less elastic and their faces tend to slide towards their necks. Nevertheless, highly developed atherosclerosis can occur in quite young people, so that it seems right for the present to consider the arterial lesions as a real disorder of the tissues and not simply a result of ageing.

There is another possibility which is now being vigorously investigated, and which may be connected with the great increase of coronary thrombosis in recent times. This concerns the composition of the blood. It is sometimes supposed that thrombosis, the blocking of the blood vessels, is in all respects similar to the clotting of blood in the test tube. Nothing could be farther from the truth. Some eighty years ago small formed elements, much smaller than the red blood cells, and measuring some 2–3μ in diameter, were detected in shed blood; they are now known by the name of blood platelets. Normally, platelets circulate in the blood as discrete bodies, but a thrombus which is essentially an agglutination of platelets can be formed from flowing blood by deposition on the vessel wall. Afterwards, fibrin and blood cells such as the leucocytes and red cells collect in connexion with the platelets.

The electron microscope gives a very interesting and clear picture of the structure of platelets. They are found to consist of membrane-bounded material containing vesicles and granules, much like the cytoplasm of an ordinary cell, but without a nucleus. When agglutination occurs the platelets become closely applied to one another but the actual physical nature of their adhesion is unknown. It is certainly not due to dissolution of the platelets to form a structureless mass. The bounding membranes at least at the beginning of the process remain intact, though the granules are gradually discharged.

Platelets are now known to contain considerable amounts of adenosine triphosphate and it has recently been shown that adenosine diphosphate causes their agglutination. These observations have been carried one stage further, and it has been shown that adenosine monophosphate inhibits the agglutinating action of adenosine diphosphate. Investigations of the properties of the platelets by biochemical means correlated with observations in the electron microscope proceed apace, and it is conceivable that in a few years we shall have a clear picture of what happens during the interesting process of thrombus formation.

It would take me too far into the minutiæ of pathology to discuss the ways in which the production of thrombi may be linked at various stages with the changes which occur in the arterial wall, apart from the ultimate thrombus formation which may block the vessel. But I hope I have been able to show you how a chronic disease, of such widespread distribution that no one in this room is free from it, is being investigated by the accumulation of data from observations on man and his environment, by biochemical investigation on man where this is possible, on animals as a substitute for man, and *in vitro*. These investigations are coupled with the use of the most modern histological and microscopical techniques.

It is perhaps noteworthy that the greatest advances of medicine during the past century have been in the realm of infectious disease. These successes were based on the fundamental discoveries which were made by Pasteur, Koch and others of the relation of micro-organisms to disease processes. Many chronic diseases, of which arterial disease is one, have not as yet been shown to have any comparable unifying thread to connect them. They are at the stage of examination by the collection of data from whatever source they can be gleaned. If in the future a clear picture of what happens during the development of arterial disease is obtained, I hope that the person who records the last observations will not be credited with making a 'break-through'. We hear too much of 'break-throughs' in these days, for in my opinion such language tends to obscure the fact that all of us depend on the work of our predecessors. This to my mind is nowhere more clearly demonstrable than in the steps that lead to the elucidation of the complicated changes of pathological processes.

EDUCATION OF A SCIENTIST*

By SIR CHRISTOPHER INGOLD, F.R.S.

LANCASTER is to have its own university, and is thus to become one of the nurseries of the country's future leaders. So, Lancaster will soon be wrestling with the strategic problems of higher education; and, less bound by the chains of tradition even than universities established only a few decades ago, it will be taking, not just a part, but also, in its early freedom (which it will never get twice), a pioneering part, in the desperately urgent task of adapting our approach to higher education to the rapidly changing times, in fact, to the present scientific revolution. This is a matter in which the Lancastrian Frankland Society is bound to interest itself.

I have referred to the contemporary 'scientific revolution', a terminology which compares it, by implication, with the mechanical revolution of 150 years ago. In his *Outline of History*, H. G. Wells declares that Napoleon, despite the great space given to him in history books, had no permanent effect on history, comparable with that of, say, James Watt and other pioneers of the mechanical revolution, whose work transformed industry and changed the whole character of life in all civilized countries, permanently. It seems clear that the present scientific revolution will prove as lasting, and as profound. Already, though it is yet young, we see it contracting the world, transforming war, deeply affecting international relations, accelerating social change in developed and in developing countries, pervading industry and hence the economic foundations of civilized life, and modifying almost everything—what we eat, wear, and do—in our daily lives. All this has happened at a tremendous speed, all within the past forty years, and mostly within the past twenty. The massiveness of this uprush of science and its applications, and the suddenness with which it has gathered momentum, are well brought home to one in the computation that 90 per cent of the scientists who have lived since the world began are alive to-day.

So the scientific revolution is with us, and we have had plenty of time to recognize it. What then should be our correspondingly adjusted attitude towards education, in particular, towards the critical matter of the balance of the educational disciplines? I think that everybody knows the answer, though some may be more willing to express it than others. In any event, my answer is that, while all the well-trodden paths of learning are desirable vehicles of education, and are deserving of devotees, the classics, modern letters, and the sciences, education in science is in a class apart. It is not only desirable, as a part of modern culture; it is also essential, much of it for those few on whose foresight our future will depend, much of it for the many whose scientific work will build our future, and some of it for everyone who would comprehend something of the world in which he has to live.

Some science for everyone! If there was ever any health in what has for so long been accepted as the two cultures, there is none now. Reconsideration of our educational methods is overdue.

The reconsideration must begin at school. Our traditional schools have done more directly than any other national institution to create and maintain the two cultures, through the specialization allowed, and even enforced, by their division into 'sides', of which at least one, often one to which a special prestige is supposed to attach, is innocent of science. The Russians, more realistic, and less fettered by tradition than we are, have not made that mistake. Throughout the U.S.S.R., there is universal education to the age of eighteen, though at the age of fifteen the less academically minded children are transferred for their last three years to academically easier, more or less vocationally slanted schools, so that only a proportion of the original intake remains for those last three years in the essentially academic schools. But even before this parting of the ways, already at the age of thirteen every Soviet boy and girl that is born into this world alive starts physics; and at the age of fourteen, every one of them starts chemistry. In the academic schools, beyond the age of fifteen, these foundation subjects of science are continued, as is mathematics, a modern non-Slavonic language, and, of course, literature and history; and some optional extras may be taken or continued. So in the U.S.S.R., you can start a conversation on a scientific subject, let us say, for example, on the photochemistry of the upper atmosphere (the Russians are interested in the upper atmosphere), with a not-too-old stranger in the street, as you could not in England, with a fair certainty that you will be understood, and that he on his part will be well able to discuss the matter. I assert that no one in England to-day should be let out of a secondary or grammar school without a comparable introduction to physics and chemistry. These foundation subjects of science should rank among the foundation subjects of everyone's general schooling.

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* Substance of the Eighth Frankland Oration, delivered to the Lancastrian Frankland Society, Lancaster, on October 19.

My education in school and university occurred fifty years ago. I read science from firm choice. More prestige was attached to modern letters, and more still to classics. No one then, that is, around 1910, had the remotest inkling of the impending uprush of science, or of the reversals of esteem that would inevitably follow.

Next to science itself, modern languages have gained in importance from the upsurge of science. The world is so contracted, and the need to develop a sense of commonweal has become so urgent, that language barriers are a danger: we all (not just a few of us) must read, understand, and talk with our neighbours, only a few hours' flight away. Therefore, the learning, in England, of modern languages, at least two, should be a primary school study, to be commenced at ages eight and nine, as it is in a number of European countries. Intelligent parents well understand that, at such ages, the first steps in a new language can be absorbed with a sense of fun, though a few years later, competing deeper intellectual interests will reduce them to drudgery. But if any new means of communication can be established in rudimentary form while the fun of it lasts, then its use and development in the grammar school could create interests comparably deeper with those of concurrent studies.

In proposing the inclusion of sciences and of more modern languages among the foundation subjects of general education, I am, of course, asking for substantially less specialization in the schools. Now you know the answer of the schools: each schoolmaster says in effect: "If my school did not specialize, the universities would prefer the pupils of another school that did, because its pupils would have more to show in the particular subjects they would propose for study in the university". I think that this argument is unanswerable, except by a complementary policy, covering the transition from school to university, and concerned with that much-neglected process, the conversion of the school child into the university student. I want to deal with that conversion last, and, before doing so, to look at its end-result, the university student, who we will accordingly, for the present, suppose has somehow become habilitated to his new situation. Let us then look at his situation, in particular, the kind of training he will thenceforth be receiving. This is the stage at which the various fields of study, science, letters, and so on, diverge, and a choice has to be made. Accordingly, I shall, from this point on, follow the course of university education in science.

At this level, there are two ways of presenting the subject-matter of science, namely, with the spirit of science, or without it. The matter with the spirit, I shall, for short, because I have much to say on it, call 'live' science, and the matter without the spirit, 'dead' science: the simile will be clear. If you want to make a scientist, you need to teach him 'live' science, because he has first to feel, and eventually to absorb, the spirit of science.

That spirit may be defined as the urge to disclose the ever-marvellous truth around us—*ever* marvellous, because it is dynamic, changing always as approaches are pressed or broadened, and, as we conceive it, subject to change unceasing, as new wonders disclose themselves. The university lecturer in science who cannot send out that signal had better give up. But the spirit I have just defined is the spirit which impels scientific research. That is why 'live' science teaching, the only kind that can build the next generation of scientists, must be done by research workers.

I may see this myself with a special clarity because of an experience of fifty years ago, which can be told now, as I am the sole survivor among the actors concerned. At school, I enjoyed both physics and chemistry, but physics distinctly the more. At college, I studied both with equal emphasis for a considerable time. I was taught physics by expert and devoted teachers, totally dedicated to the pedagogy of physics, and asking no reward higher than the excellent examination results that most of their pupils obtained. These teachers presented physics as a system of exact laws, which one had only to learn to apply. It was so tidy that one could scarcely go wrong. I did particularly well in my examinations, and my teachers could not understand why I elected thereafter not to specialize in physics. However, as I saw the matter, physics was tidy, but dead. Lord Kelvin had finished physics. So what could one do ? One cannot live with a corpse, however tidy. The mysteries that Planck and Einstein had already touched on even at that time, that is, around 1910, were never put before me; you see, they were not required in the examination.

I was taught chemistry by research workers, whose approach was very different. One in particular, D. R. Boyd, a brilliant teacher, made us from the outset feel that we were partners with him in an attempt to understand what we could of how Nature worked. He presented his science strictly fairly, strictly truthfully, including its imperfections, never over-simplifying, never glossing over obscurities, distinguishing sharply between what was known, what might be surmised, what was dubious and what was completely mysterious, giving reasons. He would summarize complicated factual situations with a skill that made reception painless—the skill of a barrister that he was in fact qualified to be, though he had never practised at the bar, but had become a professor of chemistry instead. So, by example, he taught us the arts of judgment, of scientific appraisal, of assessing possible significance, of scientific insight; and he showed us how continually one has to exercise these mature qualities of thought in a 'living' science. The frontiers of knowledge are never far away in any science, and he revealed them to us constantly, sometimes explaining how a sector had recently been pushed back, or pointing out what needed to be done to push it back. Obviously, chemistry was alive, pregnant with advance and adventure, unfolding on all sides in new revelations and new mysteries. I did not get such good examination results in chemistry. But what did that matter ? I did receive the spirit of science, and so, for that much better reason, I elected to specialize in chemistry.

That reminiscence, which I hope you will excuse, will serve to illustrate the first three major points that I want to make about university science, namely, the sharp contrast between 'live' and 'dead' science teaching, the intimate mixing by compulsive common motivation of the former with research, and the absolute necessity for 'live' science in the training of scientists.

One does still hear teaching and research set in contrast; but that is only the hang-over from an exploded, but formerly hard-worked, though never very credible defence of the university teacher who takes 52 weeks' salary for 30 weeks' part-time work. "Some are born researchers, others are born teachers, and we need both"—so the argument ran. We in universities know just what that is worth, but the educated general public, including even some civil servants, seem not to. I recently read an official paper in which occurred the phrase, "the balance between teaching and research": and the context showed that "balance" meant balance. A no-doubt carefully educated civil servant, writing on university education (so you would expect him to know something about it), obviously did not know the first thing about university education in science. He did not know that live science teaching and research are intrinsically inseparable, and therefore cannot possibly be weighed or balanced one against the other. The rule in science is that the best researchers are the best teachers. Nearly always, they are the keenest teachers, and always, they are the most inspiring. Remember, these researcher-teachers are the individuals on whom we are wholly dependent for the production of the next generation of scientists.

A side-deduction from this is that we should not as a nation set up research institutes covering any of the main subjects of formal instruction to science students. For such institutes do not take care of the scientific succession: they sequester the men who should, by influence and example, be pointing the way to the next generation of scientists in their formative years. Research institutes of more specialized types are, of course, in quite a different case. I think that Britain is broadly sound in its policy on this matter. I could not say the same of all countries.

I might, perhaps, here slip in the explanation that my remarks on science have no concern with mathematics, which is not a science, until so applied, but is intrinsically a technique of thought, highly interesting it itself, and indispensable to many scientific, and to certain other, disciplines.

Let us now return after these digressions to our science student. Let us assume that he has been exposed for some years to 'live' science teaching (such as I had in chemistry), with the result that he well understands that science is a dynamic unfolding, and that, in that light, his interest, indeed his dedication, stands confirmed. Let us assume also that he has acquired some scientific background knowledge; how much, his bachelor's degree will show, though he will have to learn more afterwards. Finally, let us assume that he is beginning to build up a capacity for scientific judgment. All that is still not enough: he has to have technique. This involves going under a master for several years of research under personal guidance, at least to the stage which is usually marked by a doctor's degree. Some of those who eventually go farthest take an additional period of research under partial and relaxing guidance, before they consider themselves competent scientists.

Why is this apprenticeship so long ? Why does it depend on one master after another ? The answer lies essentially in the great difficulty and complexity of modern science. The arts are in a different case. The man of letters is usually self-made, and his work is strictly individual. A historian gathers scattered information, writes his book, and launches himself: he does not have to be launched by another historian. But every scientist comes out of a scientist: no modern scientist could launch himself unaided. One could write many scientific genealogies, reminiscent of the "begat" lists in the Old Testament.

Let us further consider the environment in which our scientist-to-be finds himself during this last phase of his training. Conditioned by the complexity of modern science, it is an environment of elaborate organization. Times have changed since the turn of the century when the Curies went into a dilapidated back-shed for four years, and with shovels, buckets, stone jars and beakers, isolated radium. To-day, techniques are enormously more powerful, but also enormously more complicated, diversified, and specialized. The first result of this is that even the scientific master can to-day do nothing by himself: he does not know enough: he is not skilful enough. He must enter, or gather around himself, and, with his students, work within, an organization of other scientists and technicians, having knowledge and skills complementary to his own. That is why scientists work in groups, in which constant mutual help is the tacit rule of life, and scientific cross-fertilization is accordingly universal and continuous. Scientific success goes to the strong groups, those that make themselves strong by thus harmoniously and intimately combining a maximum of complementary intellectual endowments and interests.

I hope that the new University of Lancaster will remember this when planning its scientific faculties. Science should be organized in the largest administratively feasible units. 'Little Kingdoms' are a source of weakness; and any eliminable internal frontier is a source of both weakness and waste.

I have so far discussed the situation of our potential scientist, first, as the schoolboy with science as one possibility before him, and then, later in his career, as the fully dedicated science student. I want now to fill the gap, that is, to consider his transition from the first of these two states of life to the second. Two problems are involved, one of adaptation, and one of selection; and they are closely interlinked.

Throughout most of the life of the schoolboy, the teaching to which he is exposed is necessarily of an *ex cathedra* character, and his attitude towards it is necessarily one of reasonably disciplined receptivity. He accepts his lessons as authoritative, does the prescribed homework, learns what he is told to learn, and practises the techniques in which he has been instructed.

In a university, the conditions of education are very different. The method is that of guided self-tuition. You are assumed to be a student; and receptivity will not make you one. A student must be purposefully such, with self-motivated, self-organized habits of study, and with an already mastered technique of self-tuition. He must learn in that way, with no more than general guidance, what can be gained from books. The lectures, which, as a science student, he will attend, will be directed fundamentally to the development of scientific judgment and scientific insight, things that cannot be obtained from books.

Now the adjustment of attitude required to convert the receptive schoolboy into the independently purposeful and self-organized student is profound. A new technique of learning has to be acquired. It must take time. In my opinion, a year would not be too long to allow. In general it cannot be done in universities, in which, as most are now organized, there is no spare time.

I was fortunate fifty years ago. In my last year at school I had no formal science lessons at all, though I received invaluable help from the masters. But I composed my own lessons, and prescribed my own home-work, with no more than general guidance from them. So my schoolmasters taught me the first of all academic lessons—that of how to learn: and when I later walked into a university, and was automatically treated as a fully re-adjusted student, I was one.

In the very best schools to-day, the method of work in the sixth forms is almost exclusively that of guided self-tuition. The boys that come out from these forms are students, ready for university education. But such schools are not in the majority. In most schools the children are lessoned and coached to the bitter end. When these pupils gain entry to universities, as they do in large numbers, they go in completely unadjusted, lost souls in an uncomprehended world. Only the toughest will survive unharmed. The rest will leave, some sooner, some later, all with more or less blemished records, which may never have been blemished before. It would have been better for them not to come. They constitute the main part of what is called 'student wastage': and it may not be because anything is wrong with them. Rather, it will be because they never had a fair start. They began with a handicap, from which it is extremely difficult to recover. 'Student wastage' is a term which summarizes severe discouragement to many young persons at the start of their careers, to more, indeed, than statistical returns plainly show, because the grievousness of 'wastage' is recognized, and so it is concealed as much as possible.

It is, of course, the competition for university places that creates this situation. Only the very best schools can rise above it, that is, refuse, despite parental pressure, to push into universities those of their pupils who could not get there with no more than general guidance. The fact is that many more of our population aspire to university education than have any notion of the severity of its rigours, or are constitutionally suited to withstand them.

So, between school and university, selection has to be applied. Great effort is expended in universities in trying to do this job well, that is, so to select as to eliminate 'student wastage'. Actually, the job is done badly: 'wastage' continues. The reason is that any properly acceptable candidate should satisfy on all of three criteria, on two of which one can, at the time of selection, get no information. The criteria concern academic attitude as just discussed, native capacity, and acquired information. Only the last of these three requisites has a yard-stick, that of examinations, which in fact jointly measure the pupil and his schoolmaster in some unknown proportion, and hence give no clue to capacity, or to attitude. Remember that the applicants for any one university come from all over the country, and that it is therefore quite impossible for the admitting officers adequately to know all the schools. As to interviews let me speak for myself, and say that I cannot assess either of the two vital imponderables on the basis of a half-hour interview. Most of the candidates know the right things to say, and those that do not may well do themselves less than justice. But a decision has to be made; and so, after trying everything, one comes back to the only, though woefully inadequate, yard-stick, and, more often than not, decides accordingly, with not much better than even chances of being right.

I think that there is an answer to this double problem of adaptation and selection. It is to shift the moment of selection from where it is now, between school and university, to one year after entry into the university. This partly resembles what is done in Scotland, and it is what is done in Australia. The university runs a first, relatively unselected, propaedeutic year, and then, after selection, three following years leading to an honours degree. The original acceptance is based purely on a school-leaving examination, and it commits the university for no longer than one year, at the end of which selection is applied. Under this system, anyone with a reasonably good school record, who wants a year in a university, can have one; and so many will get no more that no stigma attaches to the shortness of the period. Those short-term students will consolidate, and somewhat enlarge, their school knowledge; and, what is more important, they will gain some independence, and capacity for responsibility. But those who will be kept on to read for a degree will have this preliminary year, in which with help of staff, to lose their school attitudes, to habilitate themselves as students, and hence, if they are science students, to fit themselves to feel as partners with those under whose influence they will come in the three subsequent years. For its part, the university will have the year in which to find out all it needs to know for the purpose of rational selection.

There would be no sense in minimizing the operational size of this proposal. It means new university buildings, and new staff. The propaedeutic year, with its large numbers, and very special pedagogic objectives, quite different from those of university education as generally understood in England, could not, in science at least (and science is all I can speak for), be run by the same people as take care of the subsequent years. In any science subject, the territory, equipment, staff and form of organization of the propaedeutic year, would have to be distinct. Exactly how this is arranged is less important, but not unimportant. One way would be to leave the first-year organization in any subject as a 'junior branch' of the relevant department. Another way would be to put together the first-year organizations of all subjects, and call the result a college of the university. I think myself that the latter kind of arrangement might well turn out to be the happier.

In conclusion, let me recapitulate, now in chronological sequence, the changes in education which I think necessary in order to produce citizens adapted to the scientific age, and, in particular, to create the scientists themselves. I should like to see at least two modern foreign languages started in the primary schools, and kept up in the continuation schools. I should like to see substantially less specialization in the grammar schools, with physics and chemistry, the foundation subjects of science, made foundation subjects, in secondary and grammar schools, of everyone's general education. I should like to see the burden of competing for university places taken from the schools, and the burden of selecting on inadequate data taken from the universities, by a shift in the time of selection to one year after entry to universities on the basis of a paper requirement only. To provide for this large, relatively unselected, preliminary year would involve special organization and substantial expansion in most English universities, changes equivalent to the adding of a college to each university. During that year, the main pedagogic objective would be to produce fully habilitated students; and the main administrative objective would be to assess their potentialities as students, their intrinsic capacity, and their depth of interest, and so to select them rationally. From then on, our selected scientist-to-be must be exposed to the living spirit of science. He must become partner from the outset with his research-teachers. As he matures in the succeeding years, his share of responsibility in the partnership will grow, until, indeed, in some cases, the final responsibility becomes wholly his. Thus will the next generation of scientists be created.

I have dealt only with general national policy, which must, I think, constitute one's first approximation to the subject. The second approximation would involve detail, less well suited to a discourse such as this. It would consist in a discussion of those variations from general policy which would be necessary to accommodate the conditions in a minority of particular universities, colleges and schools. But as long as the general policy is so far from being nationally agreed, there is no sense in raising these more particular questions. Yet it is worth while now to note the general point that the variety in the English educational scene is such that one must be prepared to apply special treatment to outstanding special cases.

THE NATIONAL LENDING LIBRARY FOR SCIENCE AND TECHNOLOGY

By DR. D. J. URQUHART
Director

IN November 1956 the Research Council of the Department of Scientific and Industrial Research decided that the planning of a new national science lending library should begin. On November 5, 1962, the National Lending Library for Science and Technology was opened by the Minister for Science, Lord Hailsham, at Boston Spa in the West Riding of Yorkshire (see also p. 1019). Such in brief is the history of the Library. Its 'pre-history' includes the efforts of S. C. Bradford in the 1920's to expand the Science Museum Library and to start a national loan service. The 'pre-history' also includes discussions at the Royal Society Scientific Information Conference in 1948 and within the Advisory Council on Scientific Policy and its Library Committee.

The essential purpose of the new Library is to ensure that the practising scientists and technologists in the United Kingdom can obtain quickly whatever scientific literature they may need. The need for such an organization was underlined by the observation in 1956 that only half the Russian scientific literature which the Russians thought worth while abstracting was reaching Britain at all[1]. The reason for this was quite simply that there was no organization specifically charged to prevent this sort of situation arising.

Planning

The outline plans for the National Lending Library were prepared in 1957 by assembling the available data about the demand and output of scientific literature. A great deal of this information was obtained by treating the Science Museum Library as the prototype for the new Library and examining its experience[1-3]. The plans evolved envisaged establishing in 1962 a library which would receive then about double the volume of current literature which the Science Museum Library was receiving in 1956. The year 1962 was chosen only because it was thought in 1957 that the Science Museum Library might have a new building in 1962. The need to link the rate of development of the new library to possible developments in South Kensington arose because it was proposed that part of the stock of the Science Museum Library should be transferred to the National Lending Library.

The preliminary surveys[1] suggested that the Library might have to hold something of the order of 15,000,000 volumes in a hundred years' time, and that then the world's output of scientific literature might be about half a million volumes a year. These figures were obtained by extrapolation from the existing situation, assuming there was a finite limit to the creative capacity of a population of a given size.

In opening the Library, Lord Hailsham observed that this estimate was "almost certainly wrong as extrapolation—the process whereby these figures have been obtained—is the only thoroughly unscientific habit in which professional scientists habitually indulge". So far the only use made of these estimates was to specify that the Library would need a large site. The only other essential requirements regarding the site was that it should have access to postal facilities which would make a "by return of post" service possible.

The search for a site took place in 1958. It revealed that a suitable site with some industrial buildings on it existed in part of the Royal Ordnance Filling Factory at Thorp Arch, Boston Spa, Yorkshire, which was then going out of production. As it was found that it would be possible to adapt the buildings economically to house the Library, this site was accepted. The buildings taken over by the Library were single-story buildings with a floor area of about 130,000 square feet. The adapted buildings came into use in 1961. The only building work on new foundations required was a boiler house and two linking corridors. By the bold use of colour the original nature of the buildings has been successfully disguised. In opening the new Library, Lord Hailsham said: "It is, of course, a filling factory, and nothing could be more appropriate than to fill it with books instead of explosives; nothing is more explosive than books unless it be science or technology. It is, however, nicer than the New Bodleian in appearance, and by using the old buildings we got it cheaply, and by acquiring the old site we got it more quickly than might have been the case otherwise".

Stock

At present the library is receiving about 12,000 current serials and a further 10,000 are on order. The aim is to have all the recent scientific and technological serials published in the world which are, or perhaps should be, abstracted. The subjects covered include all branches of science and technology including agriculture and medicine. Books, as distinct from serials, form only about 10 per cent of the collection, and consist mainly of Russian books published since 1957 and English-language books published since 1960. In all, the Library contains about 350,000 volumes. This figure has been estimated from the fact that about 700 tons of literature have been moved to Boston Spa, the great majority of it from London.

The Library only collects microforms where material is not available in the original size, but it has a collection of about 125 miles of microfilm. This consists mainly of copies of the report literature issued by the U.S. Office of Technical Services and copies of *ad hoc* translations prepared in the United States of articles in Russian scientific literature.

The Library stock consists, in roughly equal parts, of : (*a*) literature which has been obtained by the National Lending Library by purchase or exchange ; (*b*) literature which was transferred to it from the Science Museum Library ; (*c*) literature which has been presented to the Library by other libraries. The flow of literature from other libraries to the National Lending Library started as a result of an invitation to libraries to transfer to it, on economic grounds, volumes which were not heavily used in the holding libraries, on the basis that the literature could always be borrowed rapidly if it was required. This appeal produced serial literature from a large number of libraries. This flow of literature is continuing, and it is proving very useful in extending backward some of the serial holdings of the Library and in duplicating more frequently used titles. For by a strange chance most copies are received of the most heavily used titles. This result was unsuspected when the appeal for literature was first launched; but it is directly related to the observation that the serial titles which were found to be most heavily used in the Science Museum Library were those which were most widely held in Britain[3].

Service

The Library only lends to organizations on an approved list of borrowers : these include universities, colleges, research organizations, Government departments and industrial firms as well as a small number of public libraries. It uses special loan request forms which cost £3 a pad of 50—the price is intended to cover the average cost of postage arising from 50 requisitions. The aim of the Library is to deal with all straightforward loan requests the day they are received, and as a rule this target is achieved.

To facilitate the use of letter post in dispatching loans the parts of about 1,500 of the most frequently used serials are bound in a light flexible binding as soon as they are received. Even where buckram binding is used publishers' volumes are split if possible to produce small volumes[4]. At present the Library is able to satisfy 75 per cent of the loan requests it receives from its own stock. Some of the requests for items not available at the National Lending Library are sent to the Science Museum Library, where that Library is likely to be able to satisfy them.

In addition to its loan service the library maintains a public reading room and it has a photocopying service. The facilities available at the Library are described in a brochure[5].

Library Techniques

The techniques used in the Library are somewhat unusual. This is partly due to the fact that the main task of the Library is to be a library for libraries, and few libraries have this problem. It is also partly due to the fact that the initial planning section consisted of three officers who had each been trained in physics and some procedures have been adopted after deliberate experiments. In developing internal procedures the aim has been to cut down records to a minimum and to obtain a maximum amount of literature with the available staff. There is, for example, no record in the Library which is called 'the catalogue'. The literature is arranged on the shelves according to its titles, and it is only possible to say whether a particular publication is available by consulting the shelves. This system permits the rapid issue of the majority of items requested, as they can be found on the shelves without consulting any records[6]. As a result of this approach to techniques Lord Hailsham said in opening the Library: "In many ways this Library breaks away from current library practice. The fact that this break has taken place in the field of scientific literature, and that the planning of the Library has been done by scientists, and that the senior staff of the Library is composed of science graduates has, of course, largely determined the form which these particular innovations have taken. But it would be a mistake to suppose that the creation of this great National Lending Library for Science and Technology would have no repercussions in other fields affecting libraries of other kinds".

Promoting the Use of Scientific Literature

In addition to being concerned with collecting and supplying literature, the Library hopes to contribute to the study of the flow of scientific information. In 1963 the Library proposes to carry out a survey of the use of serial literature. Besides using the results for administrative purposes such as deciding where duplicating is necessary or what the binding policy should be, it is proposed to study the existing system in the English-speaking world for making known the contents of serials which have not been used. It is hoped that this may throw some light on the efficiency of the present arrangements for making information available. It may, for example, reveal the relative importance of such factors as the inadequate coverage of abstract publications or the lack of awareness among potential users of how to use the guides to the literature of science.

This last factor has already been the subject of a small experiment. During September 1962 eight research students holding Department of Scientific and Industrial Research awards were invited to attend a four-week course at the Library on the use of scientific literature. The preliminary results indicate that formal training in this subject might usefully receive more attention than at present.

It is hoped that the survey on the non-use of literature may indicate which of the possible ways of improving the existing channels, which link the producer of scientific literature to the consumer, should receive priority. The survey may also indicate how important it is to develop entirely new channels based on computors. A useful computor would be one capable of reading types in all languages, analysing, translating and selecting. At the moment the input required is about ten million words a day and at present this is expanding exponentially.

The National Lending Library hopes to keep the possibility of such developments in mind and to act as a centre for innovations in the fields of librarianship and documentation. Such innovations are not only necessary in the field of scientific literature. As Lord Hailsham said: "Science and technology form, of course, the field in which the corpus of human knowledge is growing at the most rapid rate, and the need for adequate documentation, classification and bibliography is therefore greater in this field than anywhere else. But it is not the only field. The time has for a long time been ripe for technical and organizational advances in other library fields. The existence of this great new step in the field of libraries—perhaps the most important in this country ever to have been made since the foundation of the British Museum Library—embodying as it does so much scientific planning and so many revolutionary new techniques and conceptions, cannot fail to have a profound and increasing influence on libraries and librarianship generally".

[1] Urquhart, D. J., *J. Doc.*, 13, 18 (1957).

[2] Urquhart, D. J., *Intern. Conf. Sci. Information, Washington, 1948*, 287 (1959).

[3] Urquhart, D. J., and Bunn, R. M., *J. Doc.*, 15, 21 (1959).

[4] Bunn, R. M., *J. Doc.*, 18, 20 (1962).

[5] *National Lending Library for Science and Technology*, August 1962 (D.S.I.R., gratis).

[6] Urquhart, D. J., *Lib. Assoc. Rec.*, 64, 319 (1962).

CLOSURE OF THE *MERLIN* RESEARCH REACTOR

MERLIN[1] was the first, and may well be the last, industrial research reactor in the United Kingdom. It was made critical for the first time on July 16, 1959, and was officially opened by H.R.H. Prince Philip, Duke of Edinburgh, in November 1959. It achieved its full design power of 5 MW in 1961 and ceased operating on August 31, 1962. During its period of operation the reactor went divergent 675 times and about four hundred separate irradiations were performed. *Merlin* will be maintained so that it can be used again if the need for reactor irradiations increases significantly in the future.

Before and during the initial period of operations the following studies were started and techniques developed: (*a*) Sub-critical studies of different arrangements of reactor cores. (*b*) Safety studies, especially the effects of reactor power transients, and of heating by radiation experimental materials placed in the reactor. (*c*) The proving trials of the reactor. (*d*) The measurement of radiation fluxes and doses. (*e*) Fission product detection. (*f*) Reactor chemistry. (*g*) Effluent measurements and disposal.

Following these initial projects the main research programme had begun to produce significant results. Shielding studies for gas-cooled, graphite-moderated power reactors were carried out on assemblies simulating various portions of such a reactor. This was done in a *Merlin* thermal column with the graphite removed to provide a large volume, about 4 ft. × 4 ft. × 6 ft. deep, for irradiations. These investigations included measurements of the neutron distributions above a simulated reactor top-reflector where streaming along the fuel channels produces large perturbations in the distribution. The results were used for providing a computer programme for the estimation of such distributions in practical power reactors. The boiler shield design for one of the new power stations was checked, as was the attenuation produced by the complicated ducting for the coolant at the top of the boiler shield. The thermal column was also used to determine the fine structure of the thermal neutron flux in a fuel element of a new shape for future gas-cooled reactors. In order to obtain as much information as possible from such measurements the spectra of the neutrons have been characterized by means of the reaction-rates of various threshold detectors.

Another field in which it was useful to obtain information on the neutron spectra was that of radiation damage research. Investigations were made, under contract to the U.K. Atomic Energy Authority, of the basic mechanisms of radiation damage to fuel materials such as uranium oxide. Radiation damage to materials such as zirconium was also being investigated, as was the effects of radiation on the corrosion of these materials. Materials were also investigated, with regard to their impurity content, using activation analysis. These materials were of two types, those to be used in reactors and those of interest as semiconductors. A fast transfer unit was built which could transfer a sample from the centre of the reactor core to a nearby radiochemistry laboratory in about 5 sec. The research in this field was aimed at minimizing the time of any necessary chemical procedures after irradiation so that analyses, as well as the irradiation, could be carried out rapidly. Since the neutron flux at the centre of the core was high, 5×10^{13} thermal neutrons/cm^2, the sensitivity was also high.

A single-crystal neutron spectrometer was built in the Laboratory. Its operation is fully automatic; the counting-rates of the various detectors are recorded on magnetic tape. The spectrometer was to be used initially for investigations of neutron capture γ-radiation; it could also be adapted for neutron diffraction experiments. In the short time in which the spectrometer was in use the spectra of neutrons from the experimental tube was determined and the variation of the gold absorption cross-section as a function of neutron energy was measured to check the operation of the instrument.

The reactor has also been used for training personnel in reactor operations and in providing experience in reactor research. Engineers came for training from associated companies in the United Kingdom, and also from Canada, Italy, Germany, Greece and the Netherlands. Scientists came for experience from the Imperial College of Science and Technology, and Queen Mary College, London, the Universities of Birmingham and Southampton and from Greece and South Africa.

The reason for closing the facility is the present economic position of the electrical industry in general and of the nuclear power industry in particular. The heavy losses in the latter field have had their effect on the amount of money which industry is prepared to invest in long-term research on nuclear power. Reactors are relatively expensive items of research equipment, and to use them to economic advantage they ought to operate almost continuously: indeed, for many experiments continuous irradiation is essential to avoid relaxation of the effects produced.

To justify such continuous operation many more experiments were necessary and these were being discussed with the National Institute for Research in Nuclear Science on behalf of several universities, and with other bodies with the view of operating continuously from 3–5 MW starting at some date in 1963. Unfortunately, the climate in the whole of the atomic energy field deteriorated rapidly, and in view of the grave uncertainty over external contracts it was decided that the reactor should be closed down.

T. E. ALLIBONE

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[1] Allibone, T. E., *Nature*, 184, B.A. 11 (1959).

OBITUARY

Prof. Niels Bohr, For.Mem.R.S.

BY the death, on November 18, of Niels Bohr we have lost the most distinguished physicist of our time, the founder of the quantum theory of atomic structure, a natural philosopher whose profound insight enabled us to understand the complementary dual behaviour of atomic objects and who was a pioneer in explaining the internal behaviour of atomic nuclei.

Niels Bohr was born on October 7, 1885, the eldest son of Christian Bohr, professor of physiology in the University of Copenhagen. In his youth both he and his brother, Harald Bohr, the mathematician, were expert footballers. After taking his doctorate in 1911 he went to the Cavendish Laboratory at the time J. J. Thomson was putting forward his "plum pudding model" of atomic structure—a sphere with positive electricity distributed uniformly with electrons embedded in it. From the Cavendish, Bohr moved in 1912 to work for a few months with Rutherford, who in the previous year had published his theory of the nuclear atom. The difficulty of the theory was to understand why the electrons did not fall into the nucleus, and Bohr's profound contribution was to quantize the orbits of the electrons and, in particular, to show how the line spectra of hydrogen could be explained by quantized transitions between orbits. Rutherford's first reaction in March 1913 was to write, "Your ideas as to the mode of origin of spectra in hydrogen are very ingenious and seem to work out well; but the mixture of Planck's ideas with the old mechanics makes it very difficult to form a physical idea of what is the basis of it all". Bohr's ideas also explained how the physical and chemical properties of every element could be based on the atomic number, the number of units of positive charge in the nucleus. From this it also became clear how the emission of α- and β-rays from heavy nuclei would shift the nucleus two places down or up in the Periodic Table.

At the meeting of the British Association in the autumn of 1913, Bohr's ideas were discussed, and according to Bohr, Jeans's introductory survey of the application of the quantum theory to the problems of atomic constitution was "the first public expression of serious interest in considerations which outside the Manchester group were generally received with scepticism".

During the autumn of 1914, Bohr returned to Manchester with his wife, Margrethe, to succeed Darwin as Schuster's reader in mathematical physics, and for a time worked with Makower on building apparatus to investigate phenomena in the Franck–Hertz experiments on quantum phenomena in the excitation of atoms. However, the fine quartz apparatus built by a German glass-blower was ruined in an accident, and Bohr soon afterwards returned to a new chair of theoretical physics in Copenhagen. In 1920 he was appointed the first director of the newly created Institute of Physics. The Bohr Institute soon became a magnet for theoretical physicists from many countries—his visitors and students included Kramers, Heisenberg, Pauli, Darwin, Dirac, Fowler, Hartree, Mott, Landau, Peierls, and many others who in turn became leaders in their own country. Of this period Bohr has said that "a unique co-operation of theoretical physicists from many countries created, step by step, a logically consistent generalization of classical mechanics and electro magnetism and has sometimes been described as the heroic age in quantum physics. Many obstacles had to be overcome before this goal was reached and time and time again progress was achieved by some of the youngest of us".

In May 1930 Bohr visited Cambridge to give in the Scott Lectures an account of his ideas on the complementary nature of different aspects of behaviour of 'atomic objects'. He discussed how the interaction between the 'atomic object' and the measuring tools prevented unambiguous answers as to whether the atomic object was a particle or a wave. Bohr was always difficult to hear and understand, and I had the difficult task of writing a verbatim account of his lecture and sending it to him to be used for publication.

In 1936 Bohr invented the liquid drop model of the nucleus, which was later to have great importance in understanding the phenomenon of fission and of the different modes of excitation of nuclei when disturbed.

When the fission of uranium by neutrons was discovered in January 1939 and the physical character of fission elucidated by the experiments of Frisch and Meitner, Bohr was on his way to Washington and took part in the discussions on the fission phenomena. By February he suggested in a letter to the *Physical Review* that it was mainly the isotope U-235 which was fissioned by slow neutrons, while the U-238 isotope would usually absorb the neutrons without fission. This led to the realization that the isotope separation of U-235 would be necessary to produce a fast chain reaction.

When Denmark was invaded in 1940, Bohr felt it his duty to remain there to protect the refugee scientists at his Institute. In the autumn of 1943, however, it became known that Hitler's policy towards the Danish Jews was to change and that Bohr, whose mother was of Jewish stock, was to be arrested. He and his wife and four sons escaped in small boats to Sweden. In accordance with a prearranged British plan, he was then flown on October 6, 1943, in a Royal Air Force *Mosquito* to Britain; failing to get oxygen, he was unconscious for most of the flight. His son Aage, who was also a physicist and was to act as his personal assistant, arrived a few days later. Bohr was welcomed by Chadwick and many friends, including myself. Chadwick was about to depart for the United States to resume co-operation with U.S. scientists on atomic energy.

Niels and Aage Bohr joined the British Group in December 1943, and Niels was known as Mr. Nicholas Baker and his son as Jim Baker. They were provided with guards by the Manhattan Project wherever they went in the United States. The guards turned out to be most useful in preventing Niels Bohr crossing the streets against the traffic lights. Bohr paid extensive visits to Los Alamos, where he took considerable interest in the theory and design of the atom bomb and acted as a father confessor or tranquillizer to the high-powered and sometimes temperamental physicists.

As soon as Bohr heard how far the development of the bomb had gone he was struck by the political implications. He saw it not only as a great threat to the world but also as a great opportunity to bring about a saner international order. He discussed this with Sir John Anderson, who was in charge of the British atomic energy project, and in May 1944 he had an unfruitful interview with Mr. Winston Churchill. Later, in August of that year, he obtained through Mr. Justice Frankfurter an interview with President Roosevelt and urged him to inform the Russians about the existence of the bomb though not the details, "in an effort to achieve international control and to head off a fateful arms race". Drs. Vannevar Bush and J. B. Conant were also thinking at this time about the establishment of an International Control Agency with Russian membership. When the President and Prime Minister met on September 18, 1944, they considered Bohr's suggestions but decided that the project should continue to have the utmost secrecy.

The idea of the international control of atomic energy was, however, pursued by Bush and Conant and led to the Acheson–Lilienthal proposals for international control, which in the end came to nothing in the face of Russian opposition in the United Nations.

In his later years Bohr continued to devote a great deal of thought to these problems and I have on several occasions walked round and round the grounds of his "House of Honour" at Gamle Carlsberg hearing about his discussions with Sir John Anderson and an account of his interviews with Sir Winston Churchill and President Roosevelt.

During this period Bohr was a founder member of CERN and took a great interest in its development. A meeting at his Institute decided what type of accelerator should be built, and the Theoretical Physics Group of CERN was first located in Copenhagen.

The 1962 Lindau Conference was saddened by Bohr's having a minor attack of the illness which finally led to his death.

Throughout his life Bohr was greatly helped by his wife, Margrethe, a daughter of the mathematician Prof. Norlund, who presided over the "House of Honour"—a hospitable house where innumerable Institute parties were held in the great conservatory with music and talk and barrels of Carlsberg beer provided by the science-loving brewery.

Bohr received the Nobel Prize in 1922 and was elected a Foreign Member of the Royal Society in 1926. He received the highest Danish honour of the Order of the Elephant and the Atoms for Peace Award in 1953.

We will remember him above all for his humanity and his great friendliness to, and encouragement of, young scientists. JOHN COCKCROFT

NEWS and VIEWS

Wykeham Professorship of Physics, Oxford: Prof. R. Peierls, C.B.E., F.R.S.

PROF. RUDOLF PEIERLS, professor of mathematical physics in the University of Birmingham, has been elected to the Wykeham professorship of physics in the University of Oxford, and will take up his new position in October 1963. He succeeds Prof. W. E. Lamb, jun., who has been appointed Ford professor of physics in Yale University. Prof. Peierls was born in Berlin in 1907, and received important parts of his education at the Universities of Berlin, Munich, Leipzig and the Technical High School in Zurich. When he went to Manchester as Research Fellow in 1933 he was already very well known for his pioneering researches on the quantum theory of thermal conductivity and other solid-state phenomena, and for his work with Landau on quantum electrodynamics. He was at the Mond Laboratory, Cambridge, during 1935–37, where important contributions to the theory of co-operative phenomena and nuclear reactions were made. Except for a period during the War when he was engaged on the atomic energy project in the United States, Peierls has been at Birmingham since 1937, first as professor of applied mathematics, and then as professor of mathematical physics. Under his guidance and stimulating leadership a very important school of theoretical physics has developed at Birmingham.

Peierls was awarded a C.B.E. in 1946 and was elected a Fellow of the Royal Society in 1945, receiving its Royal Medal in 1959. He is a member of the Governing Board of the National Institute for Research in Nuclear Science. Besides numerous research papers in many different branches of theoretical physics, Peierls has written two highly regarded books, *Quantum Theory of Solids* (1955) and *The Laws of Nature* (1956). His transfer to Oxford is a very favourable development for that University, where the activities in experimental physics cover a wide range, which, however, is fully matched by the breadth of the new professor's interests and talents.

Zoology at Edinburgh: Prof. J. M. Mitchison

DR. JOHN MURDOCH MITCHISON, at present reader in zoology in the University of Edinburgh, has been appointed to the chair of zoology at that University. This chair, which is in addition to the chair of natural history occupied by Prof. M. Swann, has not been filled since Prof. J. H. Ashworth was transferred in 1927 to the chair of natural history. The appointment will take effect from January 1. Dr. Mitchison's undergraduate career at Trinity College, Cambridge, was interrupted by war service spent in Army Operational Research, in which he attained the rank of major. Nevertheless, he added in 1946 a first-class honours in Part 2 of the Natural Science Tripos to the first-class honours in Part 1 which he had gained in 1941. He spent the period from 1947 until 1953 in research in the Department of Zoology at Cambridge, and was awarded the degree of Ph.D. in 1950. In the same year he was elected to a Prize fellowship in his own College, Trinity, which he held until his appointment to the Department of Zoology in Edinburgh in 1953. Dr. Mitchison has also accepted several invitations to pursue his research overseas. In particular, he worked as a research associate at the University of California, Berkeley, in 1957, while holding a Carnegie travel fellowship, and in 1960 he was appointed visiting professor at the Marine Biological Station, Wood's Hole. Throughout this period, Dr. Mitchison's principal research interest has lain in the field of cell biology.

British Committee on Chemical Education

Prof. R. A. Morton, professor of biochemistry in the University of Liverpool, has been appointed the first chairman of the new British Committee on Chemical Education, established jointly by the Royal Society and the Royal Institute of Chemistry. This Committee comprises nominees from the Royal Society, Royal Institute of Chemistry, Chemical Society, Society of Chemical Industry, Science Masters' Association, Association of Women Science Teachers, Ministry of Education and the Scottish Education Department, together with several persons who have been invited to serve in their personal capacity but with special reference to their experience in diverse fields of chemical education. The secretary of the Committee is Mr. D. G. Chisman, Education Officer, Royal Institute of Chemistry, 30 Russell Square, London, W.C.1. One of the main functions of the new Committee will be to co-ordinate and make more widely known the various developments that are taking place in chemical education and generally to disseminate information on what is happening in different spheres of chemical education in Britain and overseas.

At the first meeting of the British Committee, preliminary consideration was given to the function of examinations in chemistry at school-level, especially in the light of the new approach to the teaching of chemistry that is being developed as part of the project for the improvement of school science and mathematics curricula, sponsored by the Nuffield Foundation. The desirability of ensuring that questions set in public examinations are in harmony with the new approach to the teaching of chemistry was stressed, and to this end the Committee hopes to establish co-operation and sympathy with the General Certificate of Education Examining Boards, many of whom are known to be revising their chemistry syllabuses at the present time.

Atomic Energy at Capenhurst and Aldermaston

In an adjournment debate in the House of Commons on November 19 on the Atomic Energy Establishment, Capenhurst, the Parliamentary Secretary for Science, Mr. D. Freeth, repeated his previous assurance that the reduction of output to the minimum level to keep the plant in production was being effected by a phased process over about 18 months. The level of activity at Aldermaston was unlikely to change significantly over the next 18 months or so.

Commonwealth Scholarships and Bursaries

In a written answer for the Secretary for Technical Co-operation in the House of Commons on November 22, Mr. Iain Macleod stated that attendance at the introduction courses for overseas students arranged each autumn by the British Council, including special courses in London for Commonwealth scholars and bursars, had doubled within the past three years. The Council encouraged institutions of higher education to arrange their own courses and would do all it could in co-operation with the educational authorities to extend this work. Under the Commonwealth Teacher Training Bursary Scheme, Britain had awarded 97 bursaries leading to a technical teacher qualification, and 54 bursars had completed their courses. Under a new scheme announced at the second Commonwealth Education Conference, 40–50 bursaries would be awarded annually for teachers of craft subjects, students attending a technical college for six months, and after six months practical training in industry, spending a second year in a technical teacher training college. The first intake of bursars would be offered courses in mechanical engineering, electrical engineering, constructional engineering and building. Every encouragement was being given to postgraduate scholars in science and technology to take advantage of part-time teacher training courses.

The U.S. National Science Foundation

An admirably illustrated brochure recently issued describes briefly but clearly the many-sided activities of the National Science Foundation (*National Science Foundation.* Pp. 32. Washington, D.C.: National Science Foundation, 1962). The way in which the Foundation supports research, its varied programmes in support of education in the sciences, its work in the field of scientific information, in assessing the potential and need for national research and its support for international scientific activities are summarized. Included also are an outline of the organization of the Foundation and a brief list of publications giving further information on particular activities.

Scientific Research in Schools

The fifth annual report of the Royal Society Committee on Scientific Research in Schools indicates the progress being made by the Royal Society to foster scientific research projects by science teachers in schools. During the year covered by the report, the number of research projects increased to seventy, involving sixty-one schools. This represents an increase of seven projects and five schools over the fourth report. The Council of the Royal Society again made available a grant of £2,000 for the provision of special equipment and apparatus. During the year, the U.K. Atomic Energy Authority offered the Committee a grant of up to £1,000 per annum for the purpose of providing grants to school science teachers requiring special equipment to pursue research of interest to the Authority: the first contribution by the Authority will be made early in 1963. A gift is also acknowledged of £105 from Griffin and George, Ltd., which is to be transferred to the Science Masters' Association for the development of laboratory apparatus for schools. All the work included in the various projects was carried out with the specialist advice and assistance of Fellows of the Royal Society and of others, all of whom have generously given their help, and the Committee gratefully acknowledges the valuable help given by these advisers. Five papers have been published in learned publications by science teachers concerned with projects supported by the Committee. Appended to the report is a list of the science teachers concerned with their scientific research projects, arranged alphabetically according to schools.

British Education

THE second edition of Prof. H. C. Dent's *British Education* gives a concise but comprehensive account of the evolution of education in England and Wales, Scotland and Northern Ireland and of contemporary developments up to and including the new universities (Pp. v+70+17 plates. London: Longmans, Green and Co., Ltd. Published by the British Council, 1962. 2*s*. 6*d*.). The balance between primary, secondary and further education of all types is fairly held and all aspects are reasonably presented. With a select, but fully representative, bibliography and well-chosen illustrations it provides not only a lucid popular account but also one which the serious student may well find convenient to have at hand.

Technical Translating in Canada

THE optimistic claims that machine translation will be practicable in the foreseeable future are strongly criticized in a paper that was written by D. A. Sinclair, chief translator of the National Research Council in Canada, for the Committee on Science and Astronautics of the U.S. House of Representatives (*Man or Machine. An Assessment of the Possibilities for Closing the "Translation Gap"*. National Research Council of Canada, Ottawa, 1961). As an alternative, Mr. Sinclair proposes a comprehensive scheme for the selection, training and employment of scientific and technical translators, taking into account existing conditions in North America. Much of this would seem to be directly applicable to Great Britain, where the demand for better translations is similarly great, and it is interesting to note that French-speaking Canada has a flourishing translating profession that exemplifies many of the features recommended by Mr. Sinclair. Neither the selection nor the training of suitable candidates should provide insuperable difficulties, but employment at adequate remuneration is quite a different problem. In Canada, competent translators are said to earn less than secondary school and college teachers.

Kodak Research and Development

THIS year the Kodak Research Laboratories celebrate their jubilee. To mark the occasion, the Eastman Kodak Company has issued an attractive and informative brochure describing the scientific work carried out in these laboratories in the fields of chemistry, physics, and photographic science and technology (*Research at Kodak*—Illustrated Brochure. Pp. 20. Rochester, New York: Eastman Kodak Co., 1962). The research programme covers: (1) fundamental research, in which the objective is new knowledge; (2) exploratory research, in which basic information is utilized in the search for new materials, processes and techniques; (3) early-stage development, in which work is directed towards new materials and processes for general sale and for scientific and military applications. Members of the research staff are encouraged to write for scientific journals and more than 2,300 scientific papers have been published since the laboratories were established, including among them outstanding contributions in the field of photosensitive polymers and in the theory of the photographic process. Techniques used in the organic chemistry laboratory include nuclear magnetic resonance, electron-spin spectroscopy, ultra-violet and infra-red spectroscopy, and gas chromatography. In physics, methods of sensitometry, psychophysics, radiometry, spectrophotometry and colorimetry together with mathematical analysis have been successfully applied to the Company's problems, while fundamental research has been carried out into the effects of turbid media and atmospheric conditions on the attenuation and scattering of light. Kodak scientists are given freedom to conduct their far-ranging exploratory research for new methods of obtaining monochrome or colour images and for new applications of photography. This type of research has produced such products as simplified photomechanical printing plates, a colloidal transfer system for copying printed matter, an emulsion-coated elastic base for use in the detection of stomach, breast and intestinal cancer, and a unique photographic paper which can be processed in less than one second.

Mount Stromlo Observatory

AFTER site testing intensively in New South Wales since 1958 and more generally since 1951, it has now been announced that a final choice of site has been made for a Field Station of Mount Stromlo Observatory of the Australian National University. The Field Station is to be at Siding Spring Mountain near the town of Coonabarabran about 200 miles northwest of Sydney. The mountain has a flat top, and being one of the highest peaks (altitude, 3,820 ft. above sea-level) in the area, it has a large open horizon on all sides. Services to the region seem adequate. It is hoped to erect the new 40-in. reflector, a 26-in. reflector (now at Mt. Bingar) and a new 16-in. reflector at this site. It is hoped at a later date to move some of the telescopes now at Mount Stromlo to the Field Station and the 50-in. reflector now at Mount Stromlo may be the first to be moved, though the 74-in. reflector will not be moved. It is hoped to make the Field Station the chief observing station of Mount Stromlo while keeping the Department of Astronomy of the Australian National University, workshops and offices in their present place. Further site testing is now in progress to find the best possible observatory site in the whole of Australia. The aim here is to have a suitable site for suggestion should sufficient finance for a 120-in. reflector become available. Further details can be obtained from the Mount Stromlo Observatory, Australian National University, Mount Stromlo, Canberra.

Prestressed Concrete

IN September the Cement and Concrete Association issued a booklet by M. R. Hollington entitled *An ABC of Prestressed Concrete* (Pp. 56. London: Cement and Concrete Association, 1962). The object of this excellent and informative publication is ". . . to provide the designer and the contractor with some concise information about prestressed concrete and its constituent materials". As the title implies, the subject-matter is arranged alphabetically and the scope is revealed by these headings: anchorages, anchor stressing, bond, cap cables, codes of practice, compacting factor test, composite construction, concordant tendon profile, concrete testing, cover, creep, deflexion, ducts, elastic deformation, end-block design, fire resistance, formwork, frictional losses, grading of aggregate, grouting, high-tensile alloy steel bars, indented and crimped wire, jacks, joints, kern, load testing, losses of prestress, mix design, notation, overloading, partial prestressing, permissible stresses, portland cements, post-tensioning, precasting, prestressed concrete designs, pretensioning, proof stress, quality control, relaxation, shrinkage, spacers, standard bridge beams, steam curing, strand, transfer, transformed tension profile, ultimate strength of beams, vebe test, vibration, wire, and Young's Modulus. This *ABC* is a most useful contribution to the literature of prestressed concrete and will be found to be of great practical value to all concerned with the use of normally reinforced concrete as a structural material. While assuming that the reader possesses a basic knowledge of the theory of prestressing, the explanations under each heading are clear and concise. It is pointed out that working efficiency of any prestressed member is dependent on a number of variables the quantitative assessment of which is often necessary by the structural engineer. In this connexion numerical values given to these factors in this booklet are based on the British Standards *Code of Practice CP 115 : 1959, The Structural Use of Prestressed Concrete in Buildings*. References are made to experimental results obtained at the Research Station of the Cement and Concrete Association, and footnotes direct the reader to relevant British Standards and other works on this important subject.

The Ciba Journal

THE *Ciba Journal* for Autumn 1962 (No. 23) includes a note on demonstrations of advances in photochemistry shown in colour on the 'Eidophor' screen at Zurich last summer, Dr. K. Hofmann's remarks on molecular medicine delivered at the dedication last May of Ciba Summit's Development Research Centre, and some account of the Goregaon Centre for fundamental biological chemical research, near Bombay, to be completed next spring. The new institute will co-operate fully with university and Government research institutions in India and other parts of Asia. There is a note outlining the differences in structure and behaviour between 'Doriden' (or glutethimide) and thalidomide, and an account of the scope and activities of the Summit Scientific Information Centre, in which Dr. F. Knill stresses the need to limit mechanization to the purposes which justify its expense and effort: the human factor is still important. Among shorter articles there is a description of the Snowy Mountains hydroelectric and irrigation scheme.

Bibliography of Gas–Liquid and Liquid–Liquid Interfaces

THE Research Department of Joseph Crosfield, Ltd., have made a handsome present to the scientific world with their new bibliography of published work on gas–liquid and liquid–liquid interfaces. The compiler is Dr. D. W. Stephens, and he has done a very thorough job: there are more than 2,600 references. The topics covered include some in which solid surfaces are involved also, a large number of papers on contact angles being cited. The work since 1890 is very thoroughly dealt with, and very little seems to have been missed; some references have dates as recent as 1961. Rayleigh's classical work is noted; but perhaps wisely, since the bibliography is intended for present-day research workers, the early history is lacking; there is no mention of Thomas Young, Laplace or Dupré, and Clerk Maxwell's comprehensive article "On Capillarity", in pre-1914 editions of the *Encyclopædia Britannica*, is not mentioned. This is a pity, since it contains an admirable summary of the very early work, even back to Leonardo da Vinci. But one cannot have everything, and this bibliography is so comprehensive that it will save research workers and writers of books untold hours of searching in the literature. In an introduction, Prof. J. T. Davies directs attention to the alarming and self-defeating increase in the rate of publication of papers. One example may be quoted: more than 100 communications on the effect of surface films on the rate of evaporation of water, a subject which assumed technical importance only a few years ago, are cited. But bibliographies such as this, easy to use and concise, arranged under both authors and subjects, do help to overcome this ever-increasing plague which now besets scientific literature, in a most helpful way. The publishers' address is: Joseph Crosfield and Sons, Ltd., Bank Quay, Warrington, Lancashire.

Genesis of Volcanic Pipes by Fluidization

IN 1961 the Russian geologists, V. I. Mikheyenko and N. I. Nenashev, published a remarkable demonstration that the diamond-bearing pipes of Yakutia were intruded as a relatively cold, gas-charged mush of solid-rock fragments, and not, as hitherto supposed, predominantly in a water-enriched magmatic state. An English translation of this report has recently appeared (*International Geology Review*, 4, 916; 1962). Although these diamantiferous pipes and dykes were emplaced in late Mesozoic times, the absolute age of the kimberlites of which they are formed, established by potassium : argon ratios on the component phlogopite mica, is not less than 660 million years. In other words, the formations were not molten within 500 million years of the time of their intrusion, but were emplaced from depth as a cold, fluidized, aqueous slurry which in its ascent disrupted from the country-rocks the many types of xenolith which the pipes contain. These observations, coupled with the recent Russian discovery of diamonds in a xenolith of plagioclase-bearing eclogite-like rock of crustal type, may materially affect geological thinking on the origin of diamonds and on the deeper structure of the continental crust. Contemporaneously, emphasis has been placed on the importance of fluidization or 'gas-tuff streaming' in a series of recent papers by Dr. E. H. Francis on the Carboniferous volcanic vents in the Midland Valley of Scotland, the latest of which is on "Volcanic Neck Emplacement and Subsidence Structures at Dunbar, South-East Scotland" (*Trans. Roy. Soc. Edin.*, 65, No. 2. Pp. 41–58 + 2 plates. Edinburgh: Royal Society of Edinburgh, 1962. 8*s*. 6*d*.). Sixty years ago, Sir Archibald Geikie drew comparisons between the Scottish necks and the diamond pipes of South Africa. The former have never yielded a diamond; but it is nevertheless of interest to find genetic conclusions drawn from field observations in Scotland so closely paralleled by the results of geochronological research on the diamantiferous diatremes of Siberia.

Pinus radiata

Pinus radiata, although a tree of little importance in its native habitat of southern California, has established itself as a most important exotic, particularly in the southern hemisphere. Wherever climate and soils are suitable, it has given outstanding results. It occupies a very prominent position in New Zealand forestry, supplying about half the total cut of home-grown timber. In addition to lumber, it is the sole raw material used in the New Zealand pulp industry. Thus, besides home consumption, this exotic is also providing produce for export. The successes achieved are all the more remarkable because the *radiata* stands are only 40 years old or less. Similar results, although on a smaller scale, are being obtained in Australia and South Africa, and it is likely that much of Chile's pulp industry will be dependent on this pine. Success is also being obtained in Spain, where the increment is much higher than obtained from the indigenous conifers. The recent Food and Agriculture *Forestry and Forest Products Studies*, No. 14, by C. W. Scott, is a remarkably complete monograph on *P. radiata* (Pp. xii + 328. Rome: Food and Agriculture Organization of the United Nations; London: H.M.S.O., 1962. 17*s.* 6*d.*; 3.50 dollars). It begins with a detailed description of the tree in its habitat and then as an exotic. Sylviculture, management, pathology, utilization and marketing are all considered, and there are ten appendixes and a good bibliography. The illustrations are excellent. It is fortunate that a person of C. W. Scott's experience was found to prepare this monograph. Not only is he familiar with *P. radiata* in Chile but he is also a forester of great experience who knows what to record in a book to make it of real value. Throughout the monograph one appreciates the balanced outlook of the author in such statements as "The whole future of *P. radiata* and all other exotics depends on so handling them that they remain healthy and free from disastrous epidemics or soil deterioration".

The Society for the Study of Growth and Development

THE following have been elected officers of the Society for the Study of Growth and Development: *President*, John G. Torroy (Harvard University); *Secretary*, William A. Jensen (University of California); *Treasurer*, Dr. Marcus Singer (School of Medicine, Western Reserve University); *Editor of the symposium volume*, Dr. Michael Locke (Western Reserve University); *Member of the Executive Committee*, Dr. Clement Markert (Johns Hopkins University).

The twenty-second annual symposium of the Society will be held at the University of Connecticut during June 17–19. It will be devoted to the general topic, "Cellular Membranes in Development". Further information concerning the Society or the symposium can be obtained from Dr. William A. Jensen, Department of Botany, University of California, Berkeley 4, California.

The John Murray Travelling Studentships in Oceanology and Limnology

THE following awards have been made by the Council of the Royal Society of John Murray travelling studentships for research in oceanography and limnology: to M. V. Angel of the Department of Zoology, University of Bristol, to enable him to extend his experience and knowledge of marine plankton and apply the techniques developed for investigating *Daphnia* endocrine systems to marine plankton in R.R.S. *Discovery* during the International Indian Ocean Expedition; to P. Herring of the Department of Zoology, University of Cambridge, to enable him to investigate the control and functions of the vertical distributions of zooplankton in R.R.S. *Discovery* during the International Indian Ocean Expedition; to Dr. A. Prakash of the Fisheries Research Board of Canada to enable him to carry out work on the dynamics of plankton blooms, with particular reference to efficiency of primary production in tropical waters, and other studies within the framework of the International Indian Ocean Expedition; to G. Topping of the Department of Oceanography, University of Liverpool, to enable him to carry out general chemical work and to study the distribution and variation of trace elements in sea-water aboard R.R.S. *Discovery* during the International Indian Ocean Expedition.

Announcements

H.R.H. PRINCE PHILIP, DUKE OF EDINBURGH, has been admitted as a Royal Fellow of the Australian Academy of Science in Canberra.

DR. E. VAN TONGEREN has joined Mr. M. D. Frank in the management of the North-Holland Publishing Co., Amsterdam.

A SYMPOSIUM on "Equatorial Geophysics", sponsored by the International Union of Scientific Radio and the Interamerican Union of Geodesy and Geophysics, will be held at the Instituto Geofisico del Peru during February 4–8. Further information can be obtained from Albert A. Giesecke, Instituto Geofisico del Peru, Apartado 3747, Lima.

THE fourth omnibus conference on "The Experimental Aspects of Nuclear-Magnetic-Resonance Spectroscopy" will be held at the Mellon Institute, Pittsburgh, Pennsylvania, during February 28–March 2. Further information can be obtained from the chairman, C. W. Wilson, Research and Development Department, Union Carbide Chemicals Company, South Charleston, West Virginia.

THE third international symposium on "Quantum Electronics", sponsored by the Office of Naval Research, the Institute of Radio Engineers and the Société Française des Electroniciens et des Radioélectriciens, will be held at the Unesco Palace, Paris, during February 11–15. Further information can be obtained from the Third International Symposium on Quantum Electronics, 7 rue de Madrid, Paris 8.

A COURSE of lectures and practical work on "The Immunological Assay of Insulin", organized by the School of Clinical Research and Postgraduate Medical Teaching, University of Cambridge, will be held in the Department of Biochemistry, University of Cambridge, during December 17–18. The course is designed for research workers and hospital laboratory workers interested in the assay of insulin in body fluids, tissue extracts, etc. The fee for the course will be two guineas. Further information can be obtained from the Secretary, the Medical School, University of Cambridge.

THE first Australian conference on "Electrochemistry", sponsored jointly by the Royal Australian Chemical Institute, the University of Tasmania and the University of New South Wales, will be held in Sydney during February 13–15 and in Hobart during February 18–20. The following topics will be under discussion: (1) Sydney, solid-state chemistry, thermodynamics of electrolytes, electroanalytical methods, applications (plating, anodizing), corrosion and theory of boundary layers; (2) Hobart, electro-winning and electro-refining, electrode processes, fused salts, fuel cells and non-aqueous electrochemistry. Further information can be obtained from Dr. Felix Gutmann, Physical Chemistry Department, University of New South Wales, P.O. Box 1, Kensington, New South Wales.

THE SUB-DEPARTMENT OF ANIMAL BEHAVIOUR, UNIVERSITY OF CAMBRIDGE

IN 1950, the Cambridge University Department of Zoology established an Ornithological Field Station at Madingley, about four miles from the centre of the City. The object of this was to provide aviary and other facilities for the investigation of bird behaviour, with special reference to the relative influence of two factors affecting the development of behaviour in the individual. These two factors are, on one hand, the innate disposition of the animal—in broad general terms, its 'instincts'—and on the other its ability to profit by experience, that is to say, the degree to which it can display the various types and degrees of learning. Birds are of particular interest from this point of view in that they combine high learning ability with elaborate instinctive behaviour patterns to an extent which is probably unique in the animal kingdom. The Field Station was from the first under my general direction, with Dr. R. A. Hinde as curator. As the work developed, experiments were extended to a number of species of mammals in addition to birds and the title was changed to the Field Station for the Study of Animal Behaviour. In 1960, the University established a Sub-department of Animal Behaviour as part of the Department of Zoology, with me as director and Dr. Hinde as assistant director of research. It was envisaged that there would ultimately be 8–10 other workers. Of these, three or four would be rather senior, holding relatively long-term research grants or appointments—the total scientific strength of the Station being completed by research students and senior visiting workers from other universities. In addition, there would, of course, be an appropriate technical staff.

This development, and the experience of a number of years, made clear the necessity for a permanent building in addition to the temporary accommodation which had sufficed hitherto. The earlier stages of development of the Field Station had been made possible by generous help from various sources, particularly the Nuffield Foundation and the Josiah Macy Foundation of New York. When the more extensive needs became known, the former again came most generously to our aid and gave the University a grant of £50,000, of which £30,000 was to be allocated to the immediate capital needs and the remaining £20,000 to be devoted to the current expenditure over a period not exceeding seven years. The Rockefeller Foundation generously followed this up with a grant of £23,330 of which £5,830 is to be used for the purchase of scientific equipment and £17,500 for more general expenses of research over a five-year period.

The site consists of about 4½ acres of field and woodland, the whole fenced with vermin-proof fencing. Within this area are outdoor pens and enclosures, a monkey house for the accommodation of four groups of seven or eight monkeys, each with extensive indoor and outdoor quarters. Madingley Wood

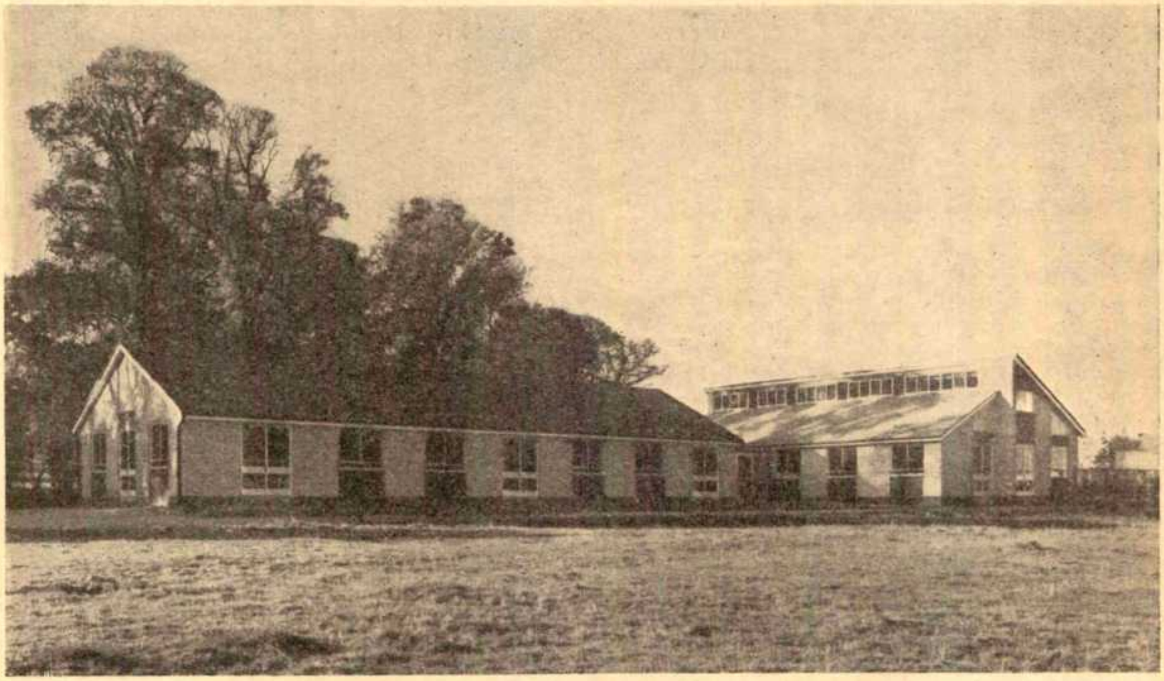

Fig. 1. The Sub-department of Animal Behaviour, University of Cambridge

(38 acres), which is a biological preserve of the University, and the lake in Madingley Park are also available for certain research projects. There are 60 small and 12 large aviaries and a large number of indoor bird-rooms and flights, as well as two sound-proof rooms especially constructed for the isolation of experimental birds from those frequencies which constitute the essential basis of bird songs and call notes. An admirable building (Fig. 1), designed by Mr. Peter Bicknell, of Messrs. Hughes and Bicknell, Cambridge, has now been erected and the Sub-department occupied it in August 1962. The new building is divided into approximately twelve individual research rooms, dark room, histology room, office, accommodation for full equipment of sound-proof cages, garage, workshop, etc. There are also three large rooms, one of which is at present being used as an isolation room for primates and birds, while the others accommodate small mammals and fish. The roofs are pitched, and the whole of the roof space is utilized. In one block the two roof slopes are split at the ridge to give a continuous clerestory, lighting small workrooms. In the other block there is a continuous loft 80 ft. × 30 ft. uninterrupted by roof timbers, which can be used for experiments requiring a long indoor runway or large choice chambers. There is also a house for the senior assistant in charge.

It is intended that the first main line of investigation of workers in the Sub-department should continue to be the instinct/learning problem, particularly as exemplified in the study of sensitive periods in learning. Such a heading includes several lines of work which the experience of the past few years has shown to be the most promising. With birds it would include studies on the ontogeny of new motor actions, and the continuance of studies on song-learning and vocal imitation and their sensory and motor control. Studies on the ontogeny of species-characteristic behaviour, particularly work such as that on the analysis of the role of experience in nest-building, will be continued. Studies on mammals will, as hitherto, centre round the mother–offspring relation, and the work with the now well-established colony of rhesus monkeys is proceeding. The second main field of investigation can broadly be described as 'motivation'. This, of course, interdigitates with the first—for example in the analysis of song patterns, endocrinology (particularly of nest-building) and studies of changes in responsiveness to a constant stimulus.

The third main aspect of the work can broadly be called physiology. Studies on the hormonal control of the cycle of reproductive behaviour, especially of nest-building in canaries, has already been mentioned, but a new development is the initiation of neurophysiological investigations. While much is known about the neurophysiological changes concerned with learning in mammals, the neurophysiological understanding of the essential mechanisms involved in what are generally known as 'instinctive actions' is, by comparison, meagre in the extreme. When we come to birds, in which these instinctive actions are far more obvious and highly developed than they are in the mammals, neurophysiological information is almost non-existent. The application of new techniques of central stimulation seem to offer great promise in this field.

In the past, workers in the Sub-department have been fortunate in being able to maintain close contact with colleagues in related fields. Thus we have had close co-operation with members of the Department of Experimental Psychology, under Prof. O. Zangwill, and especially with the workers on primates there—Dr. L. Weiskrantz and his associates. Also within the University, contact has been maintained with the Department of Physiology (Sir Bryan Matthews), and we look forward to future collaboration with the new Department of the Physiology of Reproduction under Prof. A. S. Parkes. Outside Cambridge, we have worked closely with Dr. John Bowlby of the Tavistock Clinic, who is investigating mother/infant interaction in man. We hope that such co-operation will be increased and extended as a result of the new developments because we believe that ethology has a special part to play in the establishment of mutually profitable relations between the various behavioural sciences.

W. H. THORPE

PROBLEMS OF COMMUNICATING SCIENTIFIC IDEAS

A *TENTATIVE Study of the Publication of Original Scientific Literature**, issued by the Abstracting Board of the International Council of Scientific Unions, like the study of *The Foreign Language Barrier in Science and Technology* †, recently published by Aslib, is concerned with the availability of original publications in foreign languages. Both are based on fresh surveys, but, except in respect of courses in Russian for scientists, neither adds substantially to the findings of the report, *Scientific and Technical Translating and other Aspects of the Language Problem*, issued in 1957 by the United Nations Educational, Scientific and Cultural Organization (see *Nature*, **180**, 1170; 1957). However, the Aslib report refers to this earlier and much more comprehensive report. The report from the International Council of Scientific Unions is based on a questionnaire sent early in 1960 to 260 selected periodicals, mainly in Europe, but 86 did not reply. The survey did not pretend to give a representative sampling of original publications, but in view of the fact that a sampling of 1,000 periodicals for the Unesco survey indicated that some 61·5 per cent of the papers were in English and only 17·4 per cent in French and 2·2 per cent in German, the selection of 72 French periodicals and 33 German compared with 39 British and 63 United States seems to rob the findings of some substance. Russian periodicals, which in the Unesco survey accounted for some 10 per cent, were not included, though this language was in 1957 competing for the second place, and Japanese was then challenging French for fifth.

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* Conseil International des Unions Scientifiques, Paris. Bureau des Resumes Analytiques. *A Tentative Study of the Publication of Original Scientific Literature.* Pp. 11 + 16 tables. (Paris: Conseil International des Unions Scientifiques, 292 rue Saint-Martin, 1962.)

† *The Foreign Language Barrier in Science and Technology.* (A study based upon three investigations carried out in the Research Department by Moira Phillips.) Pp. vi + 57. (London: Aslib, 1962.) 12*s*.

This survey, however, was not primarily concerned with language: it was equally concerned with the selection of papers for publication and with financial aspects, on which later, however, the only firm conclusion drawn was that no law of political economy appears to have any validity in this field of publication. Only in Canada did some effort appear to have been made to organize scientific publication financially and there is a very wide variation in the price demanded of the scientific subscriber for 10,000 words of scientific literature. Sometimes a single periodical has widely different subscription rates for different categories of subscribers; even within one country and one scientific discipline, rates may vary widely with no apparent explanation. The mean value for commercial publishing seems to be higher everywhere than the mean value for periodicals owned by non-profit concerns or societies, though a few exceptions can be found.

Submission of papers to a referee was practised by 71 per cent of the periodicals, but was not universal, especially outside the United States; 72 per cent of the periodicals publish authors' abstracts, but only 40 per cent decline to publish a paper unaccompanied by an abstract written by its author. Only 38 per cent of the periodicals appeared to be aware of the *Guide for the Preparation of Synopses*, in spite of the efforts made since 1949 by Unesco and the Abstracting Board of the International Council of Scientific Unions to distribute this and make it known; only a minority (6 per cent) of editors have taken steps to make it known to the authors of papers, while only a very few have framed and distributed their own rules.

The Aslib report is based on three studies carried out by Miss Moira Phillips, of the Aslib Research Department, but the introduction, while rightly stressing that in the immediate future an increasing proportion of the world's scientific literature will be in Russian, Chinese and Japanese, in urging the importance of a knowledge of these languages, and of cover-to-cover translations, appears to underestimate both the outstanding importance of English itself as a medium of communication and of the economic and other considerations which will tend to encourage publication in one or other of these four major media as against nationalistic considerations. The first of the three studies sought to establish how many hours of tuition were necessary to enable a scientist to acquire sufficient knowledge of Russian grammar, construction and vocabulary to understand Russian papers at least on his own subject. The second was a study of cover-to-cover translations with the view of seeing to what extent it would be feasible to meet the demand for translations by cover-to-cover publications. In this, Dr. D. J. Urquhart comments that the number of *ad hoc* translations made before a cover-to-cover translation is no measure of the use of the cover-to-cover translation, which is a disseminating medium in its own right. The third study was of the participation and co-operation in the *Commonwealth Index of Unpublished Scientific and Technical Translations*.

Summarizing the three studies, Mr. C. W. Hanson concludes that language barriers separate English-speaking scientists and engineers from about one-third of the world's scientific and technical literature, to which learning to read foreign languages, and especially Russian, at present offers the most direct approach. A reading knowledge of Russian sufficient to enable a scientist to make sense of a paper on his own subject can be acquired with about 50–200 hours of tuition, and it would be feasible to provide sufficient courses in the United Kingdom for about one-quarter of the annual output of graduate scientists and engineers. While the demand for cover-to-cover translations of individual articles is concentrated on a few periodicals, the production of some additional cover-to-cover versions would be justified as providing ready-made translations of individual articles which may be needed. Generally, the demand for translations of individual articles is too thinly scattered over a large number of periodicals to justify a massive programme solely as a means of meeting demand. The need for translations to meet individual wants will continue, and co-operative indexes and pools provide a means of avoiding duplication of effort. The *Commonwealth Index of Unpublished Scientific and Technical Translations* operates at a profit to the community even when judged on the criterion of avoidance of duplication alone and has scope for growth. A study of the literature supports the view that the progress already made and the magnitude of the rewards of success justify major efforts in machine translation, although comprehensive machine-translation services comparable in adequacy and cost with those provided by human translators are unlikely to be achieved in less than a decade.

DANGERS OF DRINKING SEA WATER

THE salt content of body fluids is about 1 per cent. In the open ocean the salt content of the sea is about 3·5 per cent. In health, when food and water consumption is adequate, the salt concentration in the body is kept relatively constant by the kidneys; it varies, but only within a small range. In a healthy individual suffering from deprivation of water the concentration of salt in the urine does not average more than 2 per cent. This difference in salt content between the body fluids and sea water, and the physiological inability of the kidney to excrete more than a certain proportion of salt in the urine, form the basis of the generally accepted view that drinking sea water does harm. It introduces a hypertonic solution into the circulation, water is withdrawn from the tissues to restore the osmotic balance between the tissues and vascular system, the blood volume is increased, and the kidney is called on to excrete the excess fluid. The net result is progressive dehydration of the tissues, leading to disturbances in the acid-base balance, a rise in the non-protein nitrogen of the blood and the plasma protein concentration, a reduced cardiac output, thirst, and, in due course exhaustion, collapse and death.

This view was challenged by Dr. Alain Bombard who crossed the Atlantic Ocean in 1952 on an inflatable raft, taking more than two months for the voyage and relying chiefly on sea water and fluids expressed from fish to quench his thirst. The opinion he reached after these trials on himself was that people should begin drinking sea water as soon as possible before dehydration starts but in small quantities only to avoid nausea and diarrhœa. Whatever fresh water is available should be carefully husbanded, and, if there is none, fluids should be expressed from fish by squeezing them in plastic bags or towels.

Bombard's experiments were followed by Dr. G. Aury, principal medical officer of the French Navy, who, in 1953 and 1954, carried out experiments on volunteers subjected to shipwreck conditions, he himself taking part. Aury described the experiments as being successful; the sea water was readily drunk by the volunteers, they suffered from no serious complaints, and, when the experiments were over, they were able to resume their duties immediately. The experiments lasted only 2–4 days.

A German physician, Dr. Lindemann, made no less than three voyages across the Atlantic ocean, in a canoe and then in a collapsible boat. He reached diametrically opposite conclusions to those of Bombard and Aury. Sea water, he held, should never be drunk; fluids could only be expressed from fish by means of a press; and no fish should be eaten if no fresh water is available.

In 1959 the question of drinking sea water came before the Maritime Safety Committee of the Inter-Governmental Maritime Consultative Organization. The experiments of Bombard and Aury, and the wide publicity they had received, created much confusion about the drinking of sea water, and the impression had spread that the dangers of drinking sea water had been grossly exaggerated. The Maritime Safety Committee felt that an authoritative opinion was required on what had become a controversial question and asked the World Health Organization for its views. Pending a firm opinion to the contrary, however, the Committee urged that no Governments should advocate the drinking of sea water by shipwrecked mariners.

The World Health Organization convened a meeting of five internationally recognized experts, Surgeon Captain F. W. Baskerville (United Kingdom), Dr. J. Fabre (Switzerland), Dr. H. Laborit (France), Prof. R. A. McCance (United Kingdom) and Prof. A. V. Wolf (United States). They considered the effects of drinking sea water under three headings: effects on the bowel, on the body as a whole and on the mind*. Because of the well-known cathartic effects of salts, drinking sea water is likely to lead to intestinal discomfort, if not to frank diarrhœa. This effect varies in different individuals and is more likely to follow if large amounts are ingested. On the body as a whole, the effect of sea water is to overload the circulation with salt, which can only be excreted by drawing on the body water and so dehydrating the body even more. If the salts are not excreted the effect will be equally harmful because of the increase in the concentration of salts in the body fluids. Finally, the evidence shows that even small amounts of sea water affect some individuals unfavourably, while large amounts have been shown to lead to mental disturbances and even suicidal impulses.

The experts examined the contention that a limited supply of fresh water could be made to last longer if it were mixed with sea water. This has been shown to be feasible experimentally in certain animals and is theoretically possible in man. But—the group emphasized—no acceptable evidence has ever been adduced that in man sea water can be used satisfactorily to eke out supplies of fresh water. This as yet unproved hypothesis cannot, therefore, be made the basis of a practical recommendation for saving the lives of people shipwrecked at sea.

One of the reasons for advocating the drinking of sea water is to preserve morale, which, in the circumstances of shipwreck, is likely to be low. The experts held the view that morale can be maintained if it is made clear to the castaways that death from lack of water alone is scarcely possible for several days. It has been shown from experimental work that a man can remain reasonably fit without water for six days, and men have survived without it for twice that period at sea. It has also been shown that 500 ml. (one pint) of fresh water daily, if possible supplemented by 100 g (3½ oz.) of carbohydrate, will maintain a man almost without deterioration for at least six days.

The group ended its report with advice to those who have to abandon ship, including the warnings to "*Never drink sea water*. Never mix sea water with fresh water if fresh water is in short supply. Sea water has been used to moisten the mouth, but the temptation to swallow it may be irresistible and it is better not to use it for this purpose. Never drink urine".

* *WHO Chronicle*, 16, No. 9 (September 1962).

ECONOMIC LIFE OF CHEMICAL PROCESS PLANTS

"ECONOMIC Life of Process Plant" was the subject of a symposium held under the auspices of the Institution of Chemical Engineers in London on October 30. Obsolescence of chemical process equipment is a vital factor which has to be taken into account by the engineer responsible for designing chemical plants to operate at minimum cost over a reasonably predictable time, having regard to certain constraints governed by operating conditions. The author of a paper on this subject, C. S. H. Munro, concludes ". . . that the physical durability is by no means the only determining factor in the life of chemical plant equipment and that the designer must consider very carefully the future capacity requirements and the likelihood of process change". All requirements for capacity increase of plant appear to arise after periods of more than five years ". . . which is perhaps as long as any commercial estimates might be considered valid". The argument would seem to indicate a case for increasing depreciation-rates to accord with actual chemical plant life, which, as is well known in the industry, is one of the most difficult to assess compared with many other engineering projects.

A method of determining economic optimum timing for replacing chemical plant was discussed by H. R. Fisher in his apparently new solution to this problem formulated in Terborgh's *Dynamic Equipment Policy* (McGraw-Hill Book Co., New York, 1949). H. C. D. Phillips, D A. Peters and J. A.

Hodson, of the Esso Petroleum Co., Ltd., Fawley, in a paper "... written against a background of experience in the design of refinery process plant", discussed the difficult matter of "Design Philosophy in the Economic Selection of Materials for Process Plants", including questions of selection of suitable materials of construction, also methods of calculation for carrying out economic evaluations. "The Economic Life of Chemical Plant in the Nuclear Fuel Industry" was considered by G. K. Dickson, K. J. Norman and R. R. Gunton of the U.K. Atomic Energy Authority. In the nuclear industry, established in Great Britain barely fifteen years ago, evolution has had the effect of transforming what was initially a national defence project to one based on normal commercial practice. They stated that "such a rapidly and drastically changing situation has required constant re-appraisal of the economic life of the plants involved".

"Process Plant in the Gas Industry" was the title of a paper given by C. Stott (S.E. Gas Board), emphasizing some of the problems of plant design for meeting 'base' and 'seasonal load' demands (under statutory obligation), and latterly the widespread adoption of oil gasification plant to meet such conditions and to replace outdated plant. The final paper, "The Economic Life and Size of Process Plant in Underdeveloped Countries", by A. P. Shahbenderian, demonstrated how "... it is possible to assess the economically optimum life and, in particular cases, the optimum capacity of chemical plant for situations where the market is static or the rate of growth predictable". H. B. MILNER

THE TEASEL

IT is one of the minor mysteries of modern crop husbandry that teasel growing should have survived in a restricted area of south Somerset when it has died out in all the former teasel-growing areas of Britain. About four hundred acres are now grown. Formerly, teasels appear to have been widely grown. Arthur Young reported teasel beds in Essex, where they were cultivated in rotation with medicinal plants like coriander, and there were acreages in Wiltshire and Gloucestershire. When it was that the hook-ended bract of the teasel flower head was first used to comb up the pile on cloth is not known, but the process almost certainly originated in the West Country to give the characteristic hard-wearing nap to West-Country broadcloth. It is probable, too, that the husbandry of teasel-growing was either begun, or at least received its first impetus to expansion and improvement, from the influx and settlement of the Huguenot weavers.

The broadcloth industry in the hinterland towns which teasel-growers served is now largely dead, but an isolated area around the Somerset villages of North Curry, Curry Rivel and Fivehead remains devoted to teasel husbandry. This has been examined by John L. Jones, who describes how acreage fluctuates from two to three hundred and the number of farmers participating is about fifty (*Land*, 11; Autumn, 1961). For most of them it is a crop which has been grown on the family farm for generations.

Why, then, has it survived? Where a highly specialized crop survives, climate and soil must obviously be right, and the heavy loams and moderate rainfall of south Somerset suit the teasel. Another unique aspect of the soil is that much of the land is 'teart'—in the region of the country distinguished by an excess of molybdenum, which expresses itself as a copper deficiency in cattle. This in itself may be a specific ecological factor of the teasel. More possibly, it was the economic aspect of teart land farming which may have contributed to the survival of teasel-growing. For until it was appreciated that the serious cattle sickness associated with teart land could be rectified with modern treatment, the farmers concerned would have been more interested to retain any lucrative cash crop for which their land was suited than those with sound and healthy land would have been to grow teasels. Added to this is the continuity of husbandry and the skill and specialized knowledge acquired in centuries of growing.

The superiority of the teasel—the fuller's teasel, as it was formerly called—lies in the supple hookends of the bracts or awns of the cylindrical flower-heads. To-day all the Somerset teasels are bought for the northern cloth-finishing mills, where they are used to produce the brushed effect on the best-quality face cloths. For these the action of the finest wire brushes is too severe.

The teasel crop begins as a fine seed sown in drills in the spring. The early husbandry is as for roots, and the crop is hand-hoed to keep down weeds and afterwards singled out to 4 in. apart. By the end of autumn the plants are ready for transplanting—the teasel is a biennial—and lifted with a special tool called a 'spitter', which severs the long tap root. They are dibbled on to the ploughed furrow at a rate of twelve thousand plants to the acre. The convention is 30 in. between the rows and 24 in. between the plants. During this second year the plants throw out big central stems and side branches surmounted by prickly heads, which are ripe for the teaselling knife in August.

The teasel is one of the latest crops to receive the benefits of modern crop protection, which has considerably increased the yields from the fields of the more progressive growers. Squat malformed plants producing soft teasels with numerous 'dead head' plants as well had long been a mysterious problem in the Somerset teasel beds. Long field rests did nothing to help. Then the eel worm was isolated as a major teasel pest, which explained why resting between crops for long periods had not helped. The pest was being perpetuated in the soil by other crops such as oats, mangolds and beans, and on numbers of hedgerow hosts such as cleavers and chickweed. It was also being transmitted by infested seed, and teasel seed can now be pre-treated like clover to kill the pest. The growing crop may also need dusting against the teasel leaf miner—small egg-laying black-flies which hatch maggots on the leaves and cause bacterial rots. Not so easily handled, but rarely occurring, are attacks by the cucumber mosaic virus which causes leaf mottling and stunted growths, and the fungus of violet root rot, which destroys the tap root and starves the plant of nutriment.

MAGNETIC RESONANCE

It has become customary to hold a workshop in magnetic resonance each year at Varian Associates in Palo Alto, California. At these workshops new workers are introduced to the principles and practice of nuclear magnetic resonance and electron paramagnetic resonance, while those already experienced in the fields have the opportunity to discuss new developments. This custom has been adopted by the Swiss daughter company, Varian *A.G.*, the second annual workshop of which was recently held at its Research Laboratory in Zurich during September 24–28.

Lectures and laboratory demonstrations in both subjects were offered, the lectures being given in the main building of the University of Zurich while practical work was conducted at Varian's Research Laboratory. The introductory lecture to the first part of the programme—devoted to high-resolution nuclear magnetic resonance—was given by Dr. E. Rogers of Varian Associates, who was one of the Bloch group during the early days of nuclear resonance. Dr. J. N. Shoolery, also of Varian Associates, talked about the theory of nuclear magnetic resonance spectroscopy during the following two lectures, thus providing a foundation for the interpretation by the participants themselves of high-resolution spectra. Further instruction in spectra interpretation and in spectrometer operation was given by Dr. P. von Philipsborn of the University of Zurich, Dr. R. J. Abraham of the University of Liverpool, and Dr. A. Melera of Varian *A.G.* The instrumentation of nuclear resonance spectrometers was discussed by Mr. R. Wenk of Varian *A.G.*, and the course was finished by Dr. Abraham with a lecture about recent applications of proton coupling constants in organic chemistry.

The introductory lecture to electron paramagnetic resonance was given by Dr. A. Horsfield of Varian *A.G.*, and this was followed by a lecture on free radicals in solution by Dr. A. Carrington of the University of Cambridge. Dr. J. Combrisson from the French Atomic Energy Authority at Saclay talked about the applications of double resonance and the Overhausser effect in chemistry. The principles of electron paramagnetic resonance instrumentation were given by Mr. R. Tschudin of Varian *A.G.* An account of oriented free radicals in crystals was given by Dr. A. Horsfield, and Dr. A. Carrington gave a second lecture on transition metal ions and the triplet state as studied by electron resonance. Prof. R. Adams of the University of Kansas, in closing the course, spoke of the use of electron paramagnetic resonance techniques in electrochemistry. Practical work included instruction in the analyses of electron paramagnetic resonance spectra and demonstrations with an electron resonance spectrometer were given.

A total of ninety participants attended the workshop, representing most of the countries in Europe. A ladies' programme was arranged for the wives of the conference members and a workshop dinner was held at one of the old guild-houses of Zurich. It is planned to hold another workshop in magnetic resonance in Zurich in September 1963.

OCTACALCIUM PHOSPHATE AND HYDROXYAPATITE

Crystal Structure of Octacalcium Phosphate

OCTACALCIUM phosphate (OCP), also called tetracalcium hydrogentriphosphate, $Ca_4H(PO_4)_3 \cdot 2{\cdot}5H_2O$, is frequently encountered in systems containing calcium phosphates more basic than dicalcium phosphate[1–4], and it appears to have a significant role in the chemistry of bones, teeth, phosphate fertilizers, and other precipitated calcium phosphates (see following communication). Its existence as a distinct crystalline phase rather than a hydrous 'defective apatite' has been questioned[5,6], but was established by single crystal[7] and powder-diffraction X-ray studies[8]. Its X-ray, optical, and chemical properties indicate[7] that OCP is structurally related to hydroxyapatite (HA), although the two salts are not isostructural, and that OCP is composed of apatitic layers that are parallel to the *b–c* plane and separated by hydrated layers. The structure of OCP, a preliminary account of which is given here, is in accordance with these indications. The method of preparation of OCP is described in the following communication.

The following unit-cell constants of OCP were used in the structure analysis:

a = 19·87 Å α = 89° 17′
b = 9·63 Å β = 92° 13′
c = $6{\cdot}87_5$ Å γ = 108° 57′
$Z = 2(Ca_8H_2(PO_4)_6 \cdot 5H_2O)$ Space group, $P1$ or $P\bar{1}$

The centrosymmetric space group, $P\bar{1}$, which was assumed in the analysis, was verified by the success of the structure determination. Chemical analyses do not yield constant numbers of waters of hydration, and this uncertainty reflects the ease with which water enters and leaves the OCP lattice. The cell contents listed in the tabulation were used in the final structure refinement which required 172 positional and thermal parameters for the 8 calcium, 6 phosphorus, and 29 oxygen atoms in the asymmetric unit. Eleven scale factors were included also, but the parameters of the 12 unique hydrogens were omitted, so that a total of 183 parameters was used. Altogether, 2,332 independent structure factors were utilized in the refinement; of these, 1,419 were from intensities measured photometrically and 913 from intensities estimated visually. Most of the visually estimated intensities were those of reflexions too weak to be measured photometrically.

The initial structure approximation was derived from Patterson sections, $P(U,V,W)$, at levels $W = 0$, 1/4, and 1/2. Positions in two dimensions for the eight calciums and five of the phosphates were derived mostly from a series of ($hk0$) Fourier projections. The remainder of the structure was determined by a series of three-dimensional Fourier syntheses and least-squares refinements which brought the disagreement between the observed and calculated structure factors within 12 per cent. The averages for the estimated standard deviations in the positional parameters are 0·005 Å for the calciums, 0·006 Å for the phosphorus atoms, 0·021 Å for the phosphate oxygens, and 0·045 Å for the water oxygens.

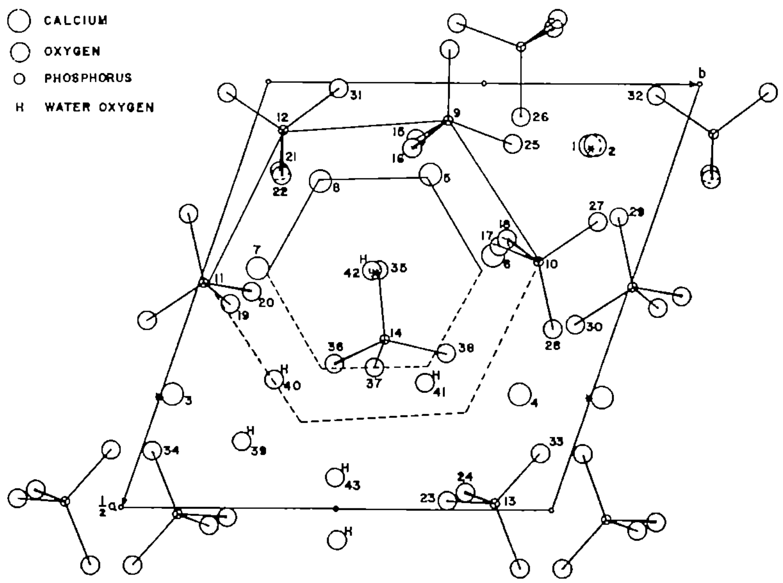

Fig. 1

The atomic parameters of OCP are listed in Table 1 and the structure, projected down the c axis, is shown in Fig. 1. The numbering of atoms in the figure corresponds with that in Table 1. The corners of the larger hexagon in Fig. 1 are the phosphorus sites as they occur in HA[*], and the corners of the smaller hexagon are the calcium sites. Only four of the sites on each of these hexagons are filled in OCP. The sites of the missing atoms are shown connected by broken lines. Four phosphorus positions of OCP closely match those of HA; but this is less true for the four calciums, particularly Ca(6) and Ca(7). A water molecule, O(41), appears to have replaced a missing calcium. The remaining four calciums of HA comprise two pairs of 'column' calciums. In OCP, one of these pairs, Ca(1) and Ca(2), is in virtually the same position as in HA, in which the two calciums lie one above the other in the position marked by the asterisk. The other pair of column calciums is split in OCP—one, Ca(3), is quite near its HA site as shown by the asterisk, and the other, Ca(4), is shifted by about 1·5 Å parallel to the b axis. The structure of OCP is a close copy of that of HA for small values of x. In the region where x approaches the value 1/4, the dissimilarities become quite apparent, and at larger values of x there is no similarity between the two salts. The region defined approximately by $1/4 > x > -1/4$ is referred to as the 'apatite layer'.

Table 1. ATOMIC POSITIONS OF OCTACALCIUM PHOSPHATE

Atom number	Parameter x	y	z
Ca(1)	0·0717	0·7929	0·0048
Ca(2)	0·0702	0·8055	0·5029
Ca(3)	0·3660	0·0246	0·0226
Ca(4)	0·3628	0·8339	0·5313
Ca(5)	0·1071	0·4490	0·7617
Ca(6)	0·2016	0·6600	0·2745
Ca(7)	0·2178	0·1250	0·7697
Ca(8)	0·1153	0·2006	0·2606
P(9)	0·0431	0·4473	0·2576
P(10)	0·2073	0·7703	0·7749
P(11)	0·2348	0·0096	0·2736
P(12)	0·0562	0·0708	0·7577
P(13)	0·4922	0·8646	0·2668
P(14)	0·3001	0·4748	0·2455
O(15)	0·0638	0·3836	0·0744
O(16)	0·0751	0·3885	0·4312
O(17)	0·1897	0·6638	0·5986
O(18)	0·1812	0·6769	0·9556
O(19)	0·2589	0·0867	0·0798
O(20)	0·2452	0·1271	0·4856
O(21)	0·1031	0·0981	0·5766
O(22)	0·1072	0·1030	0·9404
O(23)	0·4891	0·7499	0·1045
O(24)	0·4803	0·7856	0·4661
O(25)	0·0693	0·6141	0·2600
O(26)	0·0386	0·6095	0·7360
O(27)	0·1600	0·8727	0·7506
O(28)	0·2852	0·8573	0·7889
O(29)	0·1547	0·9172	0·2606
O(30)	0·2803	0·9085	0·3103
O(31)	0·0075	0·1677	0·7543
O(32)	0·0133	0·9075	0·7606
O(33)	0·4317	0·9276	0·2811
O(34)	0·4332	0·0284	0·7404
O(35)	0·2187	0·4045	0·2111
O(36)	0·3272	0·3791	0·3976
O(37)	0·3335	0·4730	0·0520
O(38)	0·3153	0·6280	0·3236
O(39)	0·4212	0·2259	0·2577
O(40)	0·3481	0·2501	0·8202
O(41)	0·3506	0·6046	0·7244
O(42)	0·2205	0·3886	0·7743
O(43)	0·4618	0·4718	0·6508

Of the remaining two phosphate ions, the one near the lower edge of the figure, P(13), ties the apatite layers together, and the other, P(14), lies in the region which in HA is occupied by the two 'hexagonal' calcium atoms which are missing in OCP. One of the oxygen atoms, O(35), of P(14) lies in the centre of the hexagon and occupies an OH^- site of HA. The other OH^- site of HA is occupied in OCP by a water molecule, O(42). Where the symbols for the phosphate oxygen, O(35), and the water molecule, O(42), overlap in Fig. 1, the asterisk depicts both the centre of the two hexagons and the OH^- sites of HA. The region bounded by the non-apatitic phosphates P(13) and P(14) is an irregular channel filled with waters of hydration. The portion of the cell defined approximately by $3/4 > x > 1/4$ is referred to as the 'water layer'.

The water molecule, O(43), shown as the pair nearest the symmetry centres at $x = y = 1/2$, appeared in the Fourier section with about one-half the electron density of the other oxygen atoms, and its temperature factor became abnormally large in the least-squares refinement. This is taken to indicate that this site was incompletely occupied in the crystal on which the X-ray intensity measurements were made.

Its layer-type structure and the similarity of part of its structure to that of HA are reflected in the chemical and physical properties of OCP (following communication). The close correspondence between the atomic positions in the apatite layer of OCP and those of HA indicates that epitaxial overgrowths of one compound on the other are very likely. The water layer, which occupies almost half the OCP unit cell, rules out consideration of OCP as a 'defect apatite' and makes formation of true solid solutions of the two compounds highly unlikely. Formation of lamellar mixed crystals of the two compounds, as described in the following communication, is more probable.

The channel in the water layer explains both the ease with which OCP loses water and the difficulty of establishing a definite degree of hydration. The formula for OCP used here is based on the assumption that the partially filled water site for O(43) is completely filled in the ideal compound. There is sufficient room at the site for a water molecule, where it is co-ordinated to eight oxygens by distances less than 3·5 Å. Complete loss of water from this site would yield the formula $Ca_4H(PO_4)_3.2H_2O$, which is sometimes given[3,4] for OCP. There is no space in the unit cell large enough to accommodate another water molecule, and it appears that earlier indications[7,8] of more than 2·5 moles of water are in error. Hayek *et al.*[3] found that two samples of OCP dried for a short time at 100° C contained 2·5 moles of water, indicating that drying at this temperature removes non-essential water without much loss of water from the lattice. In view of these results, the formula $Ca_8H_2(PO_4)_6.5H_2O$ would be a better designation for OCP because it avoids fractional moles of hydration, it represents the asymmetric unit, and it is consistent with the common name.

The asymmetric unit of OCP contains two non-water protons which may form interphosphate hydrogen bonds. One such hydrogen bond is indicated by a distance of 2·47 Å between oxygens O(20) and O(36). This hydrogen presumably contributes to the weak 2,400 cm^{-1} infra-red absorption reported for OCP (ref. 10) and for 'calcium-deficient' apatites[11]. The other interphosphate oxygen-to-oxygen distances are greater than 2·9 Å, and a second interphosphate hydrogen bond cannot be located definitely on the basis of the present information.

The great variety in the anion–cation combinations which form apatite-type structures indicates that a high degree of stability is associated with this atomic arrangement. OCP appears to be the first-established compound with this arrangement in a layer-type structure, and it is noteworthy that this structural feature persists even though it is present in only about one-half of the unit cell. Arnold[1] and Carlstrom[5] suggested that OCP is a hydrated, layer-type modification of HA. Their views that the layers are formed simply by replacement of calcium ions by hydronium ions and that the layers are normal to the *c* axis were not borne out by the structure determination.

The two most common habits of HA (following communication) are such that the forms (100) could be the major contributors to the surface area of the crystals. Since the layers of OCP also are parallel to the 100 face, the structure of the water layer may prove to be a useful model for describing the interface between the apatite crystal and the aqueous phase.

The two-dimensional phase of this work was done at the University of Amsterdam on a grant from the Rockefeller Foundation, Agriculture, with advice of Prof. Caroline H. MacGillavry of the Laboratory for Inorganic Chemistry. Mr. Abraham Kreuger of the Netherlands Organization for Pure Scientific Research (Z.W.O.) prepared the films from which the intensity data were collected.

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Crystallographic and Chemical Relations between Octacalcium Phosphate and Hydroxyapatite

MANY investigators[1,5,6,12–14] have observed that precipitated calcium phosphates that give apatitic X-ray patterns often have mole ratios Ca : P less than that of hydroxyapatite (HA), $Ca_5(PO_4)_3OH$. Most of these investigators have advanced explanations for this apparent anomaly, none of which is entirely satisfactory. On the basis of a recent determination (preceding communication) of the structure of octacalcium phosphate (OCP), $Ca_4H(PO_4)_3.2{\cdot}5H_2O$, we offer another explanation which is consistent with the crystal structures of these two calcium phosphates. We have suggested[7] that HA and OCP form crystalline interlayered or lamellar mixtures analogous in this respect to clays. Such intra-crystalline mixtures not only account for apatitic precipitates with ratios Ca : P less than that in HA, but also explain observed chemical and physical properties of precipitated basic calcium phosphates.

We have obtained further evidence in support of this explanation from X-ray and petrographic investigations of basic calcium phosphates the mole ratios of Ca : P of which ranged from 1·33, that in OCP, to 1·67, that in HA, and of products obtained from OCP by dehydration in air, hydrolysis in boiling water, and treatment with fluoride solutions. Many of the alteration processes were observed with single-crystal X-ray techniques. The basic calcium phosphates were prepared in several ways, including titrations of phosphate solutions with solutions of calcium salts, ammoniation of acidic calcium phosphate solutions, hydrolysis of dicalcium phosphate, and diffusion of solutions of calcium acetate and sodium phosphate. The temperatures of the preparations ranged from 20° to 100° C.

The crystals used in the single-crystal X-ray studies were prepared by diffusion at 50°–60° C of 0·5 M sodium phosphate solution into a 0·5 M calcium chloride solution. Both solutions were also 0·5 M in sodium acetate, as was the barrier solution; all were adjusted to *p*H 8. The optical properties of some of the crystals showed that they had partially hydrolysed.

The optical properties of OCP were measured on crystals that were prepared by slow hydrolysis at 40° C of dicalcium phosphate in 0·5 M sodium acetate solution[7].

OCP crystallizes as thin (100) blades of triclinic : pinacoidal symmetry. The blades are elongated along *c* and modified by the set {010} and the terminating set {011}. Contact twinning with (100) as the twin plane was commonly observed optically. Single-crystal X-ray patterns commonly revealed twinning with *c* as the twin axis, and this is probably the polysynthetic twinning observed optically. In both modes of twinning the (100) is the composition plane.

The crystals are biaxial (–) with a $2V$ of 50°–55° (calc. 54°). The refractive indexes measured by the immersion method at 25° C are $N_\alpha = 1{\cdot}576$, $N_\beta = 1{\cdot}583$, and $N_\gamma = 1{\cdot}585$. The optic plane is inclined to the (100), where its trace on that face is at 78° relative to *c* in acute α. The acute bisectrix lies in the quadrant defined by *a*, *-b*, *-c*. One optic axis is roughly normal to (100), which accounts for the very low birefringence of crystals in their preferred orientation. Dispersion $(r > v)$ is very weak. The specific gravity obtained by the sink–float method is

2·61 g/cm^3, and the value calculated from refractive indexes[17] is 2·59 g/cm^3.

The indexes of refraction of microscopically coarse, euhedral crystals of synthetic HA with the correct chemical composition are $n_\epsilon = 1{\cdot}640$ and $n_\omega = 1{\cdot}646$.

Preparations with ratios Ca : P between those of HA and OCP give X-ray powder-diffraction patterns with lines of both salts, and the relative intensities of the OCP lines generally decrease and those of the HA lines increase as the ratio Ca : P increases. Individual preparations may appear homogeneous under the microscope because of the uniform appearance of the crystals. Optically, however, the individual crystals display wavy extinction, symmetrical extinction without the expected conoscopic figures, or marked variation in refractive indexes from one crystal to another. In some preparations, the variation in refractive indexes is slight; but in others it extends over much of the range between OCP and HA. The optical properties of individual crystals show them to be intra-crystalline mixtures, even when they appear superficially homogeneous.

The idea that crystals of this type are lamellar mixtures of HA and OCP (ref. 7) was supported by the features of close structural similarity in the two salts (preceding communication). Proof of this idea was supplied by a single-crystal X-ray study of an optically homogeneous 'crystal' the indexes of refraction of which were between those of HA and OCP. Its zero-layer Weissenberg pattern from a c-axis setting could be indexed on the basis that it contained both salts with the b and c axes of the HA collinear, respectively, with those of the OCP.

The mean index of refraction of a lamellar mixed crystal should increase roughly in proportion to the HA content, and Posner and Perloff[18] found a nearly linear relationship between the mean index and the ratio Ca : P in their preparations. An exact relationship of this type is unlikely, however, because both non-essential water[19] and the history of the sample have considerable effects on the refractive index. The optical indicatrix does not change in a simple manner with the ratio Ca : P because the mixed crystals are very thin, the OCP component has a pronounced tendency to twin, and the conoscopic figures are rarely visible and are indistinct and composite when they can be seen. Lamellar intra-crystalline mixtures should produce indistinct conoscopic figures when viewed normal to the plate because the direction then is nearly parallel to an optic axis of the OCP component and perpendicular to the optic axis of the HA. On edge view, OCP is optically length slow and HA is length fast. In a lamellar mixture these effects should tend to compensate each other, and the birefringence should be minimum in a crystal containing about two parts of HA and one part of OCP. This mixture would have a mean refractive index of about 1·625, and crystals with indexes near this value show feeble birefringence on edge view.

The lamellar crystals are so thin that the different layers cannot be distinguished under the microscope, so it is not known how many layers are in any given crystal or whether the usual number of layers is large or small. There are two processes by which the lamellar mixed crystals might be formed: one of these, epitaxial overgrowth, might produce crystals with many layers; and the other, hydrolysis, might produce crystals with few layers.

Because of similarities between the structures of HA and OCP (preceding communication), epitaxial overgrowths of one compound on the other should occur easily. Variations in the composition of the liquid phase in the immediate vicinity of a growing crystal, as in a dropwise neutralization, could cause alternate deposition of first one compound and then the other. This type of lamellar growth could result in crystals with many layers.

Hot aqueous solutions cause OCP crystals to hydrolyse to pseudomorphs the properties of which progressively approach those of HA. Crystals produced in this manner may contain few layers, such as a layer of OCP sandwiched between two layers of HA.

It would be difficult to demonstrate that the lamellar mixed crystals can be formed by epitaxial overgrowth alone. OCP hydrolyses so readily to HA, particularly at temperatures above 50° C, that this alteration could be occurring even under conditions where the epitaxial overgrowth mechanism appears predominant.

Hydrolysis of Octacalcium Phosphate. In boiling water the progressive hydrolysis of OCP to HA is reflected in a gradual increase in indexes of refraction. At the same time, the intensities of the OCP lines in the X-ray powder pattern of the residual solids decrease, and after continued boiling the solids give sharp patterns of HA. The Weissenberg pattern of an OCP crystal which had been boiled in water for 6 h showed reflexions from both OCP and HA, with the b and c axes, respectively, of OCP collinear with those of HA. The pattern of an OCP crystal that had been in boiling water for 72 h, however, was essentially that of HA alone. The reflexions were sharp, and the pattern showed the full hexagonal symmetry of HA, which indicated that there had been considerable re-organization of the lattice during the hydrolysis.

Even after prolonged boiling, the index of refraction of the residue was never higher than 1·635. It then appears either that the hydrolysis is never completed or, more probably, that the resulting pseudomorphs contain fissures that result from incomplete closure of the lattice after loss of water and phosphate ions, and possibly calcium ions. The fissures, which probably are parallel to the (100) face of the original OCP, could be sites of entry and retention of carbonate and other foreign ions in sedimentary phosphorites, hard tissues, and other apatitic materials that may be formed from OCP, and they could also contribute to internal or 'entrapped' surface area[20]. Pseudomorphs formed by hydrolysis of OCP in the presence of carbonate ions give strong tests for carbonate.

Anhydrous dicalcium phosphate (DCPA), $CaHPO_4$, is sometimes formed as a separate phase in the hydrolysis of OCP, in agreement with phase-rule considerations of the system $CaO–P_2O_5–H_2O$ (ref. 21).

Magnesium, even in concentrations as low as 0·001 N, markedly inhibits the hydrolysis of OCP. This inhibition may result from the formation of a magnesium complex on the terminal edges of the crystals that impedes loss of water molecules and ions from channels in the 'water layers' (preceding communication) of the OCP, and may be important in the growth and development of hard tissues.

OCP probably was not the most stable calcium phosphate under any of the conditions of its preparation, even though OCP precipitated in preference to HA and DCPA. The rapid precipitation and subsequent alterations of OCP go far to explain some complications in the chemistry of basic calcium phosphates. As an example, when 3 g of dicalcium phosphate dihydrate, $CaHPO_4.2H_2O$, was added to 100 ml. of distilled water at 85° C with continuous stirring, OCP crystals were observed as a separate phase within 5 min. These crystals grew for about 15 min until the dicalcium phosphate dihydrate nearly disappeared. At about this time, aggregates of extremely fine acicular crystallites of HA appeared, and soon rounding of edges of the OCP crystals indicated that the continued precipitation of HA was maintained by dissolution of OCP. The remaining OCP was converted to apatite, mostly through hydrolysis *in situ*, when the mixture was allowed to cool and stand overnight. Thus, HA apparently was produced by two mechanisms, and its acicular or platey habit showed whether it had been produced by direct precipitation or hydrolysis, respectively. At lower temperatures, conversion of dicalcium phosphate dihydrate to DCPA, rather than formation of HA, appeared to terminate the precipitation of OCP.

Precipitates with low indexes of refraction and with ratios Ca : P near those of OCP usually are relatively large thin blades similar in morphology to OCP. As the compositions and indexes approach those of HA, the typical habit becomes rosette clusters of very small scalar crystals. Precipitates in which the ratio Ca : P was essentially that of HA consisted of stellate clusters of delicate needles and only then did the crystallites have the hexagonal habit of HA.

Apatitic crystallites in bone are present with both scalar and needle habits[22]. The co-existence of crystals of two habits is consistent with the two mechanisms of formation of HA—scalar crystals from partial or complete hydrolysis of OCP (ref. 7), and needle crystals by direct precipitation of HA. There is a possibility, however, that a few layers of OCP in the seeds from which the scalar crystallites developed may have modified the directly precipitating HA into a platey habit. These crystallites, however, are (100) plates and not distortions of the (001) platey habit of HA as has been implied[1].

Reaction of Octacalcium Phosphate with Fluoride. In aqueous systems containing low concentrations of fluoride ion, fluorapatite is the most stable calcium phosphate[23], and OCP is reported to react with fluoride to produce an apatite[24]. It is significant that this reaction also occurs *in situ*, and the resulting crystals are pseudomorphs after the original OCP. In an examination of this reaction, a single crystal of OCP was mounted on an X-ray goniometer and its zero-layer Weissenberg pattern from a *c*-axis setting was obtained after the crystal had been immersed in 0·1 N sodium fluoride solution at 45° C for different times. Only OCP reflexions were present in the pattern of the untreated crystal; both apatite and OCP reflexions were present in the pattern after 5 h of immersion, and, except for a few very faint OCP reflexions, only apatite reflexions were present in the pattern after 9 h of immersion. The apatite reflexions were less sharp than the OCP reflexions, indicating that the apatite 'crystal' was composed of oriented sub-units. The orientation of the apatite crystallites relative to the original OCP was the same as for the OCP–HA crystals described previously.

The multicrystalline character of the apatite crystal was indicated further by the fact that it fell apart while being handled for optical study. In contrast to dehydrated or hydrolysed OCP crystals, fluoride-treated OCP crystals are very soft and smear easily, and their multicrystalline character implies that they may adsorb or occlude relatively large amounts of impurities. If OCP is present in tooth enamel, fluoride treatment would increase its chemical stability but may decrease its mechanical strength.

When a 1-g sample of OCP was treated with 100 ml. of 0·1 N sodium fluoride solution at 45° C for 7 days, the residue dried at 105° C for 1 h weighed 0·844 g and contained CaO 52·9, P_2O_5 41·2 and fluorine 4·0 per cent. This composition and the loss in weight indicate that the product was an impure fluorapatite (theoretical for $Ca_5(PO_4)_3F$, CaO 55·6, P_2O_5 42·2, fluorine 3·8 per cent). It is probable that the impurities can be attributed to the multicrystalline character noted here. With the same treatment, fine-grained HA took up only 0·4 per cent fluorine, indicating that HA reacts much slower with fluoride than OCP. Similar treatment of materials with compositions between those of HA and OCP caused fluorine uptakes roughly proportional to the OCP contents calculated from the ratios Ca : P. It is concluded that, when a lamellar mixed crystal of HA and OCP is treated with fluoride solution, the resulting crystal is largely fluorapatite interlayered with HA.

Dehydration of octacalcium phosphate. On drying at temperatures slightly above 100° C, OCP loses some of its water of crystallization without apparent change in its X-ray powder diffraction pattern (preceding communication and ref. 3), although the mean refractive index increases slightly. As the drying temperature is raised, the material progressively loses water, the 18·7-Å line of OCP shifts to slightly lower spacings, the OCP lines in the X-ray pattern gradually weaken and disappear at about 180° C, and the refractive index rises to a maximum of 1·61, which is well below that of HA. Except for the 002 line, the powder pattern of material dried at 180° C is very diffuse; it resembles that of a poorly crystallized apatite, and it contains no DCPA lines. The presence of DCPA in a sample heated at 220° C, however, has been detected[7] by X-ray diffraction.

The Weissenberg patterns of a single crystal of OCP which had been dried at 180° C contained diffuse spots which corresponded in position and intensity to those of an apatite. Their positions indicated also that the *c* axis and *h*00 planes of the apatite were parallel to the corresponding elements, respectively, of the original OCP. Present also were rather sharp reflexions of DCPA the positions of which indicated that the *b* axis and 00*l* planes of DCPA were parallel to the *c* axis and *h*00 planes, respectively, of the original OCP.

The patterns also contained diffuse streaks along the reciprocal lattice lines parallel to the reciprocal axis a^* of the original OCP. The streaks indicate the presence in the dehydrated OCP of amorphous, partially oriented, 'collapsed OCP layers' that remain after destruction of 'water layers' (preceding communication) of the OCP lattice. The diffuse streaks showed a two-fold symmetry about the *c* axis, in keeping with the pseudosymmetry of OCP, the 'apatite layers' of which presumably tend to persist in the collapsed lattice, and not with the hexagonal symmetry of HA. The intensities of the diffuse streaks indicated that the collapsed OCP layers comprised a considerable fraction of the dehydrated crystal.

Since DCPA was detected by the single-crystal method but not by X-ray powder diffraction, it is possible that DCPA has been present and overlooked in many apatitic materials. The strongest line of

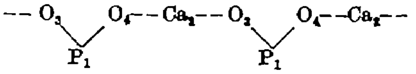
DCPA nearly coincides with the 002 line of HA or OCP which is frequently used to measure the particle size of apatitic material by line broadening. This measurement can be reliable only if DCPA, OCP and collapsed OCP are absent.

Results of previous studies[7] of dehydrated OCP were taken to indicate that the product was a defect apatite. The fact that OCP breaks down on drying into an apatite and DCPA, as shown by the single crystal X-ray investigation, makes it unnecessary to postulate the existence of a defect apatite, and the quasi-amorphous material, collapsed OCP, which is presumed to give rise to the diffuse streaks in the X-ray pattern, cannot properly be called hydroxyapatite.

The reasons for the orientations of the apatite and collapsed OCP relative to the original OCP crystals are apparent from structural considerations. The orientation of the DCPA relative to the original OCP crystal is understandable in terms of the three lattice elements of OCP (preceding communication) and DCPA (ref. 25) that lie in the plane of juncture; these are shown as follows:

OCP	DCPA
b = 9·63 Å	a = 6·91 Å
c = 6·88 Å	b = 6·66 Å
α = 89° 17′	γ = 89° 11′

Although the difference between b of OCP and a of DCPA precludes a normal epitaxy between the two compounds, there is a structural similarity which may orient the DCPA. In the notation of MacLennan and Beevers[26], DCPA has calcium phosphate chains of the type:



that are parallel to the b axis. These chains are similar to those parallel to the c axes in OCP and HA that involve the 'hexagonal' calciums (preceding communication). The ratio of the length of b of HA to that of a of DCPA is close to 3/2, which may be conducive to the orientation.

The zero-layer Weissenberg pattern of dehydrated OCP has strong reflexions with d values of $3{\cdot}37_5$, 2·70 and 2·25 Å on the central lattice line coinciding with that of the h00 reflexions of the original OCP. These spacings coincide almost exactly with the fourth, fifth and sixth orders of a 13·50-Å lattice spacing. This was first interpreted to mean that removal of the water had collapsed the OCP layers so that they had an average spacing of 13·50 Å instead of 18·68 Å as in OCP. It was found, however, that the $3{\cdot}37_5$- and 2·25-Å spacings could be indexed as the 002 and 003 reflexions, respectively, of DCPA, and the 2·70-Å spacing as the 300 reflexion of HA. The closeness of the fit shows that the thickness of the unit cell of HA, d_{100}, is very nearly equal to 5/6 of d_{001} of DCPA. The 002 and 003 reflexions of the DCPA component were relatively strong, and the possibility that some of their intensities arose from collapsed OCP layers with a spacing of 13·50 Å cannot be ignored.

HA and DCPA, the two crystalline phases in dehydrated OCP, are the stable solid phases at the invariant point near the water corner of the system $CaO–P_2O_5–H_2O$. Treatment of dehydrated OCP with boiling water then should cause these phases to increase at the expense of the amorphous phase, and, in accord with this, 6 h of this treatment sharpened the X-ray powder pattern of dehydrated OCP. This treatment of dehydrated OCP may be a method for preparing mixtures of HA and DCPA in which each particle would contain both phases. Such a product would be useful in studies of the uptake of phosphate by plants and in physiological studies because, according to the phase rule, the presence of the second solid phase reduces the number of degrees of freedom by one. We have no evidence of the formation of intracrystalline mixtures of HA and DCPA by direct precipitation or by hydrolysis of OCP; but, in view of the stability of the two solids, such mixtures should be anticipated.

Ignition at 500°–600° C converts DCPA to a pyrophosphate. The net process for OCP, then, could occur according to either of the equations:

$$4[Ca_4H(PO_4)_3 . 2{\cdot}5H_2O] = 2Ca_5OH(PO_4)_3 + 3Ca_2P_2O_7 + 11H_2O \quad (1)$$

$$4[Ca_4H(PO_4)_3 . 2{\cdot}5H_2O] = 4Ca_3(PO_4)_2 + 2Ca_2P_2O_7 + 12H_2O \quad (2)$$

Half the phosphate would be converted to pyrophosphate in the reaction of equation 1, but only a third in that of equation 2. Gee and Deitz[27] determined the pyrophosphate contents of several apatitic preparations with different ratios Ca : P that had been ignited at 600° C for 60 h. The experimental points shown in Fig. 2 of their paper are poorly represented by the line labelled 'equation 4' which corresponds, in effect, to our equation 1. They fall very close, however, to a line defined by the condition that all the OCP in the precipitates reacted according to equation 2. It appears from their results that little of the calcium pyrophosphate produced according to equation 2 reacted at 600° C. with the original HA (that is, in the HA layers) to form more tricalcium phosphate, but that this reaction did occur above 800° C. This behaviour is more readily understandable in terms of crystals which are interlayered than ones in which the acidic groups are distributed homogeneously in the lattice as they should be if the crystals were defect apatites or solid solutions.

Detection of octacalcium phosphate by X-ray diffraction. An unsuccessful attempt was made to use the intensities of the 100 (18·68 Å) and 700 (2·67 Å) lines in the powder pattern of OCP as quantitative measures of the amount of OCP in precipitates of intermediate composition. The intensities of these lines varied irregularly and disproportionately with change in the ratio Ca : P. For example, the pattern of a sample with mole ratio Ca : P of 1·52 contained neither line, whereas the 18·68-Å line was obvious in the pattern of a physical mixture of 5 parts of OCP and 95 parts of HA which had a mole ratio Ca : P of 1·65. Since these lines, which are the strongest lines distinguishing OCP from HA, did not appear in the pattern of a sample that contained about 50 per cent OCP, it is not surprising that OCP is not detected in bones and teeth from their X-ray patterns even if it is present.

The reasons for the faintness of the distinguishing lines of OCP are not far to seek. The lines arise from planes parallel to the platey direction of the crystals, so that the intensities of lines of very thin crystals, such as those in bones and teeth, would be expected to be low. Lamellation and twinning also are parallel to the platey direction, and so decrease further the effective particle thickness. Furthermore, loss of water from OCP crystals may also lower the intensity of the distinguishing lines.

Since X-ray powder methods are not reliable for the detection of OCP in its mixtures in poorly crystallized materials, suitable chemical or physical methods for its detection would be highly advantageous. Such a test might be based on differences in the reactions of OCP and HA with fluoride ions.

Infra-red absorption. Posner, Stutman and Lippincott[13] report infra-red absorption data on synthetic 'calcium-deficient' apatitic materials in which the intensity of the peak at about 3,400 cm^{-1} is inversely proportional to the ratio Ca : P. This relationship is interpreted to indicate that the positive charges of the missing calcium ions are supplied by hydrogen ions that form interphosphate hydrogen bonds in the apatite lattice. Winand, Dallemagne and Duyckaerts[11,16] disagree and state that the frequency of the absorption band is too high to be caused by a hydrogen bond of the type $O_3PO{-}H\cdots OPO_3H$; they suggest that the absorption is due to water adsorbed by the crystals.

An alternative explanation, which is consistent with interlayering in these materials, is that the intensity of the infra-red absorption peak is proportional to the amount of OCP in the preparation and so inversely proportional to the ratio Ca : P. Because of its water content, OCP has a broad absorption peak in the 3,400 cm^{-1} region[10].

Winand, Dallemagne and Duyckaerts[11] report a weak absorption at 2,400 cm^{-1} for some of their calcium-deficient apatitic materials. This absorption could be caused by the shortest interphosphate oxygen-to-oxygen distance in OCP which is 2·47 Å (preceding communication). Although these results indicate that the materials studied by Winand and co-workers contained OCP, the 2,400 cm^{-1} peak may have arisen from DCPA. Samples treated in a hydrothermal bomb[16] should not contain OCP, but may contain DCPA and HA.

Causes for low ratios Ca : P. It is apparent that at least two compounds, OCP and DCPA, are difficult to detect by X-ray powder diffraction in the presence of apatite, and each may contribute to low ratios Ca : P in apatitic materials. Collapsed OCP may behave similarly. The present results do not preclude the existence of calcium-deficient or defect apatites, occluded phosphate, solid solutions, or dubious crystalline compounds which have been suggested to explain the observed variations in the ratio Ca : P in apatitic materials, but to a large degree they make it unnecessary to hypothesize the existence of such materials. Surface adsorption certainly influences both the ratio Ca : P and the water content when the crystallites are small, as they are in bone and dentine. The crystals used in this investigation, however, were too large for the external surface to have had a significant effect.

The general formula for the unit-cell contents of calcium-deficient apatites, $Ca_{10-x}(H_3O)_{2x}(PO_4)_6(OH)_2$, given by Neuman and Neuman[6] has for $x > 0$ more atoms than can be contained in the unit cell of HA and, furthermore, is based on a degree of hydration of OCP that appears incorrect. The loss of two molecules of water for each mole of OCP postulated by them is in accord, however, with the reaction:

$$Ca_4H(PO_4)_3.2{\cdot}5H_2O = 3/2CaHPO_4 + 1/2Ca_5(PO_4)_3OH + 2H_2O \quad (3)$$

indicated by the single-crystal dehydration experiment described earlier. Dehydration experiments are difficult to interpret because of experimental ambiguity between waters of crystallization of OCP and non-essential water; the variability in the amount of water evolved when OCP is dehydrated, depending on whether the product contains collapsed OCP, HA and DCPA, or $Ca_2P_2O_7$ and $Ca_3(PO_4)_2$; and the uncertainty in the extent of reaction of hydrogen ions of the OCP layers with the hydroxyl ions of the HA layers. These factors prevent placing much reliance on dehydration experiments and probably account in part for the variation in the reported values for the degree of hydration of so-called tricalcium phosphate hydrate[15,20].

A general formula for lamellar mixtures of OCP and HA might be written as $Ca_{10-x}(PO_4)_6(OH)_{2-x}(H)_x.2{\cdot}5xH_2O$, provided it is not considered to represent an isomorphous series.

The several general formulæ proposed for these materials may be written $(10{-}x)CaO.3P_2O_5.nH_2O$, where n has the value 1 for the formula of Winand and Dallemagne[16], $(1 + x)$ for that of Stutman, Posner and Lippincott[14], $(1 + 3x)$ for that of Neuman and Neuman[6], and $(1 + 2{\cdot}5x)$ for the one we give above for lamellar mixtures of HA and OCP. In this formula, n includes both water of constitution and water of crystallization, and, in practice, some non-essential water may also be included. The value of n should be obtained, therefore, by difference after accurate analysis for calcium and phosphorus, and not by dehydration. In the foregoing form, the general formula clearly shows the inadequacy of the ratio Ca : P as the sole compositional description of apatitic calcium phosphates.

Crystallographic methods are unlikely to distinguish between HA, OCP, and lamellar mixtures of the two salts when the crystallites are as thin as 25–75 Å as they have been reported for bone[28,29]. These dimensions correspond to no more than five d_{100} thicknesses of OCP. It appears, therefore, that a comparative study of the chemical reactivities and solubilities of HA, OCP and their lamellar mixtures is one of the more promising means for gaining an understanding of the properties of biological and other cryptocrystalline apatitic calcium phosphates.

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SOME POSSIBLE RELATIONSHIPS OF CARBOHYDRATES AND OTHER BIOLOGICAL COMPONENTS WITH THE WATER STRUCTURE AT 37°

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IN two previous publications[1,2] some suggested conformations for polypeptides were discussed based on hexagonal arrangements of the carbonyl oxygens of the peptide bonds. One of the interesting aspects of these suggested hexagonal patterns was the very close correlation with a similar regular hexagonal arrangement of the 'second neighbour' oxygen positions in water at a temperature of about 37°. (My calculations were based on a 'first neighbour' oxygen distance of 2·93 Å at 37° as explained in ref. 2. Later, I found a brief mention by Brady and Krause[3] to a 'first neighbour' oxygen distance of 2·94 Å at 30°. This suggests a value of 2·95 Å at 37°, and would represent a 'second neighbour' oxygen distance of 4·82 Å in liquid water at this temperature, compared with our calculation of 4·79 Å.) Thus, a water layer lying over a peptide layer in this suggested conformation would have the possibilities for exact collinear hydrogen bonding between the respective oxygen atoms of the two layers at this temperature.

In view of these similar dimensions it seemed expedient to examine other biological substrates and see if their reactive groupings also permitted definite interactions with the water structure at about 37°. Because the conformations of the cyclitols have been extensively studied and are well defined, a specific example, *scyllo*-inositol, was selected to serve as a model for some of the interrelations which may exist. Many other substances have been studied in less detail, and I will mention some relevant points about them.

Scyllo-inositol and the water structure. *Scyllo*-inositol is a hexahydroxy *cyclo*hexane having the all-*trans* conformation with the six hydroxyl groups equatorial[4]. A model of this compound was constructed from the Dreiding stereomodels using the stable chair conformation for the *cyclo*hexane ring. (It was interesting to note that even in the models the boat form of *cyclo*hexane is non-rigid and allows considerable rotation around the bonds between carbons. However, once the model is forced into the chair form, this conformation is very rigid and allows no rotation about the carbon–carbon bonds. Only the hydroxyl hydrogens can rotate around the C–O axis.) The conformation of *scyllo*-inositol is given in Fig. 1. Measurements made on such a model indicate that the oxygen atoms 1, 3 and 5 form the corners of an equilateral triangle with sides which measure about 4·86 ± 0·02 Å on the Dreiding scale. This oxygen pattern has, therefore, approximately the same dimensions as the pattern formed by three 'second neighbour' oxygen atoms in the water structure at 37° (4·82 Å on a side). In a similar manner oxygen atoms 2, 4 and 6 form an equilateral triangle of the same dimensions.

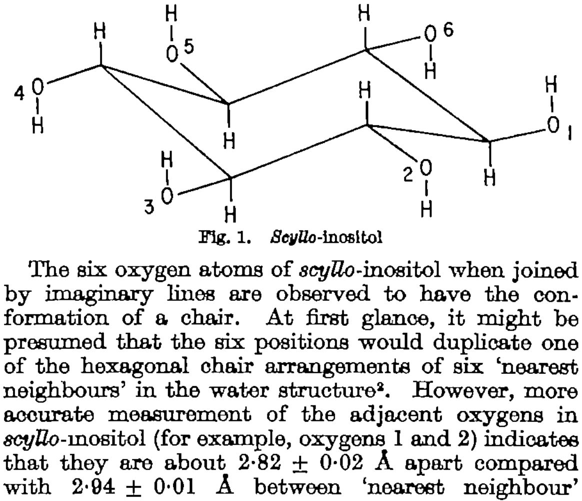

Fig. 1. *Scyllo*-inositol

The six oxygen atoms of *scyllo*-inositol when joined by imaginary lines are observed to have the conformation of a chair. At first glance, it might be presumed that the six positions would duplicate one of the hexagonal chair arrangements of six 'nearest neighbours' in the water structure[2]. However, more accurate measurement of the adjacent oxygens in *scyllo*-inositol (for example, oxygens 1 and 2) indicates that they are about 2·82 ± 0·02 Å apart compared with 2·94 ± 0·01 Å between 'nearest neighbour' oxygens in water at 37°. It was further observed that the respective 1–3–5 and 2–4–6 oxygen planes in *scyllo*-inositol are only about 0·48 Å apart, while the corresponding 'second neighbour' planes in water would be about 0·98 Å apart at 37° (Fig. 2, layers *C–D*, for example). The shorter distance between the oxygen layers in inositol and other sugars may bear some relation to the frequent occurrence of these compounds in nature as phosphate or sulphate esters; and the possibility should be kept in mind that the accommodation of these larger atoms into the water structure could require molecules with some alterations in the attaching positions to fit them into a possible stabilizing water pattern.

These possible interactions of inositols (and other sugars such as α- and β-D-glucose) with the water structure, especially with the 'second neighbour' pattern at 37°, indicate that such carbohydrates or their polymers could also interact in an orderly manner with peptide (or protein) layers arranged in

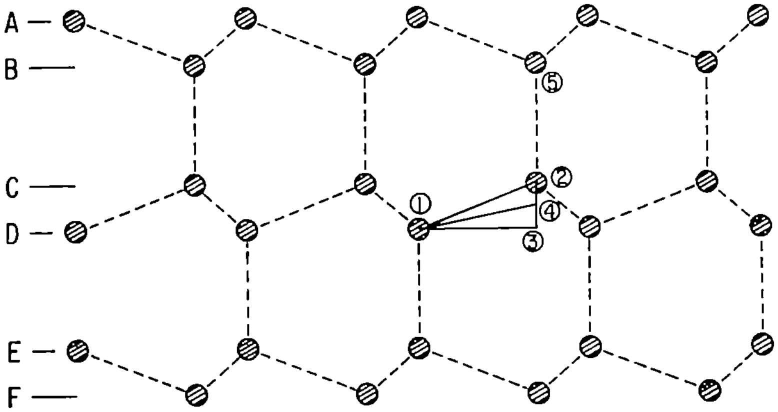

Fig. 2. Oxygen layers in the water structure (not to scale)

the hexagonal conformations which I have proposed[1,2]. In these conformations adjacent carbonyl oxygen atoms at the hexagonal corners are about 4·8 Å apart (with a possible 4·76–4·90 Å spread for the *cis-trans* interval as indicated in ref. 2); and any two corners and the hexagonal centre form an equilateral triangle with sides of about 4·8 Å. Therefore, hydroxyl groups 1, 3 and 5 of *scyllo*-inositol could form hydrogen bonds with two oxygens at hexagonal corners and have the third hydroxyl directly over a hexagonal centre. The presence of a phosphate ester, for example, on the third hydroxyl, would bring this phosphate directly over the hexagonal centre, which in some instances might have a serine hydroxyl present. (Using the Catalin space-filling models[1,2], it seems that phosphate fits well when centred over a hexagonal unit, particularly in units containing a single trans —N—H group (ref. 2, sec. 4) for bonding with the semipolar oxygen of the phosphate group. I have also looked at *myo*-inositol[4] fitted over such a hexagonal unit with phosphate on the axial hydroxyl, but exact measurements are difficult with space-filling models so this will be repeated if phosphorous atoms become available from Dreiding.)

The model also shows, as indicated in Fig. 1, that hydroxyl groups 1, 3 and 5 point upward as three parallel —O—H projection lines about 4·86 ± 0·02 Å apart. If the hydrogens of these three hydroxyl groups were bonded with three positions at similar 4·8 Å intervals (for example, two peptide oxygens and a hexagonal centre) the hydrogen atoms would be collinear with the bonded atoms for the strongest hydrogen bond[5]. The hydrogen atom could be furnished either by the sugar hydroxyl group or the enolic carbonyl of the peptide. In a similar manner the parallel projections of hydroxyl groups 2, 4 and 6, pointing downward as indicated in Fig. 1, could contact three similar positions below the *scyllo*-inositol ring. Recalling that my proposed peptide conformations allow hydrophobic and hydrophilic amide sides to the peptide structure[1,2], *scyllo*-inositol could bind two peptide layers above and below the *scyllo*-inositol ring through contacts with their respective hydrophilic amide surfaces.

Similar possibilities for bonding the hydrophilic amide surfaces of two peptide layers might be suggested for glycerol. In this molecule hydroxyls 1 and 3 can be arranged as parallel projections 4·8 Å apart, and the 2-hydroxyl group points in the opposite direction. Since the hydroxyl groups of glycerol (or *scyllo*-inositol or glucose), unlike the oxygen positions in water, will not vary appreciably in their separation distance with temperature, such bonds in glycerol could stabilize peptide structures at temperatures where uniform water contacts would be unlikely. The known utility of glycerol or glucose for stabilizing biological systems or viable tissues during freezing and thawing[6] could be partially explained by the suggested interactions based on our proposed hexagonal conformations.

We are now in a position to relate some of the observations on *scyllo*-inositol and peptides to a cross-section of the oxygen layers of the water structure as indicated in Fig. 2. This section[7] represents one of the possible edge-on views of the planar layers discussed in my previous article (ref. 2, plate 1), and each of the six layers of oxygen atoms represented by *A* through *F* would be a 'second neighbour' arrangement with the hexagonal patterns of water oxygens of ref. 2. At 37° ('nearest neighbour' oxygen in water = distance 1–2 = 2·94 ± 0·01 Å), layers *C* and *D* (or *A–B* or *E–F*) would be about 0·98 Å apart (distance 2–3) for the tetrahedral angle (109° 28′). Layers *B* and *C* (or *D–E*) would be 2·94 ± 0·01 Å apart (distance 2–5). If we now replace certain of these aqueous oxygen layers by my suggested peptide (or protein) conformations having similar hexagonal carbonyl oxygen patterns, the following results may be observed. Peptide conformations replacing layers *A* and *C* would have only a single 'second neighbour' aqueous oxygen layer, *B*, between them with a separation distance of 0·98 + 2·95 = 3·93 Å. The same distance would apply to each situation such as *B–D*, *C–E* or *D–F*, where two protein layers are separated by one 'second neighbour' oxygen layer. However, if the two peptide layers replace oxygen layers at intervals allowing two 'second neighbour' layers between, then the peptide layers may be separated by different distances. Thus, two peptide layers with their hydrophilic carbonyl oxygens in layers *A* and *D* (and their respective 'hydrophobic' or side-chain surfaces above and below these layers) would be about 4·91 Å apart at 37°, while two peptide layers replacing oxygen layers *B* and *E* would be about 6·88 Å apart. Although both the *A–D* and *B–E* peptide situations contain two 'second neighbour' aqueous oxygen layers between their carbonyl oxygen layers, the *B–E* situation could represent a possible interaction of water layers *C* and *D* with the enolized form of the peptide carbonyl groups, and the *A–D* situation suggests the interaction of the keto-form of the carbonyl oxygen with water layers *B* and *C*. If such possibilities exist, forces which might influence keto–enol transformations (for example, the transfer of phosphate from a sugar to a serine hydroxyl in the centre of a hexagonal peptide unit or vice versa) could conceivably operate to induce contractions or expansions between protein layers in liquid water.

If we consider the *A–D* and *B–E* peptide situations stabilized by water layers in Fig. 2, and attempt to fit the two 'second neighbour' oxygen planes of *scyllo*-inositol (1–3–5 and 2–4–6 oxygen planes, Fig. 1) into this water picture, then the distance between the planes and the general chair conformation is closer to the *C–D* spacing of water oxygens in the

B–E peptide situation. Referring to the triangle 1–2–3 in Fig. 2, with 'nearest neighbour' distance 1–2 = 2·94 ± 0·01 Å, and the angle 2–1–3 = 19° 28′, the projection of 2 on the *D* plane gives calculated values for the projection distance 1–3 = 2·77 ± 0·01 Å and for the interplane distance 2–3 = 0·98 Å. From the Dreiding models of *scyllo*-inositol it will be recalled that the distance between its two oxygen planes was 0·44–0·48 Å. Using the 0·48 Å distance and designating it as 3–4 on our small triangle (Fig. 2), the calculated distance 1–4 becomes 2·82 Å and the line 1–4 nearly bisects the 2–1–3 angle (angle 3–1–4 = 9° 48′, calculated). The distance 1–4 agrees well with the measured distance (2·82 ± 0·02 Å) between adjacent oxygens 1 and 2 in the models of *scyllo*-inositol, or what we might call the 'nearest neighbour' oxygens of *scyllo*-inositol. Since the points 5, 2 and 4 are apparently on the same straight line, it seems likely that *scyllo*-inositol is capable of maintaining approximately the same collinear hydrogen bonding possibilities between the suggested peptide layers *B* and *E* as would be afforded by water layers *C* and *D* at 37°, although the *B* and *E* layers could conceivably be about 0·5 Å closer together with *scyllo*-inositol. In this respect it is interesting to note that if hydroxyls 3 and 6 of *scyllo*-inositol (Fig. 1) are oxidized to keto-groups, then, in the resulting model, oxygens 1 and 2 (or 4 and 5) are now about 2·96 Å apart. (These estimates were measured from the chair form of the 3,6-diketo compound. The reference by Allinger and Freiberg[8] would indicate that in 1,4-*cyclo*hexanedione the boat form may predominate in inert solvents although no comment was made about interacting solvents, such as water.) This distance is very close to the 'nearest neighbour' oxygen distance in water (1–2, Fig. 2). At the same time the distances 2–4 or 1–5 in the 3,6-diketo-compound (cf. Fig. 1) are still about 4·84 Å apart, reasonably close to 'second neighbour' water oxygen distances. These four oxygens would then be capable of interchanging almost exactly with four water oxygens in the *C–D* layers (Fig. 2) although keto oxygens 3 and 6 would be far removed from their corresponding hydroxyl positions before oxidation.

General considerations. One other item in Fig. 2 may deserve special mention. The projection of oxygen position 2 on the *D* layer gives the distance 1–3 = 2·77 ± 0·01 Å. This distance is equivalent to the observed oxygen–oxygen distance in *ortho* dihydroxybenzenes (estimated at 2·76 Å, Drieding models) and may be very close to the oxygen–oxygen distance between enolic hydroxyls in vitamin C (2·78 Å from Dreiding models using conjugated carbon) or between adjacent methoxy oxygens in coenzyme *Q* (ref. 9). The distance between oxygens in *meta*-dihydroxybenzenes is about 4·76 Å in the Dreiding models. This is the same value as the calculated distance between the two carbonyl oxygens of a dipeptide bond in the *cis* configuration[2]. For a *meta*-amino-phenol the nitrogen–oxygen distance would be about 4·80 Å (Dreiding scale), and this may also represent the approximate distance between the 2-amino- and 6-hydroxy-groups in guanine. Therefore, these nearly planar aromatic systems with 1,2 or 1,3 substituents may also contain the possibilities for orderly interaction with our suggested protein conformations.

I have examined and measured other biologically active compounds, and in many instances the reactive groupings show similar interactions with the water structure. This present model of the water structure was made from the Dreiding tetrahedral carbon atoms, and as a consequence all measurements had to be transposed by a ratio of about 12 to 6·4 to fit oxygen distances in water. Trying to determine the position occupied by an entire steroid molecule, for example, under these conditions is very difficult, although the oxygen positions can be assessed. To avoid needless measurements and conversion errors, we have ordered appropriate tetrahedral atom units for assembling the oxygen positions of the water structure at 37° on the same scale as the other Dreiding models (1 cm = 0·4 Å) and complete with the resonating hydrogen positions on each bond. Not only will such a water model allow direct comparisons, but it will also enable one to assess more accurately the relation of axial hydroxyl groups in sugars, steroids and antibiotics to the water structure or my suggested peptide layers within that framework. It will also be interesting to see whether paraffinic hydrogens, for example, might interfere with the resonating hydrogen positions in the water structure at 37° and thus produce changes in the water stability at that particular site.

I would like to emphasize in conclusion that the measurements presented were made for the most part with the Dreiding stereomodels, and they are subject to inaccuracies inherent in the physical assembly and measuring devices[10]. However, the general results of the analysis of the *scyllo*-inositol case certainly suggest some interesting relationships with the water structure at about 37°.

These relationships have also permitted some speculations about feasible bonded interactions of carbohydrates with the peptide linkages of one or more peptide or protein layers in the conformations which I have proposed for consideration[1,2]. The bonded interactions are such that when small differences do occur in the measured oxygen–oxygen distances they are still within the limits of the 4·76–4·90 Å interval which was tentatively set up between adjacent carbonyl oxygens of a dipeptide for the *cis-trans* interval. In fact, small differences observed from the models may be real differences corresponding to preferential ability to react with *cis* or *trans* peptide arrangements. Such possibilities will certainly have to be kept in mind for amino sugars and amino-containing antibiotics where N—O or N—N distances might be slightly larger than the corresponding —O—O— distances in structures containing only hydroxyl groups, and —N—H distances in amino-groups are also slightly longer than —O—H distances in alcohols (Dreiding models). In a similar way a selected axial hydroxyl (for example, in *myo*-inositol versus *scyllo*-inositol) may set the stage for intra-layer instead of inter-layer reactions with protein or peptide surfaces. Our expanded model of the water structure should enable us to observe such three-dimensional differences directly.

I thank Drs. R. B. Kelly and P. F. Wiley for several helpful discussions on carbohydrate conformations and stability factors. I also thank Dr. E. L. Schumann for his advice.

Note added in proof. The water structure illustrated in Fig. 2 is based on the tetrahedral arrangement existing in the so-called hexagonal phase of ice. An arrangement corresponding to the diamond cubic phase of ice would also be possible. These two ice arrangements are discussed by Dowell and Rinfret[11]. An additional distinguishing feature of the two phases lies in the placement of six 'near neighbour' oxygens. In the ice hexagonal phase, six 'near neighbour' oxygens form chairs in one direction (for example, the horizontal dimension in Fig. 2) but boats in the other direction (the vertical dimension in Fig. 2). In the diamond cubic phase of ice, six 'near neighbour' oxygens always are present in the chair form. Cortisone, for example, shows different methyl group interactions with water arranged in these two possible phases.

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POLAROGRAPHIC ACTIVITY OF PROTEINS AS A FUNCTION OF THE DILUTION OF AQUEOUS SOLUTIONS

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TROPP *et al.*[1] were the first to point out that the polarographic activity of proteins is considerably affected by the state of protein molecules in solution, namely, that increased dilution causes changes in the molecular structure of proteins, and the number of polarographic active groups. The possible reliable polarographic characterization of different proteins as resulting from this dependence was the subject of our investigations. In what follows I give the results of my efforts at polarographic characterization of α_2-globulins from guinea pig serum.

Investigations were made with guinea pig serum. Blood was drawn from the heart after fasting for 18 h, and centrifuged after standing at room temperature for 30 min. α_2-Globulins were separated by continuous electrophoresis (Shandon apparatus) in a borate buffer, *p*H 8·6, at 450 V. For testing the purity of the α_2-globulin fraction the eluates containing α_2-globulin and the corresponding sera were subjected to paper electrophoresis.

Eluates obtained by continuous electrophoresis were assayed for α_2-globulin by the micro-Kjeldahl method[2], and dilutions afterwards prepared containing 120, 200, 400, 600, 800 and 1,000 mg per cent.

Polarographic analyses were carried out using a radiometer Copenhagen *PO*3 apparatus. The basic electrolyte was aqueous solution containing equivalent ammonia, 0·1 equivalent ammonium chloride and 10^{-3} moles $[Co(NH_3)_6]Cl_3$. Into the polarographic vessel were placed 0·9 ml. basic electrolyte and 0·1 ml. α_2-globulin solution of known concentration. The potential applied between the electrodes was 1–2 V. The sensitivity of apparatus was 1/3,000, the dropping time of mercury 3·5 sec, at room temperature.

α_2-Globulins were eluted under the above conditions into two test-tubes (eluates I and II). The electrophoretic mobility of the respective bands of these eluates, namely, of the α_2-globulins from the corresponding sera, was identical. The polarographic waves of α_2-globulin, at concentrations studied, are given in Fig. 1. The average wave-heights are given in Table 1 and Fig. 2.

Polarographic analysis showed that the proteins in two eluates did not behave identically in some of considered concentrations. The average heights of polarographic waves of α_2-globulin in eluate I at concentrations 12, 20 and 40 mg per cent were higher than those of corresponding ones in eluate II (Table 1). The difference is statistically significant only at concentration of 20 mg per cent (P = 0·005).

The heights of the polarographic waves of α_2-globulin in both eluates increase with concentration rising to 20 mg per cent; after that they fall (Fig. 2). At concentration 40 mg per cent the wave height of the protein in eluate I is considerably lower than that at a concentration of 20 mg per cent ($P = 0·05$). This decrease becomes considerably slower as the concentration changes to 60, 80 and 100 mg per cent.

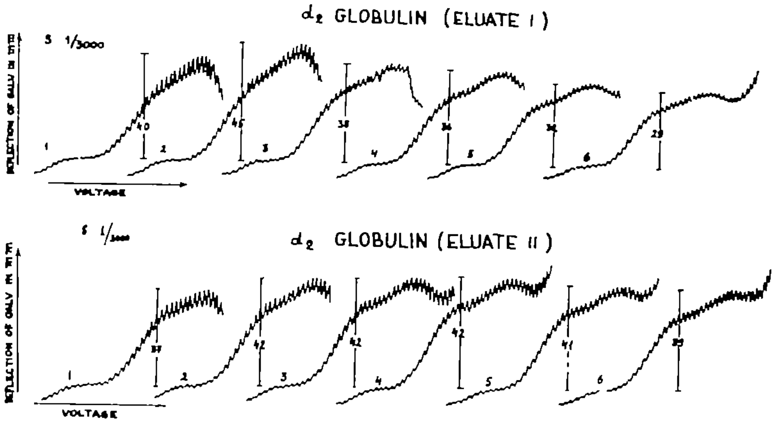

Fig. 1. Polarographic waves of α_2-globulins (eluates I and II) at concentrations. (1) 12; (2) 20; (3) 40; (4) 60; (5) 80; (6) 100 mg per cent. Initial voltage, −1 1 V

These results show that the polarographic activity of α_2-globulin falls as the concentration increases from 20 to 100 mg per cent, that is, in this region it is proportional to dilution. It follows that the number of groups capable of polarographic activity is higher in solutions of lower protein concentration. This phenomenon can only be explained by assuming that with the dilution the structure of the protein molecule changes, with the result that more hitherto inactive groups become free to react. Tropp *et al.*[1] also used

Table 1. AVERAGE HEIGHT (MM) OF THE POLAROGRAPHIC WAVES OF NATIVE α_2-GLOBULINS

Protein concentration (mg per cent)	12		20		40		60		80		100	
α_2 Globulins	M ± *S.D.*	*S.E.*	M ± *S.D.*	*S.E.*	M ± *S.D*	*S.E.*	M ± *S.D.*	*S.E.*	M ± *S.D.*	*S.E.*	M ± *S.D.*	*S.E.*
Eluate I	44·7±5·19	±1·84	49·0±3·65	±1·88	48·5±6·21	±2·35	39·5±3·31	±1·25	36·1±3·62	±1·37	34·1±3·76	±1·54
Eluate II	39·5±1·91	±0·72	43·4±2·76	±1·04	42·4±4·43	±1·37	40·0±5·72	±2·34	37·8±4·81	±1·97	33·0±5·56	±3·21

this hypothesis to explain the higher polarographic activity of certain dilute protein solutions. They concluded: "Welche Rolle das Wasser bei diesen Verdünnungserscheinungen spielt, wissen wir noch nicht sicher. Wir sehen jedoch, das jeder Verdünnungsgrad der untersuchten Eiweiß systeme polarographisch einen anderen Zustand des Eiweiß individuum aufzeichnet".

It is now known, however, that the stability of the secondary and tertiary structure of the protein molecule depends on its medium[3]. Media of high dielectric constant, for example, water, may cause a disruption of the hydrogen bridges of the protein molecule, and cause an unfolding of the molecule. The fact that this process implicates the liberation of the polarographically active groups may mean either that these groups were engaged in the formation of the secondary and tertiary structure or that they were unable to react owing to inaccessibility. The polarographic activity of α_2-globulin at high dilutions, namely, at concentrations of 12–20 mg per cent, was directly proportional to protein concentration (Fig. 2). This may mean that at such dilutions the protein molecule was unfolded, with all reactive groups free, so that the activity depended on concentration alone.

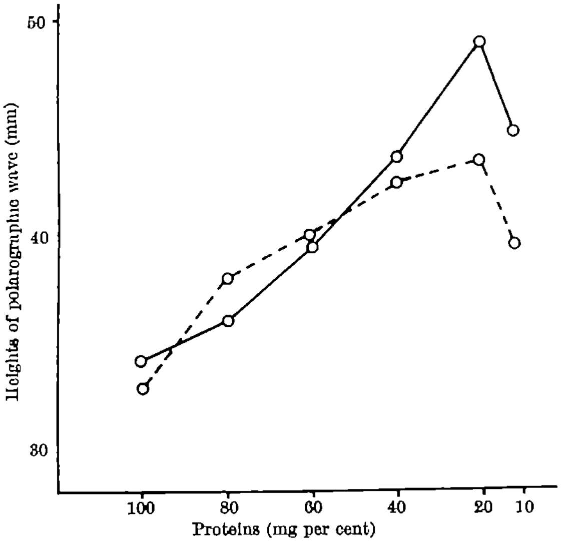

Fig. 2. Dependence of polarographic activity on the dilution of the α_2-globulin solution. —, eluate I; - - -, eluate II

It is now considered that the predominant force causing the folding of the protein molecule is the tendency of the non-polar amino-acid residue in the side-chains to assume the internal, hydrophobic part of the molecule, and the polar side-chains to enter the aqueous phase[4]. It might therefore be expected that at high dilutions the non-polar groups, including cysteine (considered mainly responsible for the polarographic activity of proteins), may appear at the surface of the molecule.

The two α_2-globulin eluates of identical electrophoretic mobility in a veronal buffer, *p*H 8·6, displayed different polarographic activity at certain concentrations. It is significant that this difference was most marked at higher dilutions (12–40 mg per cent). In view of the fact that these two proteins differ at lower concentrations in the number of groups available for polarographic activity, while at higher concentrations they appear to contain the same number of such groups, it follows that the number of structurally bound groups is higher in the latter case. This is only possible if different structures are involved.

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UNITAGE AND SUGGESTED DESIGNATION OF ANGIOTENSIN ACTIVITY

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BEFORE pure angiotensin[1] was available it was necessary to use arbitrary units of measurement to designate its biological activity. Several unitage systems were suggested[3,4,6,7,9,12,15–17], one no better than the other. They were based either on blood pressure increase in trained, unanæsthetized dogs or on comparison with the action of crude samples of peptide designated as 'standard' in anæsthetized animals or isolated muscle strips.

This multiplicity of units has led to confusion when attempting to compare data from one laboratory with those of another. The purpose of this article is to indicate, where possible, the relationships of different unitage systems to each other and to pure synthetic angiotensin. In the future, it is recommended that activity be designated in terms of weight of pure isoleucine[5]—or valine[5]—angiotensin II or the asparagine[1] derivatives.

Dog pressor assay[10], rat pressor assay[8,13], and isolated uterine and ileal myotropic assays[8] have been described. The preparation of the perfused rat hindquarters is a modification of a method by McQueen[11] and will be described in detail. Female rats (250 g) were killed either by a blow on the head or by ether. The abdominal aorta was quickly ligated just distal to the origin of the renal arteries. A nick was made distal to that. The aortic wall was elevated by a hook at the end of a 20-gauge needle, and the blood vessel cannulated almost to the bifurcation of the aorta. The inferior vena cava was then cut, and the hindquarters perfused with saline to flush out blood. This procedure took about 5 min. The rectum and uterus, after ligation, were removed, the rat transected at about the level of the third lumbar vertebra and immediately perfused at a constant rate by a Sigmamotor pump, with Krebs–Henseleit solution containing 10 per cent washed human red blood cells, and aerated with 95 per cent oxygen, 5 per cent carbon dioxide. Krebs–Henseleit solution alone led to rapid development of œdema, while the same solution containing dextran quickly abolished the vascular response. When 10 per cent red blood cell suspension was used, the preparation responded well for approximately 3 h, even though some œdema developed as shown by a doubling in weight. The rest of the procedure was identical with that use by McQueen[11].

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In a few experiments, hypertensive patients from the research ward of the Cleveland Clinic Hospital were used for assay. An 18-gauge needle was introduced into the brachial artery and connected to a Statham pressure transducer, model *P* 23 *D*. A slow infusion of 5 per cent dextrose in water was given intravenously through the contralateral cubital vein, and the angiotensin samples, diluted in 5 per cent dextrose in water, were rapidly injected via the infusion.

Three-point assays were conducted, as previously described for dogs[10], using a standard angiotensin preparation of Green and Bumpus[7] as a reference sample. This was then compared with different samples of synthetic isoleucine^{5}–angiotensin II synthesized in our laboratory[2], a synthetic sample of valine^{5}–angiotensin II, and four commercial preparations of asparagine^{1}–valine^{5}–angiotensin II ('Hypertensin', Ciba), lot numbers 32-654-1, *E* 6127 *B*, Prod. No. 8883.00 and 8595/8. The last four samples were kindly supplied by Dr. Schwyzer[17] of Ciba, Ltd., Basle, and were reported by him possibly to contain residual amounts of ammonium acetate. For this reason we have not expressed biological activities in terms of nitrogen content of the sample. The purity of the peptides was checked by paper electrophoresis. The strips were developed using 14·8-V/cm paper with a *p*H 2·1 formic acid–acetic acid buffer[19].

The pressor activities, expressed as Green and Bumpus units[7] per μg peptide, found for the different angiotensin preparations are presented in Table 1. Each figure in Table 1 is an average of 10–15 determinations with the standard deviation shown in parenthesis. Since the number of trials on human patients was limited (four experimental subjects), these results are presented as ratios to indicate only the relative activities of the different samples. As can be seen there is no difference between the valine^{5}– and isoleucine^{5}–angiotensin octapeptide, which agrees with the results reported by Schwyzer and Turrian[18]. The two peptides show no difference in the rat pressor assay, the dog pressor assay, the rat hindquarter assay and human assay. In contradistinction, some of the samples of asparagine^{1}–peptide show less activity than the aspartic^{1}–peptides. This difference may be due to differences in purity.

In an attempt to correlate the different unitage systems used, Dr. L. T. Skeggs kindly supplied a lyophilized sample of his standard, which he reported to contain 22 Goldblatt units[20]. Also, Dr. O. M. Helmer kindly supplied a solution which he reported to contain 10 Kuether and Haney units per ml.[8]. Other standard preparations used in earlier work[3,4,14,15] are no longer available.

Table 1

	Rat pressor*	Dog pressor*	Rat hindquarter pressor*	Ratio of human pressor†
Valine^{5}–angiotensin II	11·1 (±1·68)	9·5 (±1·36)	12·2 (±3·35)	1
Isoleucine^{5}–angiotensin II	11·4 (±2·02)	12·0 (±0·96)	12·0 (±2·12)	1
Ciba 32–654–1 asparagine^{1}–valine^{5}–angiotensin II	7·5 (±1·06)	7·2 (±1·92)	7·3 (±1·53)	0·83
Ciba *E*6127*B* asparagine^{1}–valine^{5}–angiotensin II	8·5 (±1·77)	8·8 (±0·26)	9·7 (±0·87)	0·87
Ciba 8883.00 asparagine^{1}–valine^{5}–angiotensin II	11·1 (±0·4)	10·1 (±0·3)	—	1
Ciba 8595/8 asparagine^{1}–valine^{5}–angiotensin II	8·5 (±1·22)	9·3 (±0·2)	—	0·87

* The activities are reported as Green and Bumpus units (ref. 7) per microgram peptide. The weight of the Ciba preparations was that reported on the ampule.

† These activities are reported as the ratio of each peptide to the activity of isoleucine^{5}–angiotensin II, which was set arbitrarily equal to 1.

From the average of four assays, one Green and Bumpus unit was found equivalent to 0·18 Goldblatt units. This is in agreement with data presented by Kuether and Haney[8], using a pithed rat preparation. From the average of seven assays, one Green and Bumpus unit is equivalent to one Kuether and Haney unit. These authors had reported that in the pithed rat preparation, one Green–Bumpus unit is equivalent to 1·16 Kuether–Haney unit. The ratio of the oxytocic unit of Schwarz, Masson and Page[16] to the Green and Bumpus unit was found to be 7·1 ± 0·2 in 20 comparative assays of isoleucine^{5}– and valine^{5}–angiotensin II. This ratio was found to be 7·7 ± 0·3 in 20 assays for asparagine^{1}–valine^{5}–angiotensin II. These ratios agree with the results previously reported[5]. Finally, the Paladini unit has been reported by him[12] to be equivalent to 0·0135 Goldblatt units.

Since synthetic angiotensin is now available, we recommend that investigators report the dosage of angiotensin in μg of synthetic valine^{5}– or isoleucine^{5}–angiotensin. 1 μg of pure angiotensin II is equivalent to about 12 Green and Bumpus units, 2·2 Goldblatt units, 12 Kuether–Haney units, and 163 Paladini units as average figures. Angiotensin is obtainable from Ciba under the trade name of 'Hypertensin. Ciba' which is asparagine^{1}–valine^{5}–angiotensin II, and contains an inert diluent. Of the four batches we have tested (Ciba Lot No. 32–654–1, No. *E*6127*B*, No. 8883.00, and No. 8595/8), three have shown about 75 per cent of the activity expected from the weight of peptide given on the vial. Electrophorograms of these peptides, shown in Fig. 1, indicate the presence of other peptides. One lot (Ciba 8883.00), containing mannitol as a diluent, showed biological activity similar to our purest synthetic angiotensin.

We suggest that since angiotensin amide is obtainable from Ciba this be used as a standard. We believe the homogeneity of such samples should be verified by paper electrophoresis as described here. We also recommend that all dosages be reported as μg of synthetic peptide, and the use of arbitrary units be discontinued.

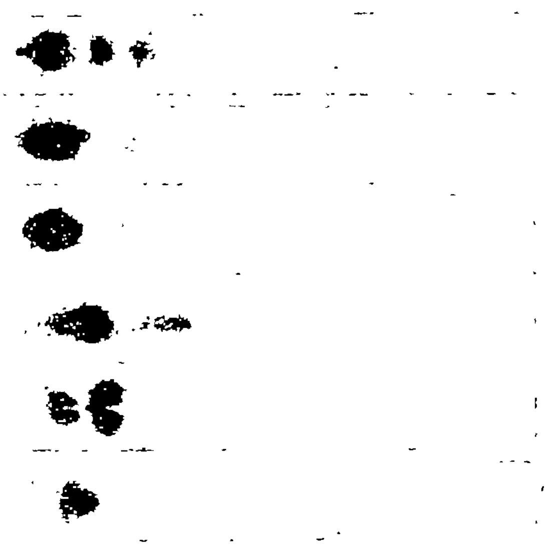

Fig. 1. Electrophoretic patterns of different angiotensin preparations

Top to bottom: (1) Val^{5}–angiotensin II amide (lot No. 32–654–1, Ciba); (2) Val^{5}–angiotensin II amide (lot No. 8888–00, Ciba); (3) Val^{5}–angiotensin II amide (lot No. 8595–8, Ciba); (4) Val^{5}–angiotensin II amide (lot *E*6127*B*, Ciba); (5) Val^{5}–angiotensin II (Ciba, reported by Dr. Schwyzer to contain a side-product, β-aspartyl–Arg–Val–Tyr–Val–Hist Pro–Phe–OH); (6) Ileu^{5}–angiotensin II (a preparation by Arakawa, ref. 2)

In a recent review, Schwyzer and Turrian[18] reported specific activities higher than we have been able to obtain. A synthetic sample of asparagine^{1}–valine^{5}–angiotensin II, assayed by Dr. Deodhar, Mount Sinai Hospital, Cleveland, Ohio, gave a figure of 3·3–3·5 Goldblatt units or 17·8–19·4 Green and Bumpus units/μg peptide. Another sample of the same peptide was reported by Schwyzer *et al.*[17] to have an activity of 4·8 Goldblatt units or 27·1 Green and Bumpus units. At present we have no explanation for these high and widely scattered figures, especially as we have repeated the assays in our systems, using the same peptide samples, and our results all fall within the range of biological variation. Skeggs[21] reported that angiotensin II prepared from horse blood, and purified by counter-current distribution to satisfy the criteria for a single substance, showed a biological activity of 2·4 G.U./μg. Schwyzer and Turrian[18] reported a value of 2·4 Goldblatt units or 13·3 Green and Bumpus units/μg in a sample of isoleucine^{5}–angiotensin II prepared according to Skeggs from horse plasma[20]. This seems to agree closely with the pressor activities we have reported.

This work was supported by the National Institutes of Health, grant No. *H*–96, and by a grant from the American Heart Association.

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IN VITRO INVESTIGATIONS OF THE HUMAN CHORION AS A MEMBRANE SYSTEM

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IN the course of investigations of the exchange of materials across the placenta we have been impressed by the need for a method by which we could quantitate *in vitro* the transfer and utilization of solutes by the specific placental membranes. Similar *in vitro* studies have been carried out by Ussing on frog's skin[1], by Garby[2] on human amnion, and by Crawford and McCance[3] on pig chorio-allantois.

The apparatus which we have found suitable for studies on the human chorion laeve and the inter-cotyledonary chorion of the sheep and goat is presented here.

As illustrated in Fig. 1, the apparatus consists of two cylindrical leucite blocks 75 mm in diameter and 25 mm thick. Into the opposing surfaces of each block a well has been cut, 32 mm in diameter and 5 mm deep, the membrane forming the common wall between these two wells or chambers. Each chamber communicates with a chimney (internal diameter

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* Senior research scholar, Joseph P. Kennedy, Jun., Memorial Foundation.

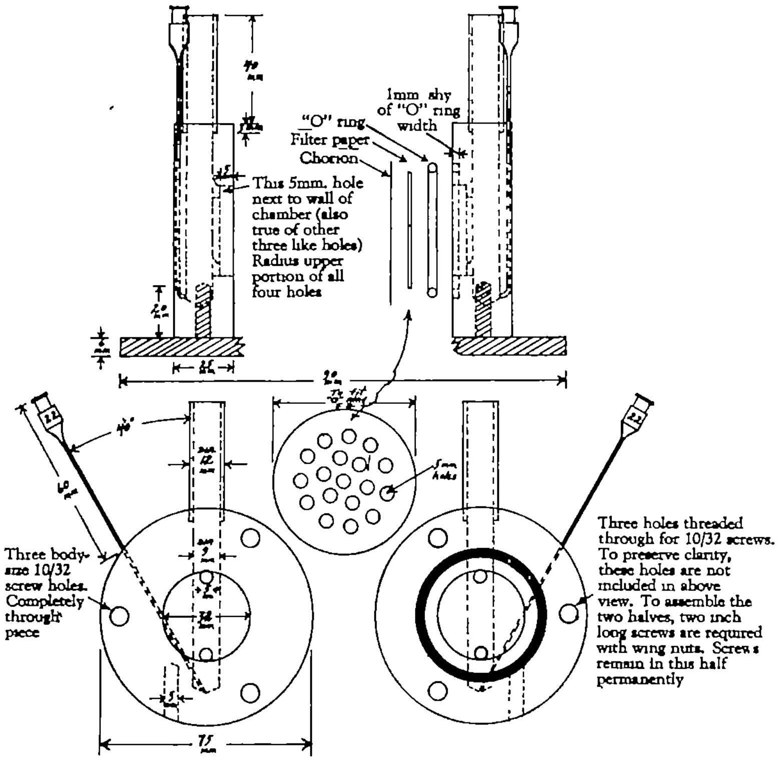
1mm shy of "O" ring width
"O" ring
Filter paper
Chorion
This 5mm. hole next to wall of chamber (also true of other three like holes) Radius upper portion of all four holes
5mm holes
Three body-size 10/32 screw holes. Completely through piece
Three holes threaded through for 10/32 screws. To preserve clarity, these holes are not included in above view. To assemble the two halves, two inch long screws are required with wing nuts. Screws remain in this half permanently

Fig. 1. Line drawings indicate the form and dimensions of the two components that support the membrane for *in vitro* study

9 mm; overall height, 105 mm), by two holes each 5 mm in diameter, in the upper and lowermost part of their common wall respectively. The chimneys provide access to the fluid in each chamber for serial sampling and a means for equilibrating the media with appropriate gas mixtures. The gases reach the base of the chimney via a spinal needle (No. 22—4·5 in. long) sunk into the leucite block for convenience during sampling at an angle of approximately 30° to the chimney. Near its tip the needle is bent at a right angle to enter the chimney. As the gas bubbles escape from the tip they rise in the chimney and agitate the medium.

Because of the fragile character of the membranes, we have found it convenient to support them on a piece of fenestrated fibre glass filter paper. By tightening the three retaining screws (2 in. long with wing nuts), which bring the two leucite blocks together, the outer margins of the filter paper and the membrane are compressed and the rubber ring gasket seals the chambers from the outside. Within the bath the apparatus is supported on a metal rack by two prongs, 5 mm in diameter, which fit snugly into two sockets, one on the base of each block.

As the first step in mounting the membrane, 10–16 holes, each about 6 mm in diameter, are punched in a fibre glass filter disk (425 mm in diameter) of Whatman *GF/B*. The disk is then soaked in Gey's medium[4] and applied to the membrane, which is trimmed to fit it. After the membrane and fibre glass disk are in place within the rubber gasket, the two chambers are clamped and filled with appropriate solutions, care being taken to fill first that chamber to which the membrane is wholly exposed. This manœuvre forces the membrane against the supporting disk and prevents its rupture by hydrostatic pressure when the opposite chamber is filled. After measured volumes of fluid have been added to the chambers, usually 9 c.c. on each side, the apparatus is submerged to the level of the chambers' upper surface in a constant temperature bath. The spinal needles are then attached to plastic adaptors for the delivery of the gases for equilibration and mixing.

The following investigations of the *in vitro* properties of the human chorion laeve illustrate the usefulness of the apparatus and the appropriateness of the dimensions of the fluid-filled chambers for this type of tissue. The medium was always the bicarbonate buffered solution of Gey and Gey[4] equilibrated with 95 per cent oxygen and 5 per cent carbon dioxide at 37° C. The carbohydrates used were obtained from commercial sources and used without further purification. The methods described by Roe[5–7] were used for the determinations of *d*-xylose, *d*-arabinose, inulin and dextran. Glucose was determined by the method of Huggett and Nixon[8]. The placental tissue was obtained within 5 min of the delivery of the infant from uncomplicated normal pregnancies. The chorion laeve was stripped manually over a wide area from the underlying amnion. The chorion, thus free of both endometrial and amniotic components, was placed on the fibre glass filter paper.

(*A*) *Permeability of the chorion laeve to polysaccharides.* Three polymers were tested, inulin, dextran fraction '10' and dextran fraction '500'. In each case the solute was added to the 'maternal' side of the chorion. The concentration of the test substance in both chambers was determined at zero time and again at the end of a timed exposure period. The chorion laeve so tested is impermeable to each of the polysaccharides.

(*B*) *Uptake of d-glucose by chorion laeve.* *d*-Glucose was added to the medium to a concentration of 5·5 µM/ml. and the concentration in each chamber determined at half-hourly intervals after time zero. As the volume of fluid in each chamber at the beginning of the experiment was known and the volume of the serial samples was fixed, the utilization of *d*-glucose from each chamber could be calculated for each interval. The results in Table 1 demonstrate that the rate of glucose utilization by the membrane was the same on the fœtal and the maternal sides; there was no apparent polarity of the membrane with respect to this function. In addition, the rate of

Table 1. GLUCOSE UPTAKE OF HUMAN CHORION
(mg/g wet weight of tissue/h)

Half-h interval	By maternal surface	By fœtal surface
1	0·86	2·47
2	2·09	1·88
3	1·71	1·86
4	1·28	1·95
5	1·56	1·89
6	1·28	1·66
7	1·36	1·57
8	1·76	1·64
9	1·67	1·65
Average	1·51	1·56

glucose uptake remained relatively constant over the 4-h test period suggesting there was no decline in the activity of the membrane during incubation.

(*C*) *The movement of d-arabinose across the chorion laeve.* The usefulness of this technique for following the transfer of a non-utilizable sugar is illustrated by the results in Table 2. In this experiment the two chambers were filled with medium containing *d*-glucose at a concentration of 2·8 μM/ml. *d*-Arabinose was present in the chamber on the fœtal side at a concentration of 40 μM/ml. As the results in Table 2 demonstrate, there is a close correlation between the amount of *d*-arabinose lost in each ½ h of the 3-h incubation period from the chamber on the fœtal side and that gained in the one on the maternal; and as the final totals indicate the quantities lost and gained are virtually equal, the difference between the two is but 0·5 μM and within the error of the method of analysis.

(*D*) *The movement of d-glucose across the chorion laeve.* When a utilizable sugar is introduced into one of the chambers, its disappearance from that chamber can be compared with its appearance in the other, and the rate of its utilization by the membrane inferred from the difference. The results of two experiments for this purpose are illustrated in Table 3. In each case the *d*-glucose concentration on the maternal side was 40 μM/ml. In contrast with *d*-arabinose, there is a marked difference between the amount lost from the chamber on the maternal side of the chorion and the amount gained on the fœtal. In the two experiments reported here the amount of glucose utilized in the 2-h incubation period is quite similar, that is, 48·5 μM in the first and 45·5 in the second.

Table 2. Movement of *d*-Arabinose across the Chorion

Half-h interval	μM lost fœtal side	μM gained maternal side	Difference
1	18·0	18·3	0·3
2	34·3	28·6	−5·7
3	44·8	45·7	0·9
4		52·8	
5	65·1	70·8	5.7
6	73·7	74·2	0·5

Table 3. Movement of *d*-Glucose Across the Chorion Laeve

Time (min)	μM *d*-glucose lost maternal side	μM *d*-glucose gained fœtal side
	Exp. 1	
0–20	36·0	9·5
20–40	52·8	20·0
40–60	54·1	21·5
60–80	76·3	30·1
80–100	97·0	40 7
100–120	99·8	51·3
	Exp. 2	
0–20	44·6	3·1
20–40	43·4	10·7
40–60	56·6	12·8
60–80	56 6	15·1
80–100		20·7
100–120	66·2	20·7

The results presented here demonstrate that when isolated in layers of cells the placental tissues can be studied *in vitro* by means of the technique described and that quantitative data may be obtained. The data indicate that over the course of a 5-h period consistent results can be obtained and satisfactory balance studies for a given solute carried out without the problems of improper mixing and non-representative aliquots or communications between the chambers and the external water bath.

The technique described appears to have a number of advantages that recommend its use for *in vitro* studies on fœtal and placental membranes, notably the absence of any unbalanced hydrostatic force on the membrane as it is supported. This feature is of particular importance for the membranes are quite fragile and easily torn, though they are readily available as tissue sheets.

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EFFECTS OF INTRAVENOUS PHOSPHOLIPID CONTAINING PHOSPHATIDYL SERINE ON BLOOD CLOTTING WITH PARTICULAR REFERENCE TO THE RUSSELL'S VIPER VENOM TIME

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INVESTIGATORS have arrived at different conclusions concerning the effect of phosphatide fractions such as phosphatidyl serine on coagulation of blood[1–4]. The clotting time of plasma mixed with Russell's viper venom has been one of the tests frequently used for evaluating the effect of phospholipids on clotting of blood. In their original work, Barkhan, Newlands and Wild[1] found that concentrations of phosphatidyl serine, which accelerated the Russell's viper venom time, inhibited thromboplastin formation in the thromboplastin generation test. In this investigation, however, increased concentrations of phosphatidyl serine were found to inhibit the Russell's viper venom time. This evidence indicated that evaluation of the effects of phospholipids on blood clotting depends to some extent on the type of test used. Despite these findings, the Russell's viper venom time has frequently been used as a guide to whether a phospholipid promotes or inhibits clotting[4]. We have found in a study of the effect of intravenous phospholipid rich in phosphatidyl serine on clotting

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Table 1. EFFECT OF INTRAVENOUS PHOSPHOLIPID ON CLOTTING TESTS

		Clotting test: mean values for 15 experiments					
		Clotting time (sec)		Prothrombin time (sec)	Prothrombin consumption (sec)	Russell's viper venom time (sec)	Platelet count No./mm.³
		Glass	Silicone				
Pre-injection		294	689	10·0	39·4	10·5	350,000
10 min after injection of phospholipid		386	3,451	17·4	21·1	6·0	250,000
Significance* of differences between means	t	3·555	8·500	12·800	4·400	10·500	1·345
	P	< 0·01	< 0·001	< 0·001	< 0·001	< 0·001	< 0·2

* Based on analysis by paired differences.

of blood that when the majority of clotting tests are inhibited, the Russell's viper venom time is accelerated.

The phospholipid used in these experiments was prepared from beef brain essentially by the method of Folch[5]. Folch fraction III was then redissolved in anhydrous ether and reprecipitated with absolute methanol. This step was repeated 2–5 times. The re-extracted phospholipid fraction was then chromatographed and identified according to the method of Marinetti and Stotz[6]. Only one major spot was detected on the chromatogram when stained with rhodamine 6*G* or ninhydrin. When stained with rhodamine 6*G* and examined under an ultra-violet light, the spot gave a bluish purple fluorescence. This spot had the same R_F value as a standard sample of phosphatidyl serine. The phospholipid content based on phosphorus analysis by the method of Zilversmit and Davis[7] was 96 per cent. Although not conclusively shown, this phospholipid fraction is considered to be rich in phosphatidyl serine[8]. Analysis in a gas chromatograph showed the fatty acid composition of the phospholipid fraction was essentially as follows: stearic acid 54·8 per cent, oleic acid 37·2 per cent, eicosenoic acid 4·3 per cent, eicosodienoic acid 1·2 per cent[8]. This phospholipid fraction was found to inhibit the activity of clot promoting phospholipid in the thromboplastin generation test[8]. The clot promoting phospholipid used in the test system was prepared from beef brain cephalin and consisted of Folch fractions IV and V combined. This phospholipid, when used at a concentration of 250 μg in place of platelets in a normal thromboplastin generation test, gave a normal curve. The addition of 5 μg of the phosphatidyl serine-rich fraction to the clot promoting phospholipid in the thromboplastin generation test inhibited thromboplastin formation.

The changes in clotting were followed in anæsthetized beagle dogs (intravenous sodium pentobarbital) after intravenous injections of the phosphatidyl serine-rich phospholipid. The phospholipid used in the intravenous investigations was dispersed in imidazole buffer (pH 7·4) containing 2 mg/ml. sodium desoxycholate[9]. The dose given to each animal was between 30 and 50 mg per kg body-weight. The following clotting tests were performed using techniques that have been described[10]. The whole blood clotting time in glass and silicone-coated tubes, the prothrombin time, the prothrombin consumption time and the thromboplastin generation test. The Russell's viper venom time was done as described elsewhere[11].

Table 2. EFFECT OF INTRAVENOUS PHOSPHOLIPID ON THROMBOPLASTIN GENERATION

Thromboplastin generation mixture*	Incubation time (min) 1	2	3	4	5	6
	Substrate clotting time (sec)					
1 part normal Al(OH)$_3$-plasma diluted 1 : 5; 1 part test Al(OH)$_3$-plasma diluted as follow :						
Pre-injection undiluted	60·0	14·0	13·0	11·8	11·5	11·5
10 min post-injection undiluted	120·0	115·0	90·0	75·0	41·0	25·0
Pre-injection 1 : 5	45·0	13·8	12·0	11·8	11·0	11·0
10 min post-injection 1:5	120·0	59·0	24·0	15·0	18·0	16·0
Pre-injection 1 : 20	34·0	14·8	11·8	11·8	12·0	11·5
10 min post-injection 1:20	80·0	49·0	13·5	12·8	12·0	12·0
1 part hæmophilia *A* Al(OH)$_3$-plasma diluted 1 : 5; 1 part test Al(OH)$_3$-plasma as above:						
Pre-injection undiluted	60·0	23·0	11·5	12·2	12·0	10·0
10 min post-injection undiluted	120·0	80·0	32·0	50·0	20·0	20·0
Pre-injection 1 : 10	65·0	20·0	16·0	11·5	10·5	10·0
10 min post-injection 1 : 10	80·0	40·0	14·0	12·5	11·5	11·0

* The mixture was as follows: brain extract 0·3 ml.; normal dog serum diluted 1 : 10 0·3 ml.; 0·15 ml. of either normal or hæmophilia *A* Al(OH)$_3$-treated plasma; 0·15 ml. of test Al(OH)$_3$-treated plasma; 0·025 M $CaCl_2$ 0·3 ml.

In each of 15 experiments, the whole blood clotting time in glass and silicone-coated glass tubes was prolonged after infusion of the material (Table 1). In every case the prothrombin time was prolonged and the prothrombin consumption time shortened. In contrast, the Russell's viper venom time was accelerated on each occasion. There was a fall of about 30 per cent in the circulating platelet count. Studies using the thromboplastin generation test showed that the samples taken 10 and 20 min after the injection contained a factor present in the Al(OH)$_3$-treated plasma or non-absorbed plasma fraction which inhibited thromboplastin generation (Table 2). The inhibiting effect disappeared when the test plasma was diluted. The undiluted 10-min post injection plasma would not correct the defect in canine hæmophilia *A* Al(OH)$_3$-treated plasma when used in the thromboplastin generation test but the diluted test plasma would (Table 2). (The canine hæmophilia *A* plasma was supplied through the kindness of Dr. K. M. Brinkhous, University of North Carolina, Medical School, Chapel Hill.)

Two possible explanations for the discrepancy between the results for the Russell's viper venom time and the other clotting tests are: first, that the intravenous phospholipid infusion accelerated clotting producing a fall in platelets, factor V and factor VIII activity and other clotting factors consumed during coagulation; secondly, that while the intravenous phospholipid inhibited clotting in terms of the majority of the tests, its effect on the Russell's viper venom time in the dosage used was one of acceleration. The results from the present investigation do not give any support to the first explanation. There was no evidence of a reduction in factor VIII activity, and it seems unlikely that there was any loss in factor V activity since there was no prolongation of the Russell's viper venom time. Furthermore, the

Table 3. CALCIUM CLOTTING TIME*, SILICONED TUBES

	Proportions of mixtures					
Normal plasma	10	8	6	4	2	0
Test plasma	0	2	4	6	8	10
	Clotting time (sec.)					
Mixture of plasma tested:						
Normal and pre-injection	180	160	130	140	150	180
Normal and 10-min post-injection	180	210	320	320	540	580
Normal and hæmophilia *B*	160	140	150	160	170	708

* Test carried out as described by Biggs and Macfarlane (ref. 12).

prolonged prothrombin time was not corrected by the addition of 10 per cent of normal $Al(OH)_3$-treated plasma[8]. There was a marked diminution in prothrombin consumption without evidence of a marked fall in platelets or factor V and factor VIII activity. Addition of the 10-min post-injection plasma sample to normal plasma in various proportions produced inhibition of clotting in contrast to the effect of adding canine hæmophilia *B* plasma to normal plasma (Table 3).

It seems reasonable to conclude that, although the quantity of phospholipid given in these studies accelerated the Russell's viper venom time, blood clotting was inhibited as judged by a variety of other tests. This evidence from *in vivo* studies suggests that the Russell's viper venom time is of limited value in trying to assess whether a particular phospholipid fraction accelerates or inhibits blood coagulation. This conclusion would seem to be in agreement with the original findings from the *in vitro* studies of Barkhan, Newlands and Wild[1]. These results are also in agreement with the observations of Silver, Turner and Tocantins[9] that the intravenous administration of phospholipid rich in phosphatidyl serine has an anticoagulant effect.

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ADVANTAGES OF IODINE-125 FOR STUDYING THYROID FUNCTION IN EXPERIMENTAL ANIMALS

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WE have found[1] that organic iodine-131, in the form of iodoprotein[2], is carried away from the thyroid gland in the lymphatics draining the gland. However, we felt that it was possible that the iodoprotein was leaking out of the thyroid follicles as a result of radiation damage to the follicles, in spite of the fact that the dose received by the thyroid (50–800 rads) was less than that which is generally believed to damage the gland[3]. We have therefore been using iodine-125 (which has recently become available from the Radiochemical Centre, Amersham, Bucks) instead of iodine-131 to label the iodine and it was thought that a comparison of the radiation doses given to the thyroid by these two isotopes would be of value. The radiation doses are determined to a certain extent by factors related to the size and to the irregular shape of the gland so that calculations have to be approximate. For the present calculation the thyroid gland of the cat has been taken as an example. Each lobe is approximately 2 × 0·6 × 0·3 cm and weighs 0·2–0·5 g.

The radiation doses given by the two isotopes (using the data[4] for emission energies, provided by the suppliers) can be compared as follows: for iodine-131 the mean β-emission energy absorbed by the tissue of the thyroid gland per decomposition (assuming that the average energy is 0·33 of the maximum energy) can be calculated to be 0·192 MeV. However, a proportion of this β-radiation escapes from the surface of the gland without being fully absorbed. A variable correction depending on the size of the tissue has to be subtracted from this figure. We estimate this loss to be of the order of 10 per cent of the β-emission energy for the thyroid gland of the cat, so that 0·173 MeV must be absorbed by the gland itself.

The internal conversion of some γ-energy to β-particles has to be taken into account. This yields a further 0·009 MeV, so that the total β-energy given to the tissue is 0·182 MeV. If the tissue contains 1 μc./g of a uniformly distributed β-emitter of long half-life, the dose-rate of ionizing radiation is 2·13 times the average energy (in MeV) absorbed per decomposition[5]. Therefore, the radiation energy delivered to the thyroid tissue by the β-emission of 1 μc. iodine-131/g is 0·388 rad/h.

The γ-energy delivered to the tissue is comparatively unimportant because the proportion absorbed is low, although this proportion is very much dependent on the geometry of the gland. The average γ-energy emitted per decomposition can be calculated from the data provided[4] to be 0·383 MeV. Using the figures which have been given[6] for γ-energy absorption in water and assuming that, for the cat, the average path of a γ-ray through the thyroid tissue is 0·3 cm, only about 0·75 per cent of the γ-energy is delivered to the tissue. Therefore, the total γ-energy emitted (0·383 MeV per decomposition) results in the delivery of ionization energy to the tissue at a rate of only about 0·006 rad/h for 1 μc. iodine-131/g.

The energy emission from iodine-125 is somewhat different. The β-emission is entirely due to a 93 per cent internal conversion of γ-energy and the energy of this β-emission is small, 0·008 MeV or less. The absorption of such low-energy β-radiation will be almost complete, even in a small gland. The effects of iodine-125 radiation on tissue have been discussed by Harper *et al.*[7]. In addition to soft β-radiation, a considerable part of the emission appears as very low energy *L*, *M* and *N* photons which are absorbed in the tissue very close to their origin and can be treated as β-like radiation. According to Harper *et al.*[7] the total energy of this β-like radiation is 0·021 MeV per decomposition. Therefore, 1 μc./g iodine-125 will deliver to the tissue a dose-rate of 0·0447 rad/h as β-like radiation. The rest of the radiation emitted consists of *K* photons and transition γ-radiation and the proportion which will be absorbed in the gland will be such that its contribution to the total dose cannot be neglected; but a considerable uncertainty is introduced here owing to the geometry of the gland. Full absorption of this γ-radiation and *K* photon X-radiation (taking its total energy per decomposition as 0·0413 MeV (ref. 7)) would mean that 1 μc. iodine-125/g of tissue would deliver energy at a rate of 0·088 rad/h. However, using the figures for γ-radiation absorption in water[4], and assuming that, for the cat, the average path of a ray through the thyroid tissue is about 0·3 cm, the proportion of the energy absorbed would be about 4·2 per cent and the energy actually delivered to the tissue would be only about 0·0037 rad/h. Thus, for a cat or similar animal, the total dose-rate to a thyroid gland which contains 1 μc./g would initially be about 0·394 rad/h for iodine-131 and about 0·048 rad/h for iodine-125. Therefore, if the same quantity of the two isotopes is injected into each of two animals, we estimate that the radiation dose given by iodine-125 over a given short period will be about 12·3 per cent of that given by iodine-131.

However, there are two other factors which must be taken into account in using iodine-125 and iodine-131. The first is that the effective biological half-life of iodine-125 is much longer than that of iodine-131 (about 27 days compared with 7 days, assuming a biological half-life for iodine in the thyroid gland of 50 days), though even for indefinite survival, an injection of iodine-125 will give to the thyroid a radiation dose of only about a half that of a similar quantity of iodine-131. The second factor is that iodine-125 is less convenient than iodine-131 for external *in vivo* counting. For *in vitro* counting, on the other hand, we have found that iodine-125 cannot be counted very efficiently with Geiger counters but by using an annular well-type NaI scintillation crystal counter (Ekco *N*618–651) we have obtained counting efficiencies for iodine-125 which are about three times greater than those for iodine-131. In addition, the slower physical decay of iodine-125 is often an advantage.

Table 1

For thyroid gland of cat	Approximate ratio $^{125}I/^{131}I$ For equal concentrations of radioactivity in the tissue	For equal counting-rates in the thyroid secretions
Initial radiation dose-rate	0·123	0·041
Total radiation dose	0·47	0·16

Lack of sensitivity of the radioactive assay procedure is often a limiting factor in biological work. Provided that a suitable scintillation counter is available, iodine-125 can be used, injecting less than one-third of the iodine-131 dose but still obtaining a comparable counting-rate, for example, in the thyroid lymph[2]. Such an injection will give, over a short initial period, only about 4 per cent of the iodine-131 radiation dose to the thyroid gland of a cat or other small animal and, even for indefinite survival, less than 20 per cent of the iodine-131 radiation dose to the gland. A comparison between the radiation effects of iodine-125 and iodine-131 on the thyroid gland of the cat is given in Table 1.

Therefore, in summary, we suggest that iodine-125 is likely to prove a valuable agent, particularly for non-survival experimental work in animals, since its unusually soft radiations make possible a high sensitivity of radioactive assay at a relatively low radiation dose to the thyroid gland. We have indeed found that iodine-125 is of much value in such work[2].

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INFLUENCE OF RABBIT POLYMORPHONUCLEAR LEUCOCYTES AND MACROPHAGES ON THE IMMUNOGENICITY OF *ESCHERICHIA COLI*

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PREVIOUS studies from this laboratory have described *in vitro* systems in which the fate of micro-organisms can be determined within phagocytic cells[1]. These experiments as well as more recent ones on the degradation of isotopically labelled bacteria within both polymorphonuclear leucocytes and macrophages indicated that within 2–3 h of intracellular residence more than 50 per cent of the trichloroacetic acid-insoluble phosphorus-32 or carbon-14 of *E. coli K*-12 was broken down into acid-soluble fragments[2]. A comparison between the two types of phagocytes revealed that the macrophage brought about a more rapid and extensive degradation of *E. coli* macromolecules. The investigations reported here were conducted in an effort to determine the influence of intra-phagocytic residence on the immunogenicity of the same organism.

Rabbit peritoneal exudate polymorphonuclear leucocytes and macrophages were collected following the injection of 0·1 per cent glycogen-saline or mineral oil respectively. The cell suspensions contained either 99 per cent granulocytes or greater than 90 per cent macrophages. Reaction mixtures were prepared containing 36–40 × 10^6 leucocytes/ml., 10 per cent fresh rabbit serum and a multiplicity of 1–3 *E. coli*/phagocyte. The tubes were incubated at 38° C on an end-over-end rotator and aliquots of the suspension removed at time-intervals of up to 120 min. 1-ml. aliquots of the complete suspension were then injected intraperitoneally into 25 g *NCS* Swiss mice. Disruption of the leucocytes prior to injection did not influence subsequent agglutinin titres. Ten days after inoculation the mice were killed and individual serum samples obtained. The sera were then assayed

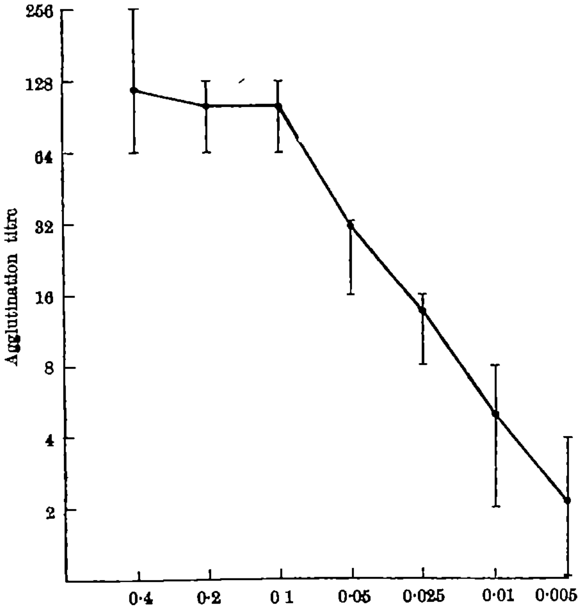

Fig. 1. Dose-response titration of *E. coli* injected intraperitoneally into *NCS* Swiss mice

for agglutinating antibody against a heat-killed (85° C/40 min) *E. coli* antigen. The rates of phagocytosis and intracellular killing were assayed on each of the incubation mixtures[1]. For each of the experiments described, more than 99 per cent of the organisms had been ingested and killed intracellularly after 20 min of incubation. This was the case with suspensions of either rabbit macrophages or polymorphonuclear leucocytes.

Initial experiments were performed on the immunogenicity of this strain of *E. coli*. Two-fold dilutions of a stock suspension of bacteria were prepared in pyrogen-free saline and injected intraperitoneally into mice. The animals were bled at 10 days and the results of the agglutination tests are shown in Fig. 1. Each point represents the mean titre of 15 animals. A relatively linear dose-response relationship existed between 0·1 and 0·005 ml. of antigen. Above this value the response became asymptotic with little increase in agglutinin titre. In all subsequent experiments 1·0 ml. of the phagocyte-*coli* suspension contained 0·1 ml. of antigen as defined in the titration experiment. This corresponded to a bacterial suspension having an optical density of 0·027 at 450 mμ with a 1·0-cm light path. Attempts at utilizing Gram-positive organisms, that is, *Staphylococcus albus* and *aureus*, *Bacillus subtilis*, for similar work proved unsuccessful since 10–100 times more bacteria were required to give a detectable agglutinin response. This number was too large to use in the *in vitro* phagocytic system and still get efficient ingestion.

Table 1. FACTORS INFLUENCING THE IMMUNOGENICITY OF *E. coli*

Pre-treatment prior to intraperitoneal mouse injection	Incubation time *in vitro* (min)	No. animals	Agglutinin titre (mean)
(*A*) *E. coli* + 10% NRS	120	12	115
(*B*) *E. coli* + 10% IRS	120	8	92
(*C*) *E. coli* + 10% IRS + PMN	120	15	3·8
(*D*) *E. coli* injected at 0 min, NRS + PMN injected 30 or 60 min later	—	10	112
(*E*) Heat-killed *E. coli* 80° C/30 min	—	18	118
(*F*) *E. coli* + 10% NRS + PMN, separated into:	60′		
1. Medium		6	< 2
2. 8,000*g* supernate		7	< 2
3. 8,000*g* pellet		7	32
(*G*) ΔK–*E. coli* + macrophages + 10% NRS	120′	12	110
(*H*) *E. coli* + macrophage + 10% IRS	120′	10	96

The influence of both the polymorphonuclear leucocyte and macrophage on the immunogenicity of *E. coli* is presented in Fig. 2. Each point represents the mean titre of 15 animals. At zero time, prior to the phagocytosis of the organisms, the titre of antibody was the same as that achieved with the administration of the bacteria alone. Thereafter, there was a progressive loss of immunogenicity of those organisms within polymorphonuclear leucocytes. After 120 min within the granulocyte the agglutinin titre of injected mice had been reduced to less than 1 : 4. In reference to the dose-response curve of Fig. 1 this corresponded to a 95 per cent decrease in the immunogenic mass of the bacteria.

The results obtained with the macrophage-*coli* mixtures were markedly different. In these circumstances there was no significant reduction in agglutinin response even after 2 h within the macrophage. Investigations have not been conducted at longer time-intervals. It should be stressed that the rate of ingestion, killing and degradation of isotopically labelled bacteria were the same in macrophages and polymorphonuclear leucocytes.

Table 1 illustrates additional studies with the same system. Groups *A* and *B* were controls in which normal and specific immune rabbit serum

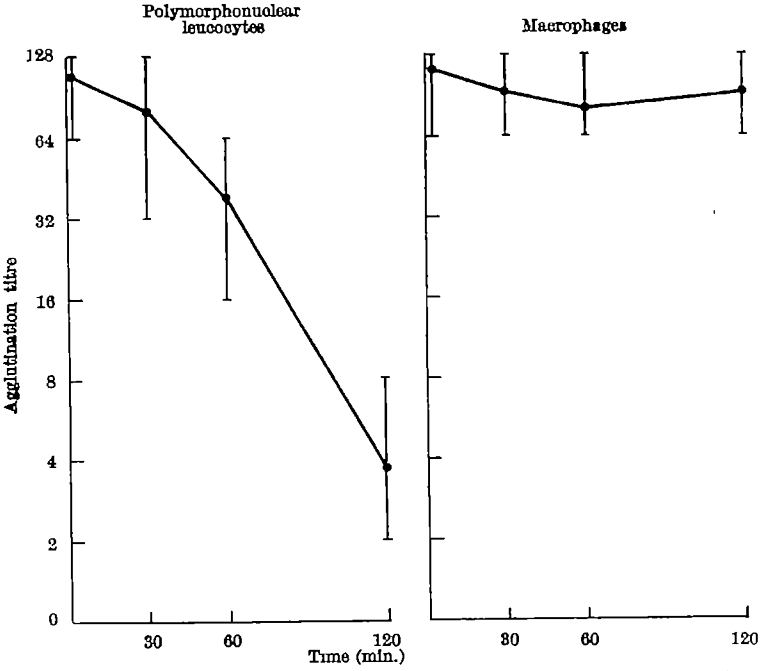

Fig. 2. Influence of intracellular residence within rabbit polymorphonuclear leucocytes and macrophages on the immunogenicity of *E. coli*

(NRS and IRS) did not alter the immunogenicity of the bacteria. The presence of immune serum in *E. coli*-leucocyte (PMN) suspensions (*C*) gave results similar to those obtained with normal serum. Experiments in which the equivalent number of leucocytes were injected intraperitoneally 30–60 min after the *E. coli* inoculum are presented in *D*. The results indicate that the rabbit cells have no influence on the antibody response of mice to *E. coli*. Group *E* shows that the use of heat-killed *E. coli* gave results similar to the viable organisms. Attempts to demonstrate the solubilization of the *E. coli* immunogen are shown in *F*. After 60 min incubation the leucocytes were centrifuged at low speed and the extracellular medium removed. The cells were then re-suspended in isotonic sucrose, homogenized and centrifuged at 8,000*g*. The residual immunogenicity was found in the leucocyte pellet, and no detectable activity was found in the soluble fraction or in the extra-cellular medium.

In view of the inability of macrophages to alter the immunogenicity of viable bacteria, additional investigations were carried out with heat-killed organisms and with viable bacteria in the presence of immune serum. Groups *G* and *H* reveal that under these conditions the macrophage was still not able to alter the agglutinogen.

The exact chemical nature of the agglutinogen of this strain of *E. coli* is not known. Preliminary observations on phenol–water extracts of the organism[3] showed that they were capable of interacting with immune mouse serum and removing agglutinating antibody. In this regard, it is likely that the somatic '0' antigens of Gram-negative bacteria are responsible for the evocation of agglutinins. Attempts at quantitating the amount of *E. coli* 'antigen' within leucocytes, by hæmagglutination-inhibition tests[4] with phenol–water extracts, have not as yet been successful.

It seems clear that a marked difference exists in the immunogenicity of *E. coli* after residence within rabbit granulocytes and macrophages. Whether this indicates a difference in the rate and extent of degradation of the responsible antigen within the two cell types is not clear. In view of the postulated role of the macrophage in preparing antigens for the stimulation of antibody synthesis[5], the formation of an immunogenic intermediate within these cells offers a second possibility. Irrespective of mechanism the results suggest that the antibody response of animals injected with certain micro-organisms may in part be related to the type of phagocyte population encountered *in vivo*. These results are in keeping with the previous data of Walsh and Smith[6] in their studies with *S. typhi*.

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ELECTROPHORETIC INVESTIGATIONS OF A CLONE OF HAMSTER FIBROBLASTS AND POLYOMA-TRANSFORMED CELLS FROM THE SAME POPULATION

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AND

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VOGT and Dulbecco have shown[1] that infection of monolayer cultures of hamster embryo cells with polyoma virus leads to the appearance of a new cell type which represents the tumour cell induced by the virus *in vivo*. Macpherson and Stoker demonstrated the same neoplastic transformation in clonal populations derived from a neonatal hamster kidney cell line (*BHK* 21), possessing many normal cell characteristics[2,3]. This system has obvious advantages for the examination of normal and cancer cells *in vitro*, and, since both the normal and transformed cells have the same rate of growth[3], comparisons may be made between cells of common ancestry and equal maturity. In previous work on the electrophoretic mobilities of normal and cancer cells[4] it was not absolutely certain that the normal and cancer cells compared were derived from the same cell type or that the higher negative charge shown by the cancer cells was not an expression of their more rapid growth. Eisenberg *et al.*[5] have shown that both neonatal and regenerating rat liver cells have an enhanced negative electrophoretic mobility, and that the greatest increase is correlated with the period of most active growth. However, in the case of liver cells, it has been shown recently[6] by Ruhenstroth-Bauer *et al.* that although the increases associated with regeneration and with malignancy are quantitatively similar, they are qualitatively different.

For the present investigation cells were grown as monolayers as described previously[2]. When nearly confluent they were re-suspended with trypsin and washed in complete medium. The cells used were those designated in the earlier communication as clone *C*13, and a transformed clone derived from them, *C*13 *TC*6. After transmission to the Chester Beatty Research Institute these cells were stored at 4° C and used within 2 days. It was found that storage for 6 days at 4° C did not affect the viability of the cells as judged by their lissamine green staining, their ability to grow on inoculation into fresh culture bottles and their behaviour when observed in a perfusion chamber on a microscope stage at 36° C. For microelectrophoresis the cells were washed thrice in 10 ml. of the electrophoresing medium, a solution 0·144 M in sodium chloride and 0·006 M in potassium chloride adjusted to pH 7·0 ± 0·2 with 0·15 M sodium hydroxide, and then re-suspended in this medium at a concentration of 4 × 10^3 cells/mm^3. The cells were introduced into an apparatus of the type described by Bangham *et al.*[7] using a micro-injection technique[8].

The results of electrophoresing three pairs of cell suspensions are displayed in the histograms of Fig. 1. Observations were made on 30 cells in the case of the normal clone and on 60 in the case of the transformed population. The C13 clone of normal hamster kidney fibroblasts behaves homogeneously in an electric field and the results obtained show a similar degree of homogeneity to that observed in erythrocytes[9] and rat thoracic duct lymphocytes[10]. The transformed clone, in contrast, shows a great heterogeneity with the emergence of cells of a higher mobility than those in the normal clone. The coefficient of variance in these populations is normally 10–15 times that of the normal clone. A similar or even greater scatter of mobilities is shown by some mouse ascites tumours[4] and by the pathological cell fraction in Hodgkin's disease[11]. The two hamster cell lines growing in parallel in logarithmic culture were first examined electrophoretically at the 160th generation and afterwards at the 280th and the 420th generations. The difference in behaviour was maintained over this period of propagation and is represented by the three pairs of observations recorded in Fig. 1. We hope shortly to examine cell lines as soon as possible after the transforming event so as to eliminate the effect of changes occurring as a consequence of prolonged growth in tissue culture.

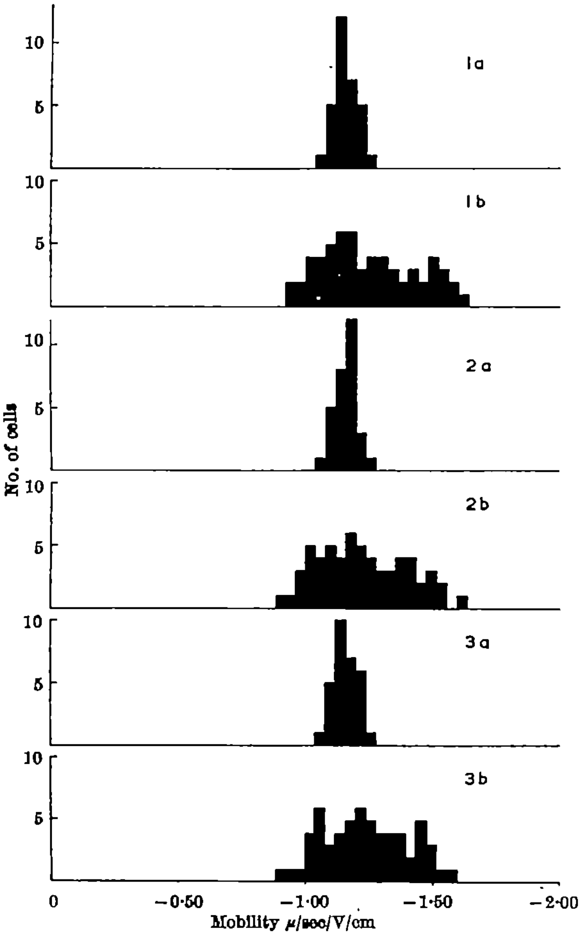

Fig. 1. Electrophoretic mobilities of normal and transformed hamster kidney fibroblasts

1a, *2a*, *3a*, Histograms of normal cell cultures; *1b*, *2b*, *3b*, histograms of transformed hamster kidney fibroblasts

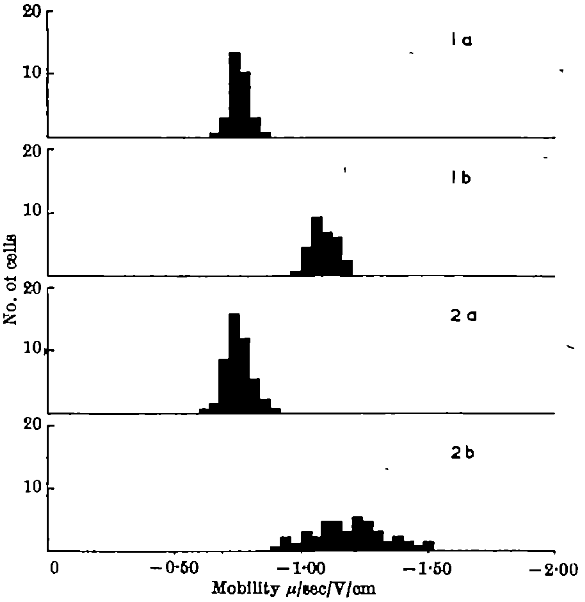

Fig. 2. Effect of neuraminidase treatment on normal and transformed cultures of hamster kidney fibroblasts

1a, *2a*, Normal and transformed cell lines after enzyme treatment; *1b*, *2b*, control incubations of normal and transformed cells using heat-inactivated enzyme

It has been shown[6,8] that among the important ionogenic groupings situated at the cell surface are sialic acid residues derived from mucopolysaccharides and mucoproteins. In an attempt therefore to assess the types of grouping which might be responsible for the cell surface charge in the C13 and C13 TC6 clones cells were examined after treatment with neuraminidase. Suspensions of 6 × 10^6 cells in 1·5 ml. were incubated for 45 min with 500 units of neuraminidase derived from culture filtrates of *V. cholerae*. The purified, freeze-dried enzyme was very kindly supplied by Dr. G. L. Ada of the Walter and Eliza Hall Research Institute, Melbourne, and the incubation was carried out under the conditions described by him as optimal[12]. The cells were then washed twice and re-suspended for electrophoresis as before. Controls were performed with heat-inactivated neuraminidase.

The result of a typical experiment is illustrated in Fig. 2. These observations indicate that in both the normal and the transformed cell lines about 35 per cent of the surface charge arises from sialic acid groupings removable by bacterial neuraminidase. Sialic acid residues have been demonstrated to be important surface charge producing groups in several other cell types, including the human erythrocyte[13], the Ehrlich ascites carcinoma[14], rat liver cells and liver carcinoma cells[6], and the EL_4 leukæmia of mice[15]. Further, suspensions of C13 TC6 cells after incubation with neuraminidase are indistinguishable in the electrophoresis apparatus from cells of the normal clone after identical treatment. This suggests the possibility that associated with the malignant transformation there is a loss in quantitative control over the synthesis of some macromolecular component of the cell surface containing sialic acid residues.

A further species of charged grouping important in determining cell surface charge is the phosphate group derived from phospholipids. It has been shown elsewhere[8] that these groups may be detected by introducing calcium ions into the electrophoresing medium. Exposed phosphate groups have approximately one hundred times the calcium binding power of carboxylic groups and the cell negative mobility is correspondingly reduced by adsorption of these divalent cations. Experiments performed with the present cell system indicated that in both the normal and transformed clones about 20 per cent of the zeta potential is derived from such phosphate groups. In this case, however, the *C*13 *TC*6 cells electrophoresed in a medium containing 0·01 M Ca^{2+} showed the same heterogeneity as in the original determinations in media containing only monovalent cations, offering support to the hypothesis that the originally observed heterogeneity was due only to changes in the sialic acid-bearing components of the surface. These experiments are in agreement with the mosaic picture of the cell surface suggested by recent studies with the electron microscope[16]. Mucopolysaccharides, mucoproteins and phospholipids probably all contribute to the observed surface charge. The results also emphasize the importance of sialic acid groupings in determining cell surface charge, and indicate that a study of the changes associated with malignancy in sialic acid containing macromolecules may lead to a better understanding of the observed differences in social behaviour of malignant cells.

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GROWTH MEASUREMENTS AND THE BIOLOGICAL INTERPRETATION OF MAMMALIAN GROWTH

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It has usually been assumed in the past that growth, or the increase in the size of animals, including mammals, is fundamentally some kind of multiplicative process. This concept has been derived from a consideration of the multiplicative nature of cell division, which is found, ideally, in a bacterial culture with an ample food supply. Also, it has been argued that secondary limitations and distortions can disturb this ideal multiplicative process, especially during the later stages of development, as a result of such factors as the limitation of nutrient materials in larger tissues, etc. In support of this concept, specific growth curves, discussed by Medawar[1] show a steadily decreasing rate of increase proportional to the mass already present, until growth stops as the mature size is reached. Further, Huxley's[2] allometry concept is based on the idea of growth as a fundamentally multiplicative process, although the allometry equation, $y = bx^k$, has been criticized since it was noted that the growth of certain tissues (for example, horn in mammals or shells in gastropods) appeared to be, theoretically, of an additive type.

Recently, fœtal growth patterns in Romney × Southdown cross and Australian Merino sheep have been examined[3,4], and the results from this work suggest a possible alternative to the multiplicative model for the biological basis of mammalian growth, at least during the pre-natal period of development.

Up until about 50 days after conception, the initiation (morphogenesis) of different tissues and parts of the body is taking place. From 50 to 140 days of fœtal age, however, the growth of the fœtus can be examined with a minimum of complications from changes in the external environment, and from changes in the internal endocrine hormone secretions, which are known to affect markedly the post-natal growth patterns although they do not appear to influence pre-natal growth to the same extent[5]. In addition, the fœtal weights increased from about 20 g to approximately 5 kg during this period and such a change, which was accompanied by marked alterations in the consistency of the organs and tissues of the body, should enable valid deductions to be made about the fundamental nature of mammalian growth during the pre-natal stages.

Fœtal weight showed the usual increasing exponential curvilinear relationship in the regression (line of best fit), on fœtal age, similar to post-natal weight changes with age prior to the point of inflexion at which the acceleration of growth becomes negative. In contrast to this pattern, the measurements of total body-length, such as straight or curved crown-rump-length, showed a linear relationship to increases in fœtal age. [Although data from other publications (for example, refs. 6 and 7) show that, prior to 50 days, a positive curvilinear increase in body-length is

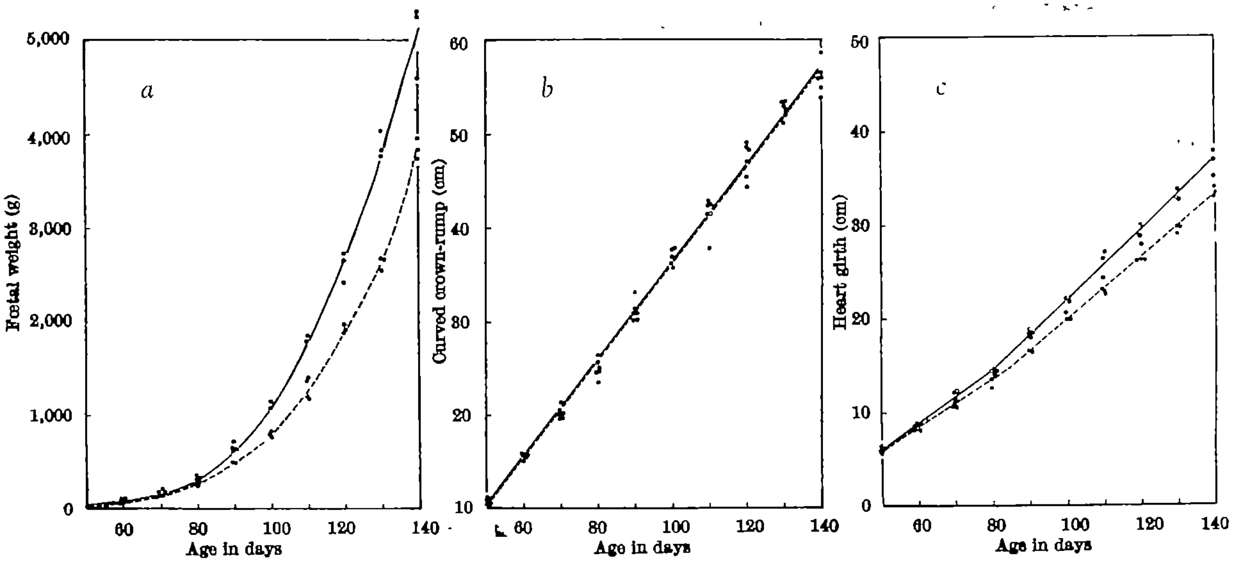

Fig. 1. Relationships between weight and age and the body-length measurements and age during pre-natal growth in sheep. ○——○, Romney × Southdown cross; ● - - - ●. Merino. *a*, Weight changes with increases in fœtal age; *b*, total body-length with increases in fœtal age. Single linear regression; *c*, heart girth with increases in fœtal age. Two linear regressions

obtained, this difference within the relationship between length and age is not unexpected for it is reasonable to suggest that the growth pattern may be different during the early stages of development.] Further, a series of 18 length measurements were taken over the surface of the body between certain fixed points, and apart from three minor exceptions, these also showed linear relationships in the regressions on fœtal age. There was, however, a marked change in the slope of several of these linear regressions at about 80 days of fœtal age, without any suggestion of a curvilinear relationship prior to or following this stage of growth. These changes in the slopes of the regression lines were most evident with measurements involving the head, the circumference of the front of the body and the lengths of the limb bones.

The general patterns shown by changes in weight and the surface-length measurements with increases in fœtal age are illustrated in Fig. 1.

The justification for these relationships has been fully discussed elsewhere[3,4]. The single linear regressions showed no obvious or systematic departures from this relationship when the means of the measurements for each age-group were plotted against fœtal age and in most instances these mean values were very close to the regression line[4]. Statistical tests were carried out to determine the significance of the x^2 terms in several of these regressions, but in all instances this component was not significant at the 5 per cent probability-level. Where two separate linear regressions were obtained, the standard errors of the regression lines showed that the change in slope was significant, at least at the 5 per cent level, but, except in three instances, there was no significant x^2 term in either of the two linear relationships. Once again, plots of the means of the measurements for each age group against fœtal age showed these values to be close to the regression lines, with no evidence of any systematic departures from the linear relationship. As a further test, where two linear relationships were obtained, the residual variances were compared with the residual variances after exponential and polynomial curves had been fitted to the data using an electronic computer. Except for two measurements of the circumference of the body, where growth results from the interaction of several different factors, the residual variances were higher after curves had been fitted and this result supports the hypothesis of a linear relationship between age and the length measurements.

The conclusion from this result is that, at 80–90 days after conception, the growth of the fœtus may change to a new constant pattern which is maintained until birth. In addition, the regression of the measurement of the length of the nose on fœtal age showed a significant curvilinear relationship up to 90 days with a positive x^2 term, but after this age the regression was linear and the slope of the line was lower than that during the first period.

These results suggest that, rather than being a truly multiplicative phenomenon, mammalian growth during the pre-natal period may be represented more accurately by the model of linear additions to existing material along the surfaces of a three-dimensional body, that is, a cubic growth pattern. Such a model should produce the weight and length changes with increases in fœtal age that have been found in this work, provided that the rate of addition of material at the surface may be altered to a new constant level at a certain stage during the growth of the fœtus.

Conversely, if a multiplicative process is assumed, it is unlikely that the decrease in the specific growth-rate is, by chance, just sufficient to give linear regressions of the surface-length measurements on age. A steady decrease in the efficiency of a multiplicative process, such as is suggested by the specific growth-curve, should produce a sigmoid shaped curve when the length measurements are plotted against fœtal age. Although a sigmoid curve may approximate closely to a linear model, especially if the point of inflexion occurs during the period at which the growth measurements are taken, there was no evidence of such curves in the investigation reported here. The increase in the fœtal weights and the marked alteration in the consistency of the organs and tissues of the body, from 50 to 140 days, should have an effect on the efficiency of a multiplicative process and produce some curvature of the length measurements.

Table 1. THE GOODNESS OF FIT OF EXPONENTIAL AND POLYNOMIAL RELATIONSHIPS TO THE SPECIFIC GROWTH-CURVES FOR THE ROMNEY × SOUTHDOWN CROSS AND AUSTRALIAN MERINO DATA

Romney × Southdown cross

Equations:

Exponential: $y = 9{\cdot}758 - 16{\cdot}47e^{-0{\cdot}1786x}$

Polynomial*

$y = -7{\cdot}4555 + 3{\cdot}0478x - 0{\cdot}22154x^2 + 0{\cdot}0061173x^3$

Analysis of variance:

	S.S.	d.f.	M.S.	F
Total	88·425214	28		
Between age groups	88·028364	9		
Residue (within ages)	0·396850	19	0·020887	
Polynomial regression	87·975724	3		0·420
Residue (between ages)	0·052640	6	0·008773	
Exponential regression	87·867365	3		1·285
Residue (between ages)	0·160999	6	0·026833	

Australian Merino

Equations:

Exponential: $y = 9{\cdot}79335 - 14{\cdot}7318e^{-0{\cdot}137754x}$

Polynomial

$y = -5{\cdot}9344 + 2{\cdot}5629x - 0{\cdot}17727x^2 + 0{\cdot}0047583x^3$

Analysis of variance:

S.S.	d.f.	M.S.	F
68·801414	28		
68·666747	9		
0·134667	19	0·007088	
68·624135	3		1·002
0·042612	6	0·007102	
68·5694054	3		2·29
0·0973416	6	0·016224	

* For both the Merino and the Romney × Southdown cross, the cubic component of the polynomial regression was significant at the 0·1 per cent level. x = Days ÷ 10 in the equations.

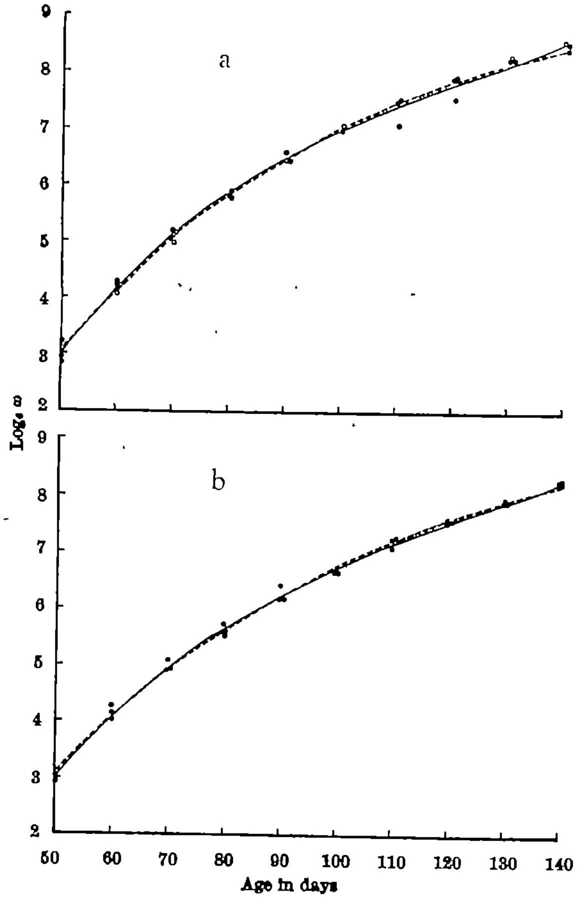

Fig. 2. Cubic and exponential equations fitted to the specific growth-curves for the Romney × Southdown cross (a) and Merino (b) fœtuses. —, Cubic curve; - - -, exponential curve

Other work supporting these relationships has been summarized by Brody[8]. Pre-natal increases in the sitting height of human fœtuses show a linear relationship to increases in fœtal age, while the corresponding weight increase is an exponential one. There is also evidence of a marked change in the growth pattern at about 18 weeks of fœtal age. Post-natal height increases in man, and increases in a number of different body-length measurements in cattle also show linear relationships to age during the juvenile growth period. In this connexion Brody[8] states that "weight (volume) growth prior to puberty tends to be self accelerating, that is, to grow in *geometric* progression —whereas linear growth, that is, terminal growth, tends to grow in *arithmetic* progression—and to plot linearly on arithmetic paper". In contrast to this pattern, post-pubertal increases in length measurements, and also total body-weight, show a declining exponential relationship to age, the equation relating weight or body-length, y, to age, x, during this period being of the form:

$$y = A - Be^{-kx}$$

Specific growth-curve and its relation to multiplicative and cubic growth models. The specific growth-curve shows a steady decrease in the relative rate of increase in weight, proportional to the mass already present, throughout the pre-natal growth period in the sheep[8,9]. Such a curve would result from either a multiplicative or a cubic model of weight increases with increases in fœtal age, and should be fitted by an equation of the form $y = a - be^{-kx}$ where y = weight and x = the age of the fœtus. An analysis of variance has been carried out to see whether such an equation does fit the data from the Romney × Southdown cross and Merino fœtuses and the results are given in Table 1 and Fig. 2. For comparison, a polynomial equation of the form $y = a + bx + cx^2 + dx^3$ has been fitted to the same set of measurements.

Table 2. EXPONENTIAL CONSTANTS AND EQUATIONS RELATING WEIGHT, w, TO TOTAL BODY-LENGTH (L) MEASURED AS STRAIGHT OR CURVED CROWN-RUMP

Sheep Breed	Equation	Significance of exponentia values deviation from 3·0	L measurement used
Romney × Southdown cross	$w = 0{\cdot}008757L^{3{\cdot}2763}$	*	Curved crown-rump
Romney × Southdown cross	$w = 0{\cdot}02836L^{3{\cdot}1707}$	*	Straight crown-rump
Australian Merino	$w = 0{\cdot}01279L^{3{\cdot}08842}$	†	Curved crown-rump
Australian Merino	$w = 0{\cdot}04032L^{3{\cdot}0096}$	N.S.	Straight crown-rump
New Zealand Romney	$w = 0\ 02474L^{3{\cdot}0075}$	N.S.‡	Curved crown-rump
New Zealand Romney	$w = 0{\cdot}06641L^{2{\cdot}8900}$	*‡	Straight crown-rump
South African Merino	$w = 0{\cdot}134L^{2{\cdot}60}$	Not tested§	Curved crown-rump
South African Merino	$w = 0{\cdot}154L^{2{\cdot}77}$	Not tested§	Straight crown-rump

* Significant at 1 per cent level. † Significant at 5 per cent level. ‡ Data from ref. 9. § Equations calculated by Malan and Curson (ref. 15). N.S. Not-significant.

It is seen that both the exponential and polynomial equations fit the data within the 5 per cent probability limits determined by the within age group variance, although for both the Romney × Southdown cross and Merino results the polynomial equation gives a slightly better fit than the exponential, mainly as a result of the high mean values for fœtal weights at 140 days.

Other relationships affecting the multiplicative or cubic interpretation of mammalian growth. Additional information can be obtained from the length/weight relationships in different breeds during the fœtal growth period, from the regressions of the $\sqrt[3]{\text{body-weight}}$ on fœtal age and from a study of the growth of individual tissues, particularly bone and muscle, which make up a large part of the weight of the fœtus.

Fœtal weight should be a function of (body-length)^3 if growth is uniform in all directions and if the specific gravity of the body remains constant during the growth period. The exponential constant relating weight to total body-length can be obtained from the linear regression of $\log_{10}$ weight on $\log_{10}$ length and these constants are given for several different breeds during the pre-natal growth period, in the equations relating to two variables in Table 2.

The average of these exponential constants is approximately 3·0; but there is some variation away from this mean value and statistical tests show this variation to be significant. The value of the exponential constant is correlated with the carcass conformation of the different breeds, for, as shown in Table 2, it tends to be higher in breeds improved for mutton production. Such breeds show a greater breadth and circumference of carcass for a standard body-length[4] and as growth in width is more rapid in these breeds, relative to growth in length, it is reasonable that the exponential constant relating weight to length should be higher. This point is stressed by the differences between the exponential constants, obtained when straight or curved crown-rump are used as the measure of total body-length. As age and weight increases, straight crown-rump increases at a greater rate than curved crown-rump on a $\log_{10}$ scale with the data included in these results, hence the resulting exponential constants are lower than those obtained with curved crown-rump. In addition, it is possible that changes in the specific gravity of the carcass during the pre-natal growth period may be greater in the breeds improved for mutton production.

The cube roots of fœtal body-weight increase with age according to the relationships shown in Fig. 3 and Table 3.

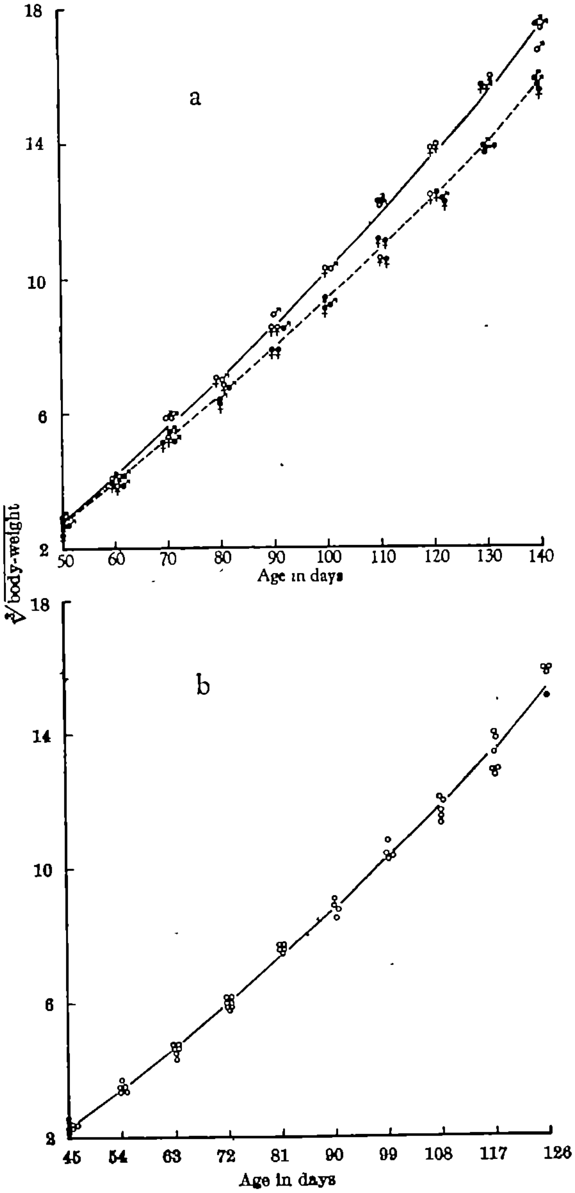

Fig. 3. Regressions of $\sqrt[3]{\text{body-weight}}$ on fœtal age. *a*, Romney × Southdown cross, – ♀♂ – and Australian Merino, - - -♀♂- - -; *b*, New Zealand Romney

If growth occurs through linear additions to existing material along the surfaces of a three-dimensional body, then the cube roots of body-weight should be linear with age provided that no changes occur in the specific gravity of the material already produced and provided that all growth-rates at the surfaces remain constant. The surface length measurements, however, show that, at least for some parts of the body there is a marked change in the growth-rate at about 80 days although the rates are constant before and after this age. Conversely, the cube roots of body-weight show a significant curvilinearity in the regressions on fœtal age for the Romney × Southdown cross, Australian Merino and New Zealand Romney breeds. This curvilinearity is not produced by a sudden change in the slope of a linear relationship at about 80 days, but is a gradual trend over the whole age range studied, and it is found in the New Zealand Romney data if the points below 54 days are excluded from the analysis. The Australian Merino data do not show a greater curvilinearity than the Romney ×

Table 3. EQUATIONS FOR THE REGRESSIONS OF $\sqrt[3]{\text{BODY-WEIGHT}}$, y, ON FŒTAL AGE, x, AND F VALUES GIVING THE SIGNIFICANCE OF THE x^2 TERM

Breed	Equations	Age period	F Value giving significance of x^2 term
(i) Romney × Southdown cross	$y = -3{\cdot}2614 + 0{\cdot}104985x + 0{\cdot}00029657x^2$	50 to 140 days	7·338 (1;26d.f.)*
(ii) Australian Merino	$y = -2{\cdot}58015 + 0{\cdot}094387x + 0{\cdot}00025133x^2$	50 to 140 days	21·97 (1;26d.f.)†
(iii) New Zealand Romney	$y = -2{\cdot}194834 + 0{\cdot}079416x + -0{\cdot}0004694x^2$	45 to 126 days	35·856 (1,53d f.)†

* Significant at 5 per cent level. † Significant at 0·1 per cent level.

Southdown cross results, as is suggested by Table 3, for the error variation about the regression line is much higher in the latter group.

This result is not consistent with a cubic growth model unless there is a change in the rate of increase during the growth period or unless there is a change in fœtal density. Fœtal density increases from about 1·03 to 1·06 between 50 and 140 days after conception[10], but this increase is not sufficient to account for the curvilinearity of the regressions. Alternatively, changes in the growth-rates, which occur at slightly different ages in different parts of the body, the different allometric growth-rates in different regions, or interactions between these two effects, could produce a curvilinear relationship between age and the cube root of fœtal weight.

Conversely, the growth of certain tissues or parts of the body may conform to a multiplicative model. Although this is not consistent with the pattern shown by the majority of the length measurements, these do not cover all parts of the body and, in addition, three length measurements do show slight curvilinear relationships in the regressions on fœtal age.

Tissue growth. Growth studies of the individual tissues should provide evidence for the interpretation of mammalian growth as a multiplicative increase in size or as an addition of new material along the surfaces of a three-dimensional body. Except for bone, hair (or wool) and horny structures produced by the epidermis, however, not enough is known of the growth of individual tissues to determine whether it is of a multiplicative or additive type. It has been suggested[11] that new muscle cells are not produced after the middle stages of gestation (about 90 days in the sheep), but the physiological aspects of subsequent increases in the size of the muscle fibres are not precisely known. Mitotic activity in the central nervous system after neurulation appears to be confined to an inner 'germinal layer' lining the central canal and brain ventricles[12], but changes in cell size, the migration of nerve cells and regional variations in mitotic activity all affect the growth pattern.

It has been known for some time, however, that the growth of hair, wool and horny structures produced by the epidermis is of an additive type. The growing region for these tissues consists of a layer of actively dividing cells, and once new cells are produced from this mitotic region, keratin formation transforms them into non-living products of the 'germinal layer'. The growth of bone, which controls to a greater or lesser extent the growth of several other tissues (notably muscle, for example, ref. 13), and has a marked influence on the size and shape of the animal, is known to occur through additions of new material at the bone surfaces and through increases in length by mitotic divisions in the epiphyseal plates[14]. Therefore, apart from the secondary resorption which occurs during growth so that the correct shape of the bone is maintained, the growth of this tissue can be said to follow the model of additions of new material on the surfaces of a three-dimensional body.

Finally, it must be emphasized that a simple theoretical model, consistent with all aspects of growth, is unlikely to be produced. General considerations lead to the conclusion that growth processes may be too complex to allow the use of one mathematical generalization to describe them. The arguments presented in this article do indicate, however, that the overall patterns of increase in body-size may conform more closely to a cubic, than to a multiplicative model during the mammalian pre-natal growth period, and also possibly during the juvenile growth period. This may, in turn, reflect the importance of bone growth in determining such increases in body size.

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A NEW ORNITHISCHIAN FROM THE UPPER TRIASSIC OF SOUTH AFRICA

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An important discovery made by the 1961–62 British–South African Expedition to the Upper Triassic rocks of South Africa and Basutoland was the partial skeleton of an ornithischian dinosaur, including the almost complete skull and lower jaw. Ornithischian remains are very rare below the Upper Jurassic; indeed, the only ornithischian material yet described from the South African Karoo, or from Triassic rocks of any age, is one very incomplete skull (*Geranosaurus atavus* Broom 1911 [1]).

The new skull (Fig. 1*A*) is almost complete but slightly crushed. The bones are covered by a thin, very hard ferruginous layer which can be removed only with the aid of a small diamond saw. Although, as yet, only the anterior region has been prepared in this manner, the general outline of the whole skull is nevertheless apparent and is shown in Fig. 1*A* by dotted lines. Complete preparation of the specimen, especially of the brain-case, will take a very long time; it has therefore been decided that a preliminary description of the skull and lower jaw should be given now, and that, for convenience, the specimen should be named.

Order: ORNITHISCHIA

Genus: *Heterodontosaurus* nov.

Derivation of name. In reference to the differentiated dentition, unexpected in an ornithischian.

Type-species. Heterodontosaurus tucki sp. nov.

Diagnosis. As for *H. tucki*.

Heterodontosaurus tucki sp. nov.

Derivation of name. In recognition of the generous help afforded to the Expedition by Mr. G. C. Tuck, managing director of the Austin Motor Co. of South Africa (Proprietary), Ltd.

Material. Only the holotype in the South African Museum (Catalogue No. *K*337), consisting of skull, lower jaw and post-cranial remains.

Horizon. In the transitional beds of the Cave Sandstone[3], below the basalt; probably of Upper Keuper age.

Locality. At an altitude of about 6,200 ft. on the mountain behind Tyindini trading store, in the district of Herschel, Cape Province, South Africa.

Diagnosis. Ornithischian with short facial region, large antorbital fenestra, and supraorbital bone projecting into orbit. Hinder part of premaxilla with three teeth of carnivorous type, the third large and caniniform; wide, deep diastema followed by about 12 close-set, highly specialized teeth in maxilla, ridged vertically in their distal halves. Predentary without teeth; first tooth on dentary large, caniniform, projecting upwards into diastema; other teeth on dentary close-set and similar to those in maxilla.

Skull. The skull is about 92 mm long, with a large antorbital fenestra (Fig. 1*A*, *ANT.F.*). The premaxilla (*PM.*) is deep below the external nares; the nasal is rather bulbous; and there is a supraorbital bone (*S.*) projecting backwards into the orbit from its anterior margin. Through the thin layer of matrix it may be recognized that the jugal possesses a flange (*J.F.*) below the lateral temporal fenestra and that the squamosal extends backwards beyond the posterodorsal corner of the quadrate.

Lower jaw. There is a small predentary at the front, separated from the dentary by a distinct suture. Behind this suture, and running dorsoventrally immediately below the first mandibular tooth, is a distinct groove; this may have served to accommodate the hinder end of the horny beak which probably covered the predentary. The lower jaw appears to have a well-developed coronoid process.

Upper teeth. The front part of the premaxilla is without teeth, but the hinder part bears three. All three are laterally compressed and slightly recurved. The first two are incisor-like, the second larger than the first; the third is much the largest and resembles the canines of therapsids and mammals. A wide bay-shaped diastema separates the premaxillary teeth from the maxillary teeth.

There are 12 teeth in the maxilla, closely packed together in a single row 31·5 mm long; their alveoli lie medially of the lateral surface of the bone. They have very long column-shaped crowns, which, seen from the side, are a little wider distally than at the alveolar border, and which curve inwards a little below. The anterior teeth are laterally compressed and possess a sharp distal cutting edge. The posterior teeth are thicker medio-laterally than the anterior and possess a correspondingly wider occlusal surface; they probably served to crush the food. The lower edges of all these teeth are more or less level, apparently because of wear; this is shown particularly well on the left side. (In Fig. 1*B* the occlusal surface has a slightly irregular appearance because the crowns of the right maxillary teeth have suffered a little damage at their distal ends.) Enamel is present on only the distal half of the outer surface of each crown, the enamelled surface being divided into two smooth depressions by a vertical ridge down its centre. The first four maxillary teeth have also secondary vertical ridges, anterior and posterior to the main ridge. This ridging produces a strikingly characteristic appearance.

Lower teeth. The predentary is without teeth. The first mandibular tooth lies near the front end of the dentary and fits into the diastema in the upper jaw; it has the shape of the canine present in therapsids and mammals, being slightly recurved, and a serrated posterior edge. Five or six mandibular teeth behind the 'canine' are missing; but seven of the mandibular teeth are fairly well preserved, and have been exposed in medial (Fig. 1*C*) and partially in lateral view. As in the case of the corresponding upper teeth, the crowns are high. Enamel seems to be present only on the inner surface, which bears a vertical ridge not far behind the anterior margin of the crown and an additional ridge farther back. This internal view of the lower teeth shows no replacement teeth at their bases.

Affinities. Differentiation of the dentition into incisor-like, canine-like and post-canine-like teeth is uncommon in animals other than therapsid reptiles and mammals.

The following characteristics, however, taken together, indicate that *Heterodontosaurus* should be placed in the order Ornithischia: (1) The presence of an antorbital fenestra (usually reduced in size in later ornithischians). (2) The form of the premaxilla (deep below external nares). (3) The presence of a supraorbital. (4) The flange on the jugal. (5) The backward extension of the squamosal. (6) The presence of a predentary. (7) The presence of a coronoid process. (8) The position of the maxillary teeth (medial to the lateral surface of the maxilla). (9) The curvature of the crowns of the cheek teeth (in anterior view, convex in the upper jaw and concave in the lower). (10) The presence of enamel on only one side of the crowns of the mandibular teeth. (11) The iguanodontid-like ridging of the crowns of the cheek teeth.

For purposes of diagnosis No. 6 is by far the most important of these.

Comparison with other ornithischians. It is obvious that the first animal with which *Heterodontosaurus* should be compared is *Geranosaurus*[1], also from the Cave Sandstone.

The latter genus, however, is very poorly known. The unique specimen consists only of a broken lower jaw (predentary and dentary) and part of the upper jaw (premaxilla and maxilla); none of the tooth crowns is preserved. Recent preparation of the type of *Geranosaurus* has confirmed the presence of a predentary in this animal. It resembles *Heterodontosaurus* in its possession of enlarged teeth ('canines') in both jaws, and in the apparent absence of replacement teeth on the medial side of the existing teeth; it differs from the new genus in lacking the large diastema in the upper jaw and in the absence of a step separating the maxillary teeth from the lateral surface of the maxilla.

Other early ornithischians (or supposed ornithischians) include *Poposaurus* from the Upper Trias of

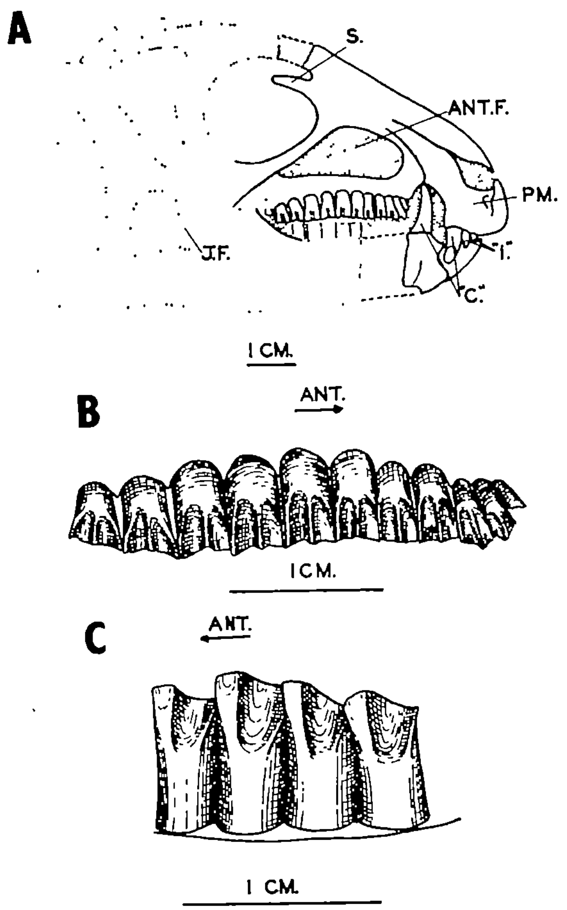

Fig. 1. *Heterodontosaurus tucki* gen. et sp. nov.
A, lateral view of the skull; *B*, lateral view of the maxillary dentition; *C*, medial view of four mandibular teeth
ANT., anterior; *ANT. F.*, antorbital fenestra; "*C.*", canines; "*I.*", incisors; *J.F.*, flange on the jugal; *PM*, premaxilla; *S.*, supraorbital

Wyoming and *Scelidosaurus* from the Lower Lias (basal Jurassic) of England. The *Poposaurus* material includes nothing of the skull; in any event, Colbert has shown[3] that *Poposaurus* is more likely to be a saurischian than an ornithischian. The only known skull of *Scelidosaurus* (in the British Museum (Natural History)) has not yet been properly prepared, but its teeth are patently quite unlike those of *Heterodontosaurus*.

Although chronologically younger than the Iguanodontidae, the Hypsilophodontidae are considered to be the most primitive family of the Ornithopoda. They include *Hypsilophodon*[4,5] from the Lower Cretaceous and *Thescelosaurus*[6] from the Upper Cretaceous. It is unfortunate that no skull of any hypsilophodontid is well known. These animals are more advanced than *Heterodontosaurus* in the smaller size of their antorbital fenestra and in certain features of their dentition: namely, in the presence of a well-developed cingulum, of more numerous vertical striations on the outer surface of the upper teeth and the inner surface of the lower teeth, and of replacement teeth medial to the lower teeth above the alveolar border. Against this, however, the hypsilophodontid dentition retains several primitive characters: *Hypsilophodon* still has five teeth in the premaxilla, the last premaxillary tooth does not appear to be enlarged, the teeth of both *Hypsilophodon* and *Thescelosaurus* are leaf-shaped, they are less elongated and less tightly packed together than in *Heterodontosaurus* (or the Iguanodontidae), and in *Thescelosaurus* enamel is present on both sides of the teeth in both maxilla and dentary. The heterodont nature of the teeth is far less pronounced in *Hypsilophodon* than in *Heterodontosaurus*.

The oldest ornithischian with which the new skull can be adequately compared is *Camptosaurus* from the Upper Jurassic. This genus, of which the skull is fairly well known, is a primitive member of the Iguanodontidae. It is like *Heterodontosaurus* in its possession of teeth which are curved towards those in the opposite jaw (the direction of curvature being as described above for the South African animal), which lack a cingulum, and which are enamelled on one side only. It is also true that both genera have a supraorbital bone of similar form. In certain features, however, *Camptosaurus* is more advanced than the new genus: the pre-orbital part of the skull is elongated, the antorbital fenestra is reduced in size, premaxillary teeth and the lower 'canine' are completely absent, the enamelled surfaces of the teeth bear many more vertical striations than are to be seen on the teeth of *Heterodontosaurus*, and there are replacement teeth on the medial side of the functional teeth. On the other hand, the teeth of *Camptosaurus* are in some respects less specialized, for they are still leaf-shaped, with relatively much lower crowns. Moreover, they were frequently replaced, so that they are not all of equal length; and this, together with the fact that they are not placed so closely together, means that they do not form a single continuous cutting edge like that of the maxillary teeth of *Heterodontosaurus*. The tooth-structure of the South African animal is altogether closer to that found in later and more specialized iguanodontids, such as *Iguanodon* itself, than to that found in *Camptosaurus*.

The Hadrosauridae of the Upper Cretaceous, though highly specialized, also show certain interesting similarities to the new genus. The individual teeth in each maxilla and each dentary are united to form a compact dental battery[7,8]. Each tooth has extremely long roots; enamel is restricted to the outer surface of the upper teeth and inner surface of the lower teeth; a single vertical ridge is present in the centre of each enamelled surface, with a shallow depression on either side; and the crowns of adjacent teeth are worn in such a way that their occlusal surfaces all lie on the same plane. In *Heterodontosaurus* the teeth do not form batteries, but all the other characters just mentioned are present and suggest a relationship to the Hadrosauridae. Replacement teeth are almost certainly present in the new specimen; but, as in the Pseudosuchia[7], they are probably lying medial to or below the roots of the functional teeth and would have become visible only when the latter had been lost or considerably worn.

Heterodontosaurus possesses characters present in each of three families of the Ornithopoda, to which sub-order it might be referred provisionally. It is clear, however, that the new genus cannot be considered ancestral to all three families. This seems to show that the main radiation of the Ornithopoda had taken place before Upper Triassic times. When the rest of the skull and the post-cranial remains of the new specimen have been prepared it may be possible to determine its relationships more certainly.

Haughton[9] gave a detailed description of the mandibular dentition of *Lycorhinus angustidens*. This animal had always been regarded as a therapsid

College, London, brought to our notice the close similarity of the dentitions of *Lycorhinus* and *Heterodontosaurus*, which appears to indicate that *Lycorhinus* was probably an ornithischian dinosaur rather than a therapsid. The mandibular teeth behind the canine' differ from those of *Heterodontosaurus*, however, in possessing a distinct cingulum. This and the nature of the wear of the teeth appear to indicate that *Heterodontosaurus* and *Lycorhinus* are generically distinct. *Lycorhinus* was found in the Red Beds; than suggested by *Heterodontosaurus*.

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FLOWERING RESPONSE OF RICE TO DIFFERENT PHOTOPERIODS OF UNIFORM DAILY AMOUNTS OF LIGHT RADIATION

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IN the recent literature on rice there has been some discussion as to the shape of the photoperiodic response curves (duration to flowering plotted against photoperiod).

One of the first to stress the importance of constructing these curves was Chandraratna[1], who calculated that the shortest duration to flowering was obtained in photoperiods varying from 9½ to 11 h according to variety: the photoperiod which gave the shortest duration was defined as the optimum photoperiod. Chandraratna found that over a limited range of photoperiods (usually part of the range of 8–13 h), which he felt included the main range of interest to agronomists and breeders, second degree polynomials could be fitted to the data. However, since his treatments in short photoperiods received considerably less light than those in long photoperiods, this interpretation was open to question.

Njoku[2] used a technique whereby all treatments received the same amount of daylight (though not total light), the differences in photoperiod all being obtained by additional periods of low-intensity incandescent illumination. He found no evidence of an increase in duration at photoperiods shorter than Chandraratna's optima, but he did not use photoperiods of less than 9 h.

The most comprehensive series of experiments and discussion of this problem, together with a summary of the earlier work, has recently been published by Best[3], who confirms the existence of an optimum photoperiod, which varies from 9 to 12½ h according to variety; but he also convincingly demonstrates, particularly when a correction for 'non-photoperiodic effects' is applied, that the data cannot be fitted to a simple equation, even over a relatively narrow range of photoperiods. Best used an ingenious correction to allow for those 'non-photoperiodic effects', which were due to the fact that the plants in very short photoperiods received less light than those in the long photoperiods. Long photoperiodic treatments were repeated using different ratios of the period of daylight to low-intensity supplementary illumination and it was found that in any given photoperiod there was a delay in flowering in the treatments receiving less light. Points of similar duration on the sub-optimal side of the curve to those on the supra-optimal side were chosen: the duration values found on the sub-optimal side were then decreased by the same amount as the difference found between high-light and low-light results of the corresponding supra-optimal photoperiod where the low-light treatment had the same daylight period as the sub-optimal photoperiod.

Undoubtedly this method does provide an approximate solution to the problem of constructing photoperiodic response curves for high-light intensities; but it suffers from the criticism that it assumes that the interaction between light intensity and photoperiod which the results demonstrate in supra-optimal photoperiods is the same as between light intensity and sub-optimal photoperiods causing similar duration. As Best points out, " . . . [the

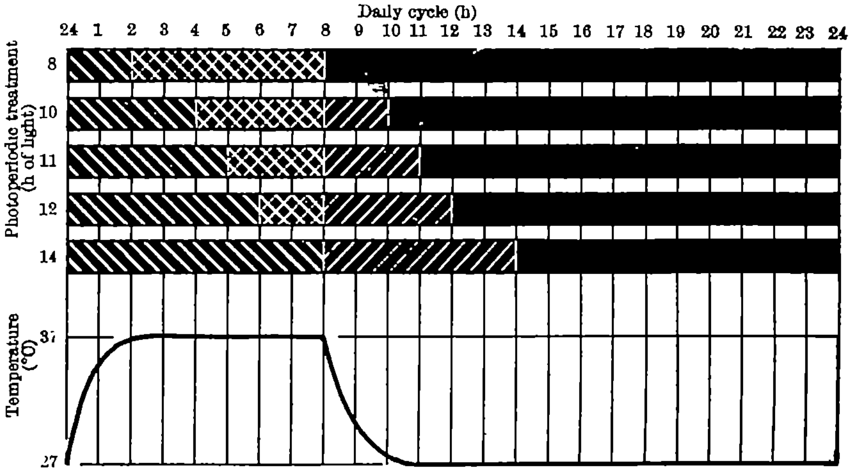

Fig. 1. Diagram showing the five photoperiodic treatments. Except for occasional minor aberrations, the temperature régime was the same (± 1·5° C) in all treatments and is shown by the graph.
Hatching downwards left to right, 640-W fluorescent light; downwards right to left, 100-W incandescent light; black, dark

Photoperiod (h)	Variety	No. of plants	Coefficient of variation with *S.E.*
8	T.C. 65	4	13·9 ± 4·9
	L. 35	3	42·4 ± 17·3
	R.C. 4	5	14·4 ± 4·6
10	T.C. 65	5	13 5 ± 4·3
	L. 35	5	9·0 ± 2·8
	R.C. 4	3	3·8 ± 1·6
11	T.C. 65	5	24·0 ± 7·6
	L. 35	4	21·5 ± 7·6
	R.C. 4	- 4	11·0 ± 3·9
12	T.C. 65	4	16·2 ± 5·7
	L. 35	5	*
	R.C. 4	1	*
14	T.C. 65	5	13 0 ± 4·1
	L. 35	5	*
	R.C. 4	4	*

* No flowering or flowering not completed.

method] is based on the comparison of sub- and supra-optimal photoperiods, in which the photoperiodic effect may be of a different nature".

In the experiment reported here we have attempted to eliminate non-photoperiodic effects by sacrificing high-light intensities and growing plants in controlled environments using photoperiodic treatments which provide the same total daily amounts of light. A full description of the growth chambers which were used will be published elsewhere; but the main details which apply to the present experiment are as follows. Main lighting in each chamber was supplied by eight 5-ft. 'white', 80-W, fluorescent tubes with internal reflectors which provided a light intensity of about 9,000 lux (falling to 7,000 lux at the extreme corners of the chamber) at the level of the top of the plants, which were kept about 30 cm from the light source throughout the experiment. Supplementary lighting was provided in each cabinet by four 25-W incandescent lamps which gave a light intensity between 200 and 500 lux depending on the position in the cabinet. (Wormer[4] carried out experiments on rice which indicated that maximum response to photoperiod is obtained with supplementary light at an intensity of about 40 lux and little or no effect is obtained by increasing the intensity above this value.) The same temperature régime was given in each cabinet and is shown together with the five photoperiodic treatments in Fig. 1.

Three pure-line swamp varieties were used: Radin China 4 (from Malaya), Lead 35 (a selection made at this station out of material from British Guiana), and Tai Chu 65 (from Formosa); the first two are members of the *indica* sub-species and the last is said variously to belong to the *japonica*, sub-*japonica* or *indo-japonica* group.

The seeds were first germinated for a week at 32° C in the dark, then single seedlings were transferred to 10-l. undrained polythene buckets containing mud and grown at ambient temperature in daylight for a further week. Five buckets of each variety were then placed in each photoperiodic treatment in the growth cabinets. (Best[3] found that no variety he investigated became sensitive to photoperiod until it was at least two weeks old.) A few plants died and the final replication numbers are shown in Table 1.

The results are shown in Fig. 2 and Table 1. It will be seen that Tai Chu 65 is the least sensitive to photoperiod in this experiment but even with this variety, which has the reputation of being completely insensitive, a delay in flowering occurred under a 14-h photoperiod. In the case of the other two varieties minimum duration occurred in the 10-h photoperiod; it will also be seen (Table 1) that it was at this photoperiod that least plant-to-plant variation occurred. This result can be related to an observation of agronomic importance based on the results of field successional sowing experiments made at this station[5]: when a photoperiod-sensitive crop is sown out of season so that flowering is delayed by long days, there tends to be an increase in the variability of flowering. Another interesting comparison between the present results obtained from plants grown in controlled environments and those grown in the field is the relative durations of Radin China 4 and Lead 35. The records for field successional sowing experiments[6] for five sites in West Africa (including Sapu 13° 29′ N. in Gambia and Nungua 5° 40′ N. in Ghana) show that, for any given sowing date, Lead 35 always flowers before Radin China 4. It will be seen that in every treatment where these varieties flowered in the cabinets the reverse is true. This comparison of results demonstrates that some other factor—which in this case may well be temperature—also affects duration and that considerable inter-varietal differences in response to this factor must exist, as well as to photoperiod.

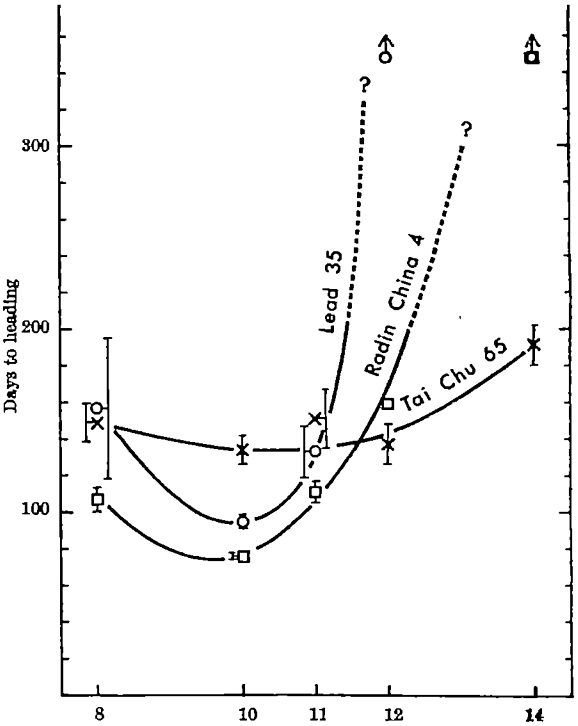

Fig. 2. Photoperiodic response curves for the rice varieties Tai Chu 65 (×), Lead 35 (○), and Radin China 4 (□). The vertical lines represent twice the *S.E.* The experiment lasted 348 days; treatments in which no flowering had occurred by this time are shown by a point at this value with an arrow pointing upwards; this symbol has also been used in the case of Lead 35 in the 12-h photoperiod where one plant out of five flowered before this time (after 215 days). Plants which were still vegetative at the end of the experiment flowered within 32 days on transfer to a 10½-h photoperiod

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LETTERS TO THE EDITOR

RADIOPHYSICS

Spectrum of the Galactic Radio Emission between 10 Mc/s and 1·5 Mc/s

As part of a general investigation of cosmic radio noise at frequencies less than 10 Mc/s the intensity of the radiation has been measured at 9·8 Mc/s, 4·8 Mc/s, 2·3 Mc/s and 1·5 Mc/s. The observations were made at Hobart during July and August in 1961 and 1962. Similar arrays of three full-wave dipoles were used at each frequency, directed to declination −42°. The bandwidth of the receivers was 4 kc/s and a combination of frequency sweeping and minimum recording was used to reduce interference from transmitting stations and atmospherics. The receivers were calibrated with noise generators.

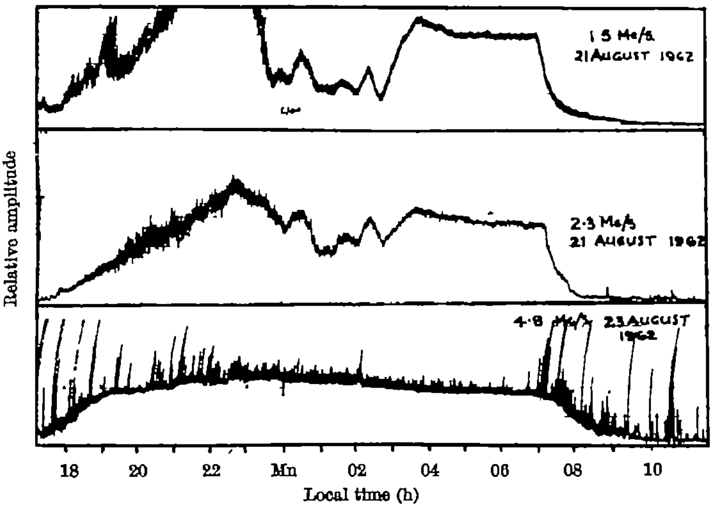

Fig. 1. Sample records of cosmic radio noise at 1·5 Mc/s, 2·3 Mc/s and 4·8 Mc/s. The first two records illustrate the effects of transmitter interference from 1900 to 2400 h and ionospheric modulation from 2400 h to 0300 h. The galactic profile appears from 0400 h to 0700 h

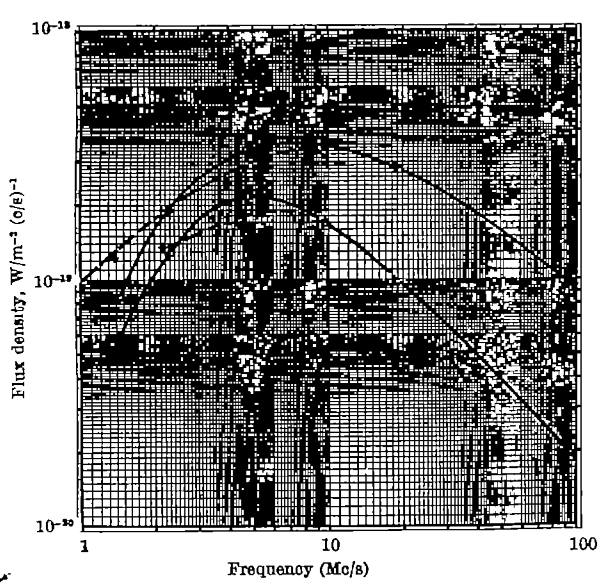

Fig. 2. Cosmic noise spectra less than 100 Mc/s. The 1956 spectra are shown dotted. Points above 10 Mc/s from ref. 2. Present observations indicated thus ○

Fig. 1 shows samples of the records at 4·8 Mc/s, 2·3 Mc/s and 1·5 Mc/s. Only records for which ionospheric modulation of the radiation appeared to be absent were used in calculating the intensities. The ionospheric critical penetration frequency, foF_2, was on a number of occasions less than 1 Mc/s and many records were obtained which reproduced the galactic sidereal profile.

Fig. 2 shows the spectra of the maximum and minimum galactic intensities. observed near 1900 R.A. and 0400 R.A., respectively. The spectrum of the maximum at frequencies less than 10 Mc/s should be interpreted with caution since high-resolution studies at 4·8 Mc/s (to be reported elsewhere) have shown much fine detail near the plane of the Milky Way. This spectrum represents an average over bright and dark regions. The spectrum of the minimum. on the other hand, is not affected significantly by spatial averaging.

Also shown in Fig. 2 are the corresponding spectra obtained in 1956[1]. It may be seen that the present spectra agree well between 2 Mc/s and 10 Mc/s. However, it now appears that the intensity decreases more below 2 Mc/s than was previously thought. In particular the 900 kc/s intensity was obtained in 1956 under rather unusual ionospheric conditions and may have to be revised in a downward direction when further results become available. It should be noted that the spectral maximum occurs at a higher frequency near the plane of the galaxy than at high galactic latitudes. In addition, at frequencies less than the spectral maximum, the intensity varies very nearly as the square of the frequency. Both of these effects may be explained in terms of absorption of the radiation in interstellar ionized gas. Their theoretical implications are discussed elsewhere[3].

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METEOROLOGY

Noctilucent Clouds in Alaska during 1962

Since the first reported sightings of noctilucent clouds in 1885, there have been many subsequent observations of them from latitudes ranging between 45° and 63·3°. The majority of these observations have been made from north-western Europe and the U.S.S.R. There appear to be only three previously reported observations of noctilucent clouds from the North American continent: two of these from Canada and one from Alaska. Vestine's observation[1] of the clouds from Meanook, Alberta (54° 37′ N., 113° 20′ W.), on July 20–21, 1933, was the first reported sighting from North America. The second reported sighting from Canada[2] was made on the night of July 23–24, 1961, from Saskatoon, Saskatchewan (52° 08′ N., 106° 38′ W.). The one reported Alaskan sighting[3] was made from Anchorage (61° 10′ N., 149° 59′ W.) on July 27–28, 1957. The lack of noctilucent cloud observations at latitudes higher than 63·3° (the latitude of Torsta, Sweden) was attributed by Ludlam[4] to the lack of keen observers at these latitudes.

Following a suggestion by Prof. Sydney Chapman, a watch for noctilucent clouds was kept this summer here (64° 53′ N., 148° 03′ W.) to determine whether the clouds could be seen at higher latitudes than previously observed. On the night of August 13–14, 1962, a noctilucent cloud display was observed from College by four independent observers The clouds were first seen at approximately 2330 A.S.T. (Alaskan Standard Time, 150° W. Meridian Time) on August 13, and were observed until 0030 A.S.T. on August 14. The display, exhibiting the characteristic billow structure, was most intense shortly after midnight at which time the clouds extended in elevation up to 11° with the azimuthal extent of the clouds being approximately 25° west to 25° east of true north. A photograph of the display seen on that night is shown in Fig. 1. This picture was taken by Mr. Yngvar Gotaas on Kodochrome film at an exposure of 90 sec at *f* 5·6. Two subsequent noctilucent cloud displays, faintly visible through the cloud cover, were seen and photographed from College, Alaska, on the nights of August 14–15 and August 16–17. The display on August 14–15 was also observed at Ft. Yukon[5] (66° 34′ N., 145° 18′ W.), and the display on August 16–17 was seen from Galena[6] (64° 46′ N., 156° 54′ W.).

Noctilucent cloud displays were also observed by the Weather Bureau personnel at Anchorage on two occasions this year[7]. Their first sighting was on May 6–7, 1962, 0005–0050 A.S.T. This display was visible up to 7° above the horizon. At the beginning of the display the azimuth extent of the clouds was 303°–009°, and toward the end of the display the azimuthal extent of the clouds was 021°–071°. Their second sighting was on the night of August 11–12, 1962. This display was first seen at approximately 2300 A.S.T. and lasted until 0115 A.S.T. The elevation angle of the upper border of the display was about 10°, and the clouds extended from north-north-west to north-north-east, approximately. According to Mr. John Gardey[7] of the Anchorage Weather Bureau, the clouds are seen at least once every year from Anchorage.

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PHYSICS

Bath-Tub Vortex

Perennially one hears controversy regarding the direction of the vortex in the drain of the bath-tub or the kitchen sink. Some claim that the direction of swirl is always the same in the northern hemisphere, and that in the southern hemisphere it is always opposite to that for the northern hemisphere. Others claim that there is no unique direction of swirl in either hemisphere.

Both schools of thought are in some sense correct. For the everyday observations of the kitchen sink and bath-tub variety, the direction of the vortex seems to vary in an unpredictable manner with the date, the time of day, and the particular household or experimenter. But under well-controlled conditions of experimentation, the observer looking downward at a drain in the northern hemisphere will always see a counter-clockwise vortex, while one in the southern hemisphere will always see a clockwise vortex.

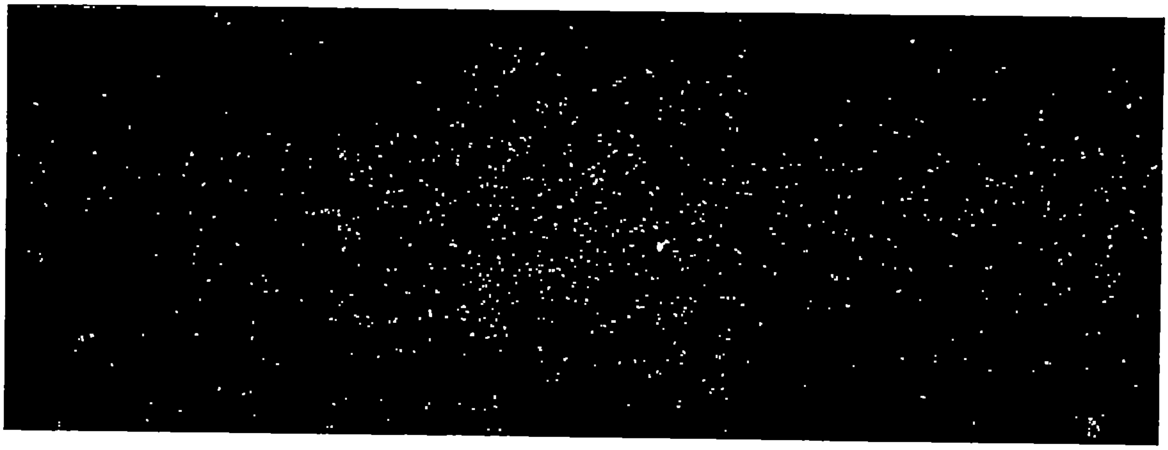

Fig. 1. Photograph of noctilucent clouds taken from College, Alaska, at 00 : 06 : 30 A.S.T. on August 14, 1962

In a properly designed experiment, the vortex is produced by Coriolis forces, which are counter-clockwise in the northern hemisphere and clockwise in the southern hemisphere.

There are two reasons for the apparent haphazard results of the everyday observations. The effective rim speed of a tank 2 ft. in diameter rotating with the Earth at Boston, Massachusetts, is only about 0·04 in./min. This tiny Earth-induced motion may be much less than other motions too small to be perceived by ordinary observation. Unless the water in the vessel is allowed to settle for many hours, for example, the residual motions from the filling of the vessel may far exceed the foregoing figure. Moreover, the very act of pulling the plug from the bottom of the vessel may inadvertently create substantial velocities. Even if many hours are allowed for settling, air currents in the room or thermal currents associated with non-uniform temperatures around the vessel may easily cause water velocities larger than that due to the Earth's rotation. All these difficulties are quite real; this was amply proved by early experiments with the apparatus described later.

The second source of confusion in the casual everyday observations is that the Coriolis forces due to the Earth's rotation may in fact be much smaller than other forces normally present. For a speed of 0·1 in./sec toward the drain, the Coriolis force at latitude 42° north is about $3(10)^{-7}$ times the force of gravity. If the vessel is not perfectly symmetrical about the axis of the drain, the asymmetrical viscous forces at the side walls and at the bottom of the vessel may produce torques larger than that due to the Coriolis force; the direction of this asymmetrical torque would, of course, vary from vessel to vessel. Even the extremely small forces of surface tension at the free surface may, owing to non-uniformities in temperature or chemical impurity, lead to torques.

A meaningful experimental demonstration of 'the bath-tub vortex' must be designed to cope with the difficulties mentioned here. Such an apparatus was made and operated near Boston for an educational motion picture entitled *Vorticity*. The tank is circular, 6 ft. in diameter and 6 in. high, with a flat bottom. The drain hole, 3/8 in. in diameter, is centrally located and flush with the bottom. A length of hose some 20 ft. long leads the water from the drain to a sump. A 'vorticity float' makes the vortex visible. This is a cross made of two slivers of wood, each about 1 in. long, pinned together with a fine wire through the centre of each. The cross floats in a horizontal plane just below the surface of the water, and a small length of wire extending above the surface of the water provides the necessary floating stability.

Before starting the experiment the end of the long hose is closed with a plug. The tank is then filled nearly to the brim with water swirling clockwise. This direction is deliberately chosen to test whether sufficient time has elapsed for adequate decay of the original motion, so that one may be confident that the swirl seen in the experiment really arises from Coriolis forces rather than from initial swirl not yet sufficiently dissipated. After filling, the tank is covered with a plastic sheet to eliminate the effect of air currents, and the room is maintained at nearly uniform temperature. About 24 h are required for settling of the motion to a degree where the residual motions are unimportant compared with those due to the Earth's rotation. The plug is then pulled from the end of the long hose and the vorticity float is delicately inserted above the drain hole. About 20 min are required for the tank to drain completely. During the first 12–15 min no rotation of the float is perceptible to the eye. At about 15 min, however, the counter-clockwise rotation of the vorticity float becomes distinctly perceptible, and gradually increases. Near the end of the experiment, when the vessel is almost completely drained, the vorticity cross turns one revolution in about three or four seconds. This is about 30,000 times greater than the effective speed of rotation of the Earth at latitude 42° N. It agrees in order of magnitude with what one would calculate theoretically on the assumption that the rotation of the float is indeed due to the Coriolis forces of the Earth.

When all the precautions described were taken, the vortex was invariably in the counter-clockwise direction.

If the plug were pulled after only about 1–2 h, the vorticity cross would begin to spin clockwise within 1–2 min after the plug was pulled, and would reach a rate much greater than the rate of counter-clockwise spin for the correct experiment. This was because the angular velocity remaining from the clockwise filling action, although not readily perceptible, was nevertheless greater than that of the Earth.

In one experiment the original clockwise swirl was allowed to settle for four or five hours before pulling the plug. An unusual result was observed. During the first two-thirds of the draining period, the vorticity float turned in the clockwise direction owing to the angular momentum of the initial swirl. After having reached a large clockwise angular velocity, the float began to slow down, ultimately came to rest, and then proceeded to accelerate in the counter-clockwise direction. This may be explained as follows. The water acquires the rotational velocity of the Earth through the diffusion of a viscous shear layer, mainly from the bottom of the tank. This viscous boundary layer on the bottom, in which the water has acquired the counter-clockwise angular velocity of the Earth, grows thicker with time. Above this boundary layer the water still retains the clockwise angular velocity associated with the initial swirl. When the vorticity float sinks to the appropriate level, it tends to reverse direction.

I am prepared to provide precise particulars of my apparatus to anyone in the southern hemisphere who wishes to perform an identical experiment for the sake of convincing any remaining doubters.

Incidentally, those who claim to have seen the direction of swirl change as a ship crosses the equator are surely pressing the case too far. At the equator the Coriolis forces vanish, and it would be virtually impossible to perform a valid experiment a short distance from the equator.

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Solution of Boundary Value Problems in Plane Strain

THIS method replaces direct solutions of the Airy relation by, in effect, linear combinations of solutions of this equation for the edge dislocation. So far its use has been investigated only in connexion with the stress fields of dislocations in finite bodies; it may well be that it is useful in other connexions as well.

This technique is limited to two-dimensional representation and is as follows: the finite body is replaced by an infinite one and the free surfaces by cuts (lines) in this body. The conditions that certain normal and shear stresses must vanish over the surfaces can then be satisfied by allowing the surfaces to deform. This representation is well known[1]. However, this deformation can be represented by two mutually independent arrays of infinitesimal edge dislocation on each cut, distributed in such a way that the required stresses vanish over the cuts. The conditions on these distributions may be represented by singular integral equations which can be solved in a number of cases of interest.

Once the various distributions have been found, the stresses are obtained by direct integration of the effect of all the infinitesimal dislocations invoked.

As a first exercise in its use, this method has been used to find the displacements in a free surface when an edge dislocation lies parallel to it. The stresses obtained by integration over the (two) distributions of infinitesimal dislocations are identical with those obtained by Head[2] using another method.

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Strengths of Sapphire Crystals

The strength of single crystals of entirely brittle materials should depend on the presence of stress raising flaws. Internally these could be formed by static dislocation arrays, voids or inclusions of foreign material. On the surface the flaws could be formed by mechanical damage or by surface steps as suggested by Marsh[1].

Surface damage appears to be the major factor influencing the strengths of fused silica and silicate glasses, and it was felt that this factor might also exert a considerable influence on the strength of brittle crystalline materials. Indeed, Dash[2] has already observed and reported the high strength obtainable with polished and etched single crystals of silicon.

Sapphire single crystal rods about ¾ mm in diameter (obtained from the Linde Co.) were flame polished by being rotated in, and traversed slowly through, an oxy-coal gas flame. The rods were then loaded to fracture in pure bending at room temperature, the applied couple measured and the maximum stress at the rod surface calculated by simple bending theory. The material appeared to be entirely elastic and frequently shattered completely at fracture.

The techniques used in flame polishing, handling and applying the bending moment were developed for the preparation of strong silica samples of similar dimensions and will be described in detail elsewhere. The main feature of these techniques was the great care taken to prevent any damage to the flame polished surface and to this end the bending couple was applied via 'Araldite' plugs cast around the rod ends. An 'Araldite'-free test length of several millimetres was left in the centre of the rod.

Fig. 1 shows the result of the bending tests. The strengths recorded after polishing sometimes were an order of magnitude greater than those of the 'as received' rod ranging from nearly 400,000 lb./sq. in. to over 1,000,000 lb./sq. in. The higher strengths are comparable with the strengths of alumina whiskers reported recently[3].

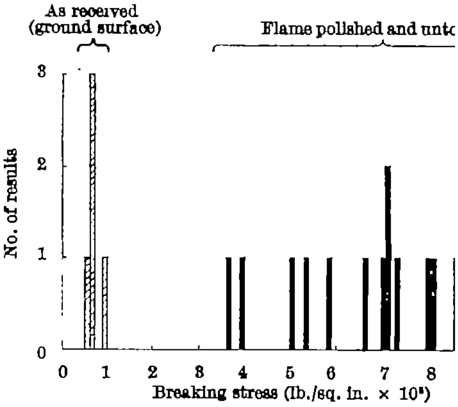

Fig. 1. Bending strengths of sapphire single

The results indicate that high streng
observed in quite large single crystals of
the effects of surface damage are removed
treatment. The phenomenon appears to b
to the behaviour of silica and silicate gl
strengths are almost wholly dependent
conditions.

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ENGINEERING

Arc Extinction in a Low-Pressure Pool Valve by a Slowly Forming

Spontaneous extinction of the arc in
pool valve has recently been given sor
attention[1]. An apparently novel type
tinction was observed when a particula
measurements was made of the values of
parameters at various instants in the
repetitive current pulse passing in the l
mercury pool valve referred to in another c
tion[2]. The valve carried half-period sinuso
pulses of a crest value of 18·3 A at a fr
50 c/s, with the cathode temperature as a
at θ_k = 25, 35, 45 and 55° C. The m
for plasma measurement is indicated in
communication[2].

During the measurements with $\theta_k = 25$
suddenly extinguished but was immediatel
by manual operation of the ignition me
the valve, and the measurements were
The curves of Fig. 1 show the results of m
of the plasma space voltage V_p, as a func
instant t in the shape of the current pul
the valve with $t = 0$ denoting the start c
soidal half-period. The deviation from no
curve at θ_k = 25° C indicates the effect on
the times preceding arc extinction and fo
re-ignition, the intervals between two
V_p, being of the order of about 2 min. Tl
in the arc leading to its extinction thus t
minutes to build up, and the after-effec
re-ignition took somewhat longer to disa
significant deviation from normal was

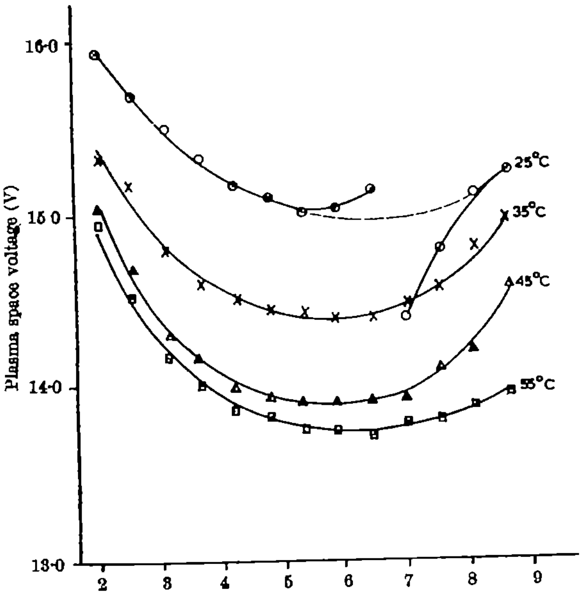

Fig. 1. Plasma space voltage as a function of the instants in the shape of the periodic current pulse passing in the valve

occur in the corresponding curves of electron density, electron temperature and arc voltage drop.

The sudden extinction of the arc was a unique phenomenon in a very extensive series of measurements, and it could not be caused to occur again. Therefore no indication can be given on the cause or on the possible mechanism of extinction of the arc.

The work was carried out in the Electrical Engineering Department of the Manchester College of Science and Technology.

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METALLURGY

250° C Internal Friction Peak in Iron

An internal friction peak, which occurs in iron at temperatures of about 250° C and frequencies of the order of 1 c/s has been the subject of some speculation[1]. The purpose of this communication is to suggest that the cause of the peak is diffusion of oxygen in iron.

According to the most recent measurements[1,2], the activation energy of the peak is 32–35 kcal/mol. Although the activation energy of diffusion of oxygen in α-iron has not been measured directly, it can be obtained from the Cottrell equation[3] for serrated yielding in a tensile test:

$$D = 10^{-9}\,\dot{\varepsilon} \tag{1}$$

where D is the diffusivity and $\dot{\varepsilon}$ is the strain-rate. Equation (1) has been verified for a number of body-centred-cubic metals[4]. Boulanger[5] has found serrated yielding in iron at a strain-rate of 6×10^{-3} sec^{-1} and a temperature of 325° C. Therefore:

$$D_0 \exp[-\Delta H/RT] = 6 \times 10^{-12} \tag{2}$$

For diffusion of interstitial atoms in body-centred-cubic metals, D_0 is typically 10^{-2} cm^2/sec (ref. 6); and ΔH, the activation energy for diffusion of oxygen, becomes 28 kcal/mol. Considering that Boulanger[5] reported the lowest temperature at which serrations appeared and not the temperature of the maximum effect, the value of 28 kcal/mol represents a lower limit on the activation energy for diffusion of oxygen in iron, and the agreement between this value and the activation energy of the internal friction peak is good. Therefore, it seems likely that the internal friction peak is caused by diffusion of oxygen.

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CERAMICS

Influence of Surface Area on the Density of Quartz heated at 1,300°–1,500° C

During an investigation of silica brick it soon became apparent that the extent of the thermal alteration of quartz was dependent on its initial particle size. A search of the literature revealed only a few fragmentary pieces of information[1–5]. Most of this information merely compared the extent of alteration of two fractions, a fine and a coarse, and stated that the finer fraction is altered to the greatest extent. The finer fraction was coarse compared with the quartz used here. Therefore a systematic, qualitative investigation of the influence of the initial surface area of quartz on the extent of its thermal alteration, as measured by density changes and X-ray diffraction, was undertaken. The purpose of this communication is to emphasize graphically the extent of this surface area effect.

For this work, well-crystallized α-quartz samples were obtained from Illinois and Pennsylvania producers. Spectrographic analysis indicated that the upper limits for lithium, molybdenum, sodium, barium and manganese were 0·001 per cent; for titanium, magnesium, calcium, iron and potassium, 0·01 per cent; and for aluminium 0·1 per cent. The surface area of each fraction was measured by the Brunauer–Emmett–Teller nitrogen adsorption method. 4-g samples of each fraction were heated in a platinum boat in a tube furnace in a current of water-saturated air (saturation temperature 30° C) flowing at 0·10 ft.^{3}/min. The samples were inserted into the furnace and removed while it was maintained at the selected testing temperature. Exposure times for each fraction ranged from 0·5 to 15 h. Density determinations were made at 35° C by the pycnometer method with *m*-xylene as the displacement liquid. Duplicate determinations agreed to ± 0·005 g/cm^3.

Figs. 1 and 2 clearly show that, for equal heat treatments, quartz samples of larger initial surface area exhibited lower densities. From X-ray diffractometer counting measurements an increase in cristobalite and decrease in quartz content was always observed when the density decreased. When the density of cristobalite (2·33 g/cm^3) was neared, no quartz could be detected. At temperatures above 1,400° C the surface effect tapers off above 1–2 m^2/g. However, at the lower temperatures the surface influence increases up to at least 5 m^2/g.

No tridymite formation was detected in any sample. The observed absence of tridymite in heat-treated, high-purity silica agrees with the findings of others[6-8].

Chaklader and Roberts[4] and Roberts[3] point out that the quartz transformation occurs as consecutive reactions: quartz→transition phase (amorphous)→ cristobalite. They found considerable amounts of the transition phase in their heat-treated quartz samples, since the rate of its formation was greater than that of its disappearance. Mackenzie[5] observed that the direct solid–solid transformation of quartz to cristobalite was equally possible.

If the rate of formation of the transition phase is greater than its disappearance, as Roberts has shown, then the decrease in density observed in Figs. 1 and 2 is due to the formation of cristobalite and transition phase mixtures. However, if the rates of appearance and disappearance are equal, as Mackenzie suggests, then the change in density is due to the formation of cristobalite alone. Regardless of the mechanism, density values still indicate the relative influence of surface area on the extent of thermal alteration. This is true since the density of cristobalite (2·33 g/cm^3) and the transition phase (2·30 g/cm^3)[3] are of the same magnitude, and a mixture of these two would have a density very nearly that of cristobalite alone.

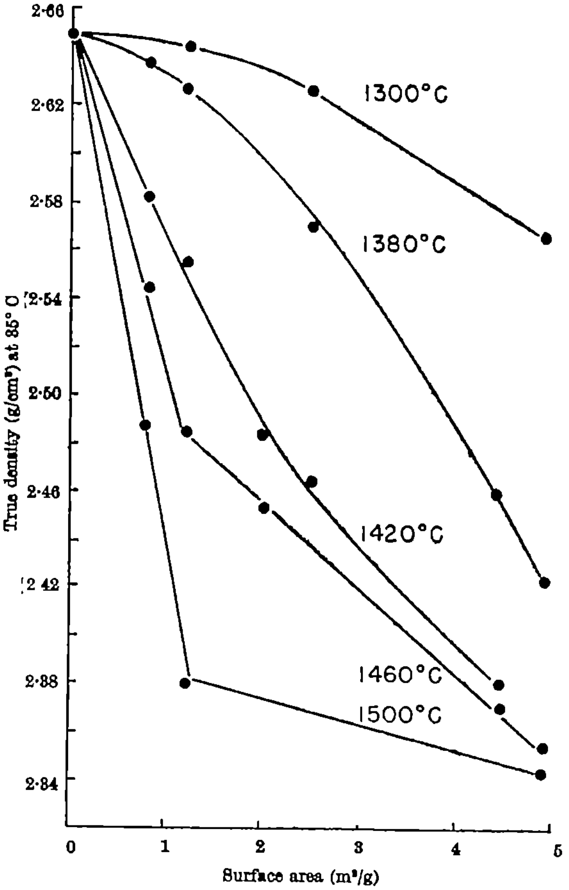

Fig. 1. Influence of the initial surface area of quartz on the true density after 2 h at the indicated temperatures

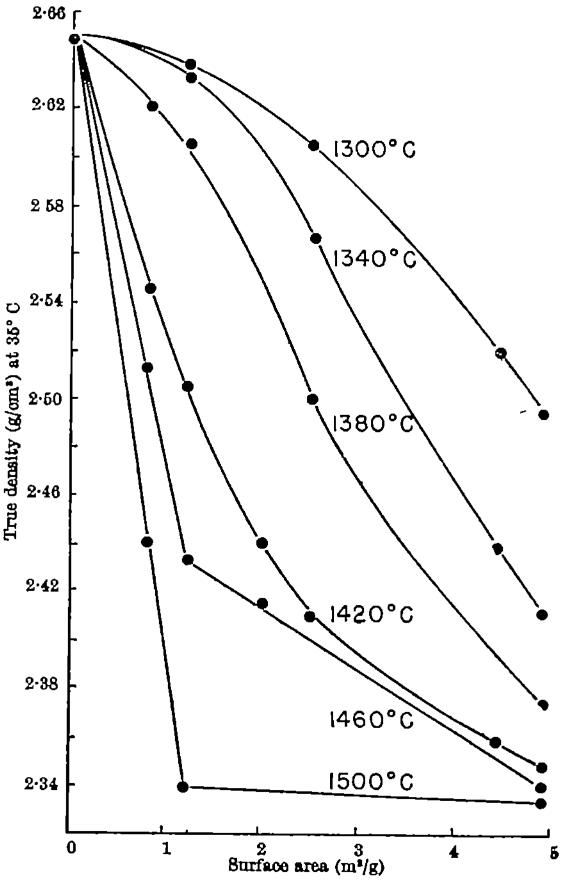

Fig. 2. Influence of the initial surface area of quartz on the true density after 4 h at the indicated temperatures

Furthermore, Roberts[3] states that for finely divided quartz the growth and subsequent decay of the transition phase becomes almost identical. It is possible to conclude from this statement that both mechanisms may prevail: direct conversion for high surface samples and indirect for samples of lower surface.

The major impurity in the quartz used here is aluminium. This probably comes from clay impurities. Grimshaw *et al.*[2] have shown that the presence of 0·7 per cent of Al_2O_3 greatly retards the transformation. Therefore, the Al_2O_3 in our samples, although in a much smaller concentration and chemically combined with silica, may slightly influence the results, but in no way negate them.

This work was carried out while I was associate chemist, Illinois State Geological Survey, Urbana.

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that “much of the so-called greywacke of the Siwaliks may, in fact, turn out to be sub-greywacke (the low-rank greywacke of Krynine) if a proper petrographic study is made. If this is the case [they say], the notion that the Siwaliks are entirely of freshwater origin may still be true, because the sub-greywackes are neither necessarily marine nor are they confined to the geotectonic belts”.

Greywacke was first described from the Siwaliks by Krynine[3], who fully accepted the palæontological evidence for a non-marine environment of deposition. The Siwalik greywackes are referred to and illustrated in Pettijohn's well-known text-book[4]. The idea, now commonly held, that greywackes are restricted to a marine environment in the orogenic belts is of quite recent origin; it is founded on hypothesis, and is contrary to observation. Examples of non-marine greywackes are to be found in the Old Red Sandstone of Lanarkshire, Scotland[5], the Pennsylvanian of Rhode Island and Massachusetts[6], and in the Eocene of Wyoming[7].

Sedimentary and tectonic environments are only two among many factors which affect the petrography (texture and composition) of sandstones. Klein[8] has recently emphasized the importance of provenance and Dapples[9] has stressed the possible effects of diagenesis. There is much evidence to suggest that greywacke (sandstone with a ‘muddy’ matrix) results from the post-depositional alteration of ‘normal’ (clean or mud-free) sand of suitable initial composition[10].

The sandstones of the Siwalik Series are characterized by a large content (35–40 per cent) of schist and phyllite fragments, and vary little in composition through a thickness of 20,000 ft. of strata. Among these sandstones, greywackes are in the minority and are only recorded from the lowest division of the Siwalik Series[3]. The inference is that all the Siwalik sandstones were deposited as ‘normal’ sands and some of the oldest of them were converted by pressure and temperature, resulting from deep burial, to greywackes[10].

The mere presence of greywacke cannot be accepted as evidence of marine sedimentation. Petrography, as a guide to depositional environment, is very much inferior to the careful use of fossils and sedimentary structures.

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The experimental techniques used in this investigation have been described elsewhere[2,3]. The accuracy and precision of the method were determined by periodic analyses of an interlaboratory strontium standard. The results of these analyses agreed closely with those reported by others, giving confidence in the accuracy of the technique. The reproducibility of measurement of the ^{87}Sr/^{86}Sr ratio was calculated from 18 analyses of the standard to equal ± 0·0015 (0·2 per cent). The measured ^{87}Sr/^{86}Sr ratios were normalized to ^{86}Sr/^{88}Sr = 0·1194 as described by other workers[2].

In order to interpret the data it was first necessary to learn something about the effect of diffusion of strontium-87 on the ^{87}Sr/^{86}Sr ratios of carbonate xenoliths. A completely recrystallized xenolith of Trenton limestone at Mt. Royal, Quebec, about 18 × 24 in. in size, was used for this purpose. X-ray fluorescence analyses revealed that the xenolith contained approximately one-half as much strontium as the igneous rock surrounding it. ^{87}Sr/^{86}Sr ratios were measured in the xenolith, in the igneous rock, and in a sample of unaltered Trenton limestone. The xenolith was found by a duplicate analysis to have preserved the ^{87}Sr/^{86}Sr ratio of the Trenton limestone, 0·712, even though surrounded by a relatively strontium-rich magma with a ^{87}Sr/^{86}Sr ratio of approximately 0·708. Since measurable diffusion of strontium-87 did not occur in a xenolith of this small size it seems very improbable that it could have been effective in bodies of carbonate rock the size of most carbonatites.

^{87}Sr/^{86}Sr ratios were measured in both carbonatites and alkaline rocks from the complexes at Oka, Quebec; Magnet Cove, Arkansas; Iron Hill, Colorado; and Rocky Bay, Montana. An example of the results of these analyses is given in Table 1.

An average ^{87}Sr/^{86}Sr ratio for Grenville carbonates and celestites was calculated from the available data[1,2–5]. The results are given in Table 1. The Oka carbonatite has been considered to be a xenolith of Grenville marble by some authors[6,7]. The results in Table 1 show that the ^{87}Sr/^{86}Sr ratio of the Oka carbonatite is identical within the analytical precision to those of the alkaline rocks of the complex and is distinctly different from those of the Grenville rocks. This indicates that the Oka carbonatite is not a xenolith of Grenville marble but rather that it is comagmatic with the igneous rocks of the complex. Additional evidence to support this conclusion is found in the fact that a rubidium–strontium age determination on a biotite from the Oka carbonatite yielded a Mon-

Table 1. STRONTIUM ISOTOPE RATIOS IN THE OKA COMPLEX, QUEBEC

Rock type	^{86}Sr/^{88}Sr	^{87}Sr/^{86}Sr
Carbonatite*	0·1195	0·7062
Okaite	0·1196	0·7065
Ijolite	0·1197	0·7059
Alnoite	0·1204	0·7074
Average for five Grenville carbonates and celestites		0·7102

* Average of three analyses, two of which were reported by Faure and Hurley (ref. 2).

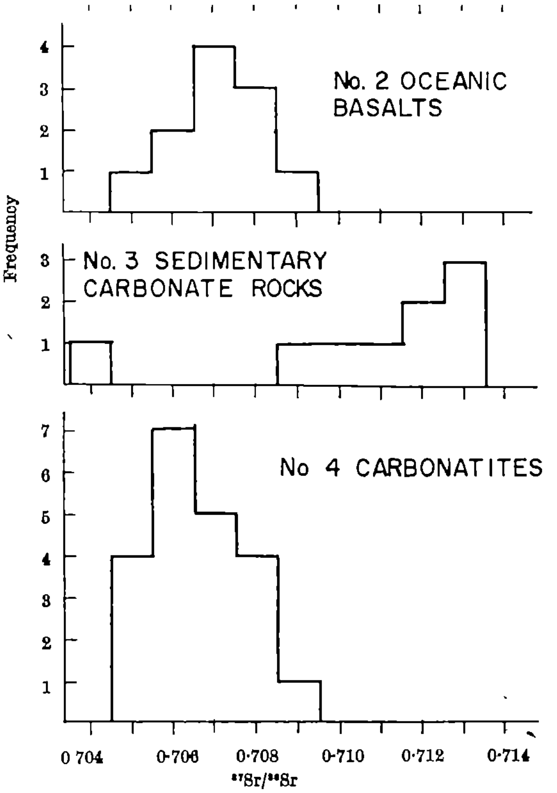

Fig. 1. Frequency distribution of ^{87}Sr/^{86}Sr ratios in various rock types

teregian age of 100 million years rather than a Grenville age of around 1 billion years[3]. The ^{87}Sr/^{86}Sr ratios of the carbonatites in the other complexes which were examined were also found to be identical within the analytical precision to those of the associated alkaline rocks.

Duplicate ^{87}Sr/^{86}Sr ratio determinations were carried out on the Spitzkop carbonatite and on the Transvaal dolomite from which this carbonatite is supposed to have been derived[8]. The ^{87}Sr/^{86}Sr ratio of the carbonatite was found to equal 0·7058 ± 0·0010 and that of the dolomite 0·7129 ± 0·0010. This evidence confirms the conclusion of other writers[9,10] that the Spitzkop carbonatite is not a mobilized xenolith of Transvaal dolomite.

^{87}Sr/^{86}Sr ratios were measured in a total of 21 carbonatites. The results of these analyses are compared in Fig. 1 with the data reported by Faure and Hurley[2] for basalts and with the data available for sedimentary carbonate rocks[1,3-5]. The mean ^{87}Sr/^{86}Sr ratio of the carbonatites was calculated to equal 0·7065 ± 0·0003.

The available experimental evidence indicates that migration of strontium-87 in carbonate xenoliths is negligible, and as shown in Fig. 1 the ^{87}Sr/^{86}Sr ratios

The spread observed in the ^{87}Sr/^{86}Sr ratios of the carbonatites is only about three times the reproducibility and is similar to the spread in the ratios of the 25 basalts measured by Faure and Hurley[2]. This appears to indicate that the source material of carbonatite is as homogeneous in its ^{87}Sr/^{86}Sr ratio as is the source material of basalt magma. In addition the ^{87}Sr/^{86}Sr ratios of the carbonatites and the associated alkaline rocks are lower than those of rocks usually found in the sial. This result supports the conclusion that the source material of these rocks is located beneath the sial.

The conclusion that carbonatites are not xenoliths of limestone does not necessarily imply that they do not contain resurgent carbon dioxide. For example, consider a magma of subsialic origin which completely dissolved a limestone and produced a carbonatite by differentiation. A carbonatite formed in this way would contain resurgent carbon dioxide, yet its ^{87}Sr/^{86}Sr ratio could be nearly the same as that of the primary magma. If carbonatites in general are formed in this way their ^{87}Sr/^{86}Sr ratios should be found to equal those of the most probable primary magma type, basalt. Yet as shown in Fig. 1 the ^{87}Sr/^{86}Sr ratios of carbonatites appear to be slightly lower than those observed in basalts. This result, if confirmed by more analyses of basaltic rocks, would indicate that carbonatites contain juvenile rather than resurgent carbon dioxide. In addition it would indicate that carbonatites are not formed by differentiation of basalt magma.

In summary, our results provide quantitative evidence which indicates that carbonatites are not xenoliths of limestone but are comagmatic with the alkaline rocks with which they are associated. The results support, but do not prove, the conclusion that carbonatites contain juvenile carbon dioxide and that they are derived from a homogeneous subsialic source which is not basalt magma.

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GEOCHEMISTRY

Cyanogen Band Suppression in Direct-Current Spectrographic Analysis

THE violet cyanogen band sequences render the region 3500–4200 Å of the spectrum of little use for analysis due to excessive interference, yet sensitive lines of many elements fall within this range[1]. When carbon or graphite electrodes are used there are two methods whereby this interference may be reduced. The first makes use of the fact that band intensity is enhanced by high arc temperatures, and consequently the phenomenon of 'selective volatilization' is utilized, the plates being exposed only during the first part of the arc burn. In this period all the more volatile elements come off and the arc temperature is depressed. The procedure was described in detail by Ahrens and Taylor[2]. The second method is to prevent the emission of cyanogen bands by arcing in an inert atmosphere. The procedure described here uses this principle and is specifically designed to involve no increase in time over normal rapid spectrographic methods.

The apparatus is very simple both in design and construction. As described, it will fit the standard Hilger and Watts routine arc and spark stand *FS*.55 and the simplest modification will adapt it for use with any similar apparatus.

The modified Stallwood jet[3] used is shown in Fig. 1. All parts are of brass, heavily chromium-plated after construction. The apparatus was made here, a simple lathe being used for both jet and stand. The body *A* is made in two pieces, silver soldered together, to which the gas inlet tubes *D* are similarly attached. The latter are bent round and joined, by means of a simple T-junction, to a single tube of the same diameter some 17 cm in length. The gas mixture is supplied via a flow-meter and short length of rubber tubing from a single cylinder of pre-mixed gases: the latter is used to overcome mixing and control difficulties. The jet unit is held in position by a second horizontal tube, some 6 cm in length, attached by a vertical thread to the Petrey table mounting of the stand. The unit can slide horizontally within the mounting to allow for thermal expansion. The vertical thread is not locked in position on the mounting, hence all the controls provided by the stand unit are unimpaired. The electrode *B* is fitted with a silica collar *C*, made from standard 'Vitreosil' tubing This can be made to fit tightly, or a shoulder turned on to the electrode to allow the collar to sit in position. The unit of collar and electrode moves vertically with respect to the jet during burn, the collar being incorporated partly for insulation. Similarly the Petrey table mounting is insulated from the arc stand case.

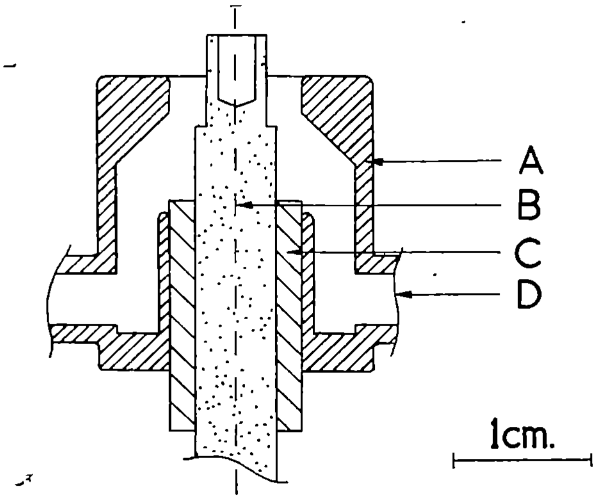

Fig. 1

Fig. 2 shows three sample spectra of identical exposure (3 min at 7·5 amp) reproduced directly from an Ilford *N*.30 plate. All are from the same powdered rock specimen containing approximately 0·1 per cent molybdenum. The region chosen is that around cyanogen band (head) 3883 Å, the most intense sequence. Case (*a*) shows the spectrum resulting from a normal arcing in air. Cases (*b*) and (*c*) are spectra produced by the apparatus described here, under different rates of gas-flow, 2 and 4 l./min respectively. Two sensitive molybdenum lines, Mo. 3,798.252 and Mo. 3,864.110, are identified for comparison.

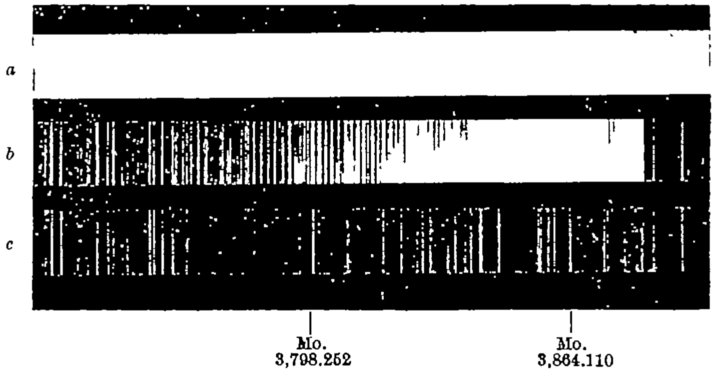

Fig. 2

The gas mixture chosen was argon/oxygen, the choice partly influenced by the cost and availability of the gases in question. By experiment a ratio of 80 per cent argon : 20 per cent oxygen was found to give line intensities strictly comparable with those in air for identical exposures. The critical flow-rate for complete cyanogen band suppression, using this mixture, was found to be 4 l./min. Annell and Helz[3] concluded that, after a comprehensive study of the properties of a direct current arc in different gases and mixtures, similar mixtures of argon and oxygen were ideal

An inert atmosphere, as well as removing cyanogen band emission from the spectrum, may possess other advantages when used in spectrochemical analysis. The work of Vallee, Reimer and Loofbarrow[4], and also Shaw, Wickremansinghe and Yip[5], indicated that the intensity of certain atom and ion lines is enhanced under these conditions with respect to air.

In their work, Shaw *et al.* also describe an apparatus developed for cyanogen band suppression using inert atmospheres. This used a partially enclosed chamber surrounding a Stallwood jet. The work included a study of the behaviour of volatile elements in an arc surrounded by an inert atmosphere, and demonstrated the value of the method in this field also. The apparatus described in the present paper was designed primarily for involatile elements analysis, where the large percentage of carbon normally mixed with the charge and the necessity for complete burn together greatly enhanced cyanogen band formation. The most important advantage of the technique described is that there is no increase in operational time, since the only departure from standard practice is the addition of silica collars after electrode preparation, just prior to arcing. The advantages of the remotely controlled, enclosed, arc stand are fully maintained. Contamination risks are slight since the jet does not come into actual contact with the electrodes, and also it may easily and quickly be removed for cleaning. The silica collars are cleaned between use by acid treatment.

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RADIOCHEMISTRY

Rapid Determination of Tungsten in Steel by Neutron Activation and γ-ray Spectrometry

A NEUTRON activation analysis method has been devised for the non-destructive, rapid determination of tungsten in steel

In the present work, varying amounts (0·01–0·1 g) of a sample to be analysed are placed by turns in the nuclear reactor *JRR*-1 for 4·5 sec to be irradiated with neutrons at a flux of 3×10^{11} neutrons per cm^2/sec and then the γ-ray spectra and the decay of nuclide formed are observed by use of a 2-in. thick, well-type sodium iodide crystal attached to a 256-channel pulse-height analyser, the sample being placed at about 1 cm above the well together with its container. Typical spectrograms thus obtained are shown in Fig. 1, the shapes of which have been found to be analogous to those obtained from a standard sample (tungstic acid powder). From the successive spectra (Fig. 1, *A*–*D*) the half-life of nuclide formed can be determined to be about 5·5 sec. Tungsten is thus identified from both spectra shape and half-life. Its amount can be determined by the comparative method as usual.

From Fig. 1, which represents a sample of 2·0 per cent tungsten content, it can be deduced that one-tenth of this concentration of tungsten would produce an adequate intensity of γ-rays. Since good results are obtained when a sample taken involves about 1 mg of tungsten, the higher the tungsten concentration, the smaller the preferable amount of the sample.

The coefficient of variation of single analyses can be deduced (from a similar experiment[1] in which ytterbium was determined by use of the formation of 6-sec ^{m}Yb) to be about 5 per cent if the activity level is suitable to the analyser used and interferences are negligible.

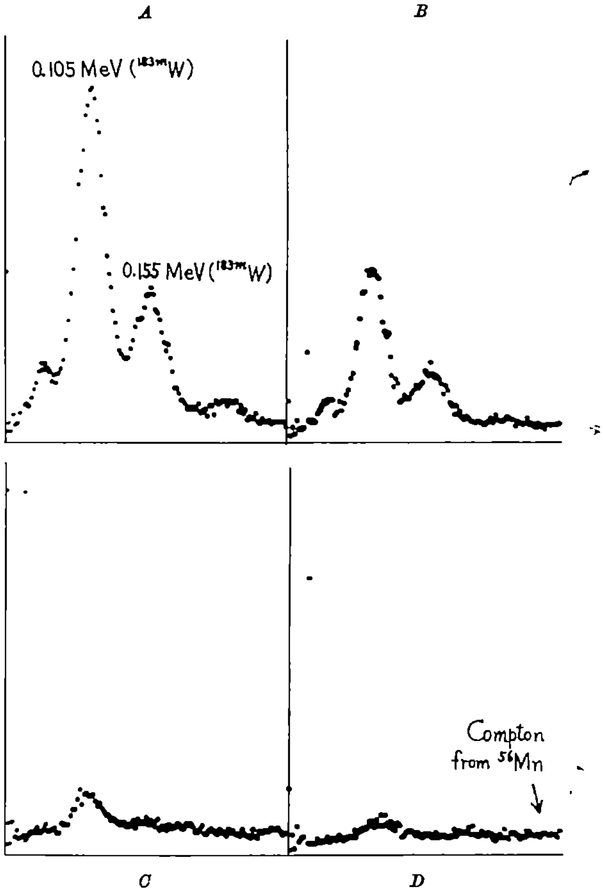

Fig. 1. Successive spectra for γ-rays of a steel sample (70·7 mg) irradiated for 4·5 sec. *A*, at 4 sec after irradiation (the pulse-height analyser was operated for 5 sec); *B*, at 10 sec (for 5 sec); *C*, at 27 sec (for 10 sec); *D*, at 47 sec (for 10 sec). The composition of this sample: Fe, 96·3; W, 2·0; C, 0·6; Mn, 0·4; Cu, 0·3; Si, 0·3; Cr, 0·12; Ni, 0·06

Possible interferences have been investigated by use of a graphical method[2] and of the effective activation cross-section of ^{182}W obtained in this work. Table 1 shows the amounts enough to interfere, at several sec after 4·5-sec irradiation, through γ-rays of nearly decuple the intensity of that from 1 mg of tungsten.

Table 1. AMOUNT REQUIRED TO EMIT γ-RAYS OF THE INTENSITY OF 10 TIMES MORE THAN THAT DUE TO 1 MG OF TUNGSTEN

Ag	1 μg	Eu	1 mg
Dy	0·01 mg	Cu	3 mg
Rh, Sc, V	0·02 mg	Ti	0·02 g
Se, In	0·03 mg	Mo	0·1 g
Er	0·1 mg	Ni	1 g
Al, Co	0·2 mg	S, Cr, Si, Fe, P, C	>1 g
Mn	0·3 mg		

I thank Prof. Shoji Makishima for advice, Dr. Fumio Aoki for his interest and Dr. Toshio Nakai, Dr. Yuichiro Kamemoto and Dr. Masao Shibuya for help with γ-ray spectrometry.

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CHEMISTRY

Luminescence in Plastics

THE luminescence of organic molecules dissolved in a variety of plastics has been examined during the course of a search for new organic optical maser materials. Most plastics are compatible with organic compounds, are optically homogeneous even at liquid nitrogen temperature, have high dielectric strength, and can, of course, be fabricated in a wide variety of forms. In some cases, notably the polycarbonates, they are hard and can be fabricated to optical flatness. Of particular interest is our finding that many aromatic hydrocarbons and dyes dissolved in most plastics exhibit phosphorescence at room temperature.

The luminescent compound can be introduced into the plastic by one or another of three methods: (1) casting from a solvent for both the compound and the plastic; (2) melting a mixture of the crystalline compound with the powdered thermoplastic while pressure is being applied; (3) polymerization to solidification of a vinyl monomer in which the compound is dissolved. Thus, aromatic hydrocarbons are introduced into purified polymers including a polycarbonate (2,2-propane*bis* (4-phenyl carbonate)), polystyrene, polymethylmethacrylate, polyvinyl acetate, cellulose acetate, ethyl cellulose, and methyl cellulose. Water-soluble dyes are introduced into polyvinyl alcohols of different degrees of acetylation and into some cellulose derivatives by the solvent technique (No. 1 above). Excitation of the luminescent plastic was carried out with the 365-mμ line of a mercury lamp or with the light from a xenon-filled electronic photographic flash lamp. Phosphorescence lifetimes were determined from the decay of emission following excitation with the flash lamp. The emitted light, modulated by a rotating sector, fell on a multiplier phototube the photocurrent of which was impressed on the vertical plates of a slow-sweep oscilloscope and the trace was photographed. In some cases, the decay deviated from a single exponential curve especially in the beginning and end of the trace and hence lifetimes were calculated from the middle portion of the trace. Phosphorescence spectra were obtained with a spectrograph and densitometer tracings were made of the photographs.

Oxygen quenches the phosphorescence of the compounds in plastics. At room temperature a luminescent plastic film which had been evacuated (10^{-5} mm. mercury for 0·5 h) exhibits a progressive decrease in phosphorescence lifetime after oxygen is introduced. On re-evacuation the phosphorescence lifetime is restored and the cycle can be repeated. In certain cases, notably polyvinyl alcohol at room temperature, phosphorescence of dyes can be observed without prior evacuation[1], presumably due to the low diffusivity of oxygen in the plastic[2], although prolonged evacuation considerably increases the lifetime. We attribute the abrupt decrease in intensity of phosphorescence of the plastic in air at the transition temperature of polyvinyl alcohol in part to the marked increase of diffusivity of oxygen in the material above that temperature. Samples are prepared by pressing a molten thermoplastic between two glass plates to which it must adhere and allowed to solidify, and phosphorescence can be excited for several weeks. The diffusion of oxygen into a phosphorescent moulded plastic object can be observed visually by the movement of the phosphorescent boundary and we are at present determining diffusion coefficients of oxygen into plastics by this method. Samples which had been quenched with oxygen at room temperature nevertheless exhibit a strong phosphorescence at liquid nitrogen temperature in the presence of ambient oxygen.

A number of aromatic hydrocarbons including naphthacene, phenanthrene, chrysene, dibenz[*a,h*]-anthracene, and fluoranthene exhibit their characteristic β-phosphorescence in plastics even at room temperature. This type of phosphorescence has previously been observed at liquid nitrogen temperature in ether–isopentane–alcohol (EPA) glass. Although this type of glass is a suitable matrix for hydrocarbons it is only useful in this limited low-temperature region. Glycerol, a classic solvent for the observation of phosphorescence of dyes[4], is suitable only in the temperature-range from about −30° C to −130° C, below which it shatters. Boric acid glass is a suitable matrix for the observation of phosphorescence at room temperature; but its preparation requires the use of elevated temperatures which usually causes some decomposition of the organic solute. The glass is difficult to fabricate in a reproducible manner, crystallizes and is sensitive to moisture[5]. According to our experiences boric acid glasses have poor transmittance in the ultra-violet region due to optical inhomogeneities and to ionic impurities. Phosphorescence of dyes has been studied in glucose glasses[6]. Such glasses are very sensitive to moisture and, depending on nuances in their preparation, exhibit strong near ultra-violet absorption, presumably due to oxidation products which are readily detected by their strong electron spin resonance signal.

The lifetime of phosphorescence of the aforestated aromatic hydrocarbons depends both on the nature of the compound and on the polymeric matrix. For a given matrix, dibenzanthracene showed the longest lifetime (1·3 sec in polycarbonate). Fluoranthene is exceptional in that it shows the same lifetime (about 0·3 sec) in a wide variety of plastics at room temperature. Chrysene, on the other hand, has a lifetime ranging from 1·1 sec in polyvinyl acetate to 0·36 sec in ethyl cellulose (degree of substitution 2·5). In cellulose acetate with the same degree of substitution the lifetime of phosphorescence of chrysene is 0·70 sec. The lifetime of phosphorescence of acridine yellow in a series of substituted polyvinyl alcohols shows a decreased lifetime with increasing degree of acetylation. In all cases lowering the temperature of these degassed samples increases the lifetime. Thus the lifetime of phosphorescence of chrysene in polystyrene at liquid nitrogen temperature, namely, 2·2 sec, is three times that observed at room temperature. These results indicate that the lifetime of phosphorescence is influenced not only by the 'local viscosity'[1,7] in the plastic (micro-Brownian movement of the polymer segments) but also by specific interaction of the compound with the polymeric matrix. Thus chrysene exhibits in polyvinyl acetate, the softest plastic at room temperature which we examined, a longer lifetime (1·1 sec) than that exhibited for other plastics.

Since diffusional bimolecular processes are suppressed in plastics they provide a convenient matrix for the entrapment of metastable species at room temperature including those produced photochemically. For example, irradiating 2-methyl anthraquinone in cellulose acetate (degree of substitution 2·44) with near ultra-violet light yields stabilized luminescent intermediates the absorption spectra of which are close to those found by flash photolysis[8] of solutions in ordinary fluids where the intermediates usually have short lifetimes. Pyrene in fluid media forms an unstable photodimer and the process is believed to be diffusion-controlled[9]. We observed the same phenomenon in polycarbonate the local viscosity of which must be several orders of magnitude greater than ordinary liquids, thus excluding, at least for this medium, the notion of a diffusion-controlled mechanism for formation of the photo-dimer. Benzophenone-sensitized phosphorescence of naphthalene was first demonstrated in EPA glass at liquid nitrogen temperature[10]. We observed this energy transfer process in polymethylmethacrylate at room temperature.

A detailed investigation of the phosphorescence of organic compounds in plastics should help in better understanding the origin of emission processes from excited species. The possibility of varying the temperature over a wide range without losing the rigidity of the matrix seems to be very attractive. Thus one can examine the effect of populating higher vibrational levels on energy transfer processes involving metastable excited states.

Perturbations of such excited species by variation in the nature of the medium can also be examined. Furthermore, the studies of phosphorescence in plastics can give new information about the microscopic rheology of the polymeric matrix itself. Thus the measurement of depolarization of phosphorescence should prove useful with regard to slow relaxation processes in plastics.

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Tensile Behaviour of Pyrolytic Boron Nitride to 2,200° C

BORON nitride is sometimes referred to as 'inorganic graphite' because its structure is like graphite with alternating boron and nitrogen atoms replacing the carbon atoms in the hexagonal structure. Its sublimation temperature (3,000° C) is somewhat lower than that of graphite. Boron nitride has been reported to have a greater oxidation resistance than graphite and is therefore of interest for some high-temperature applications.

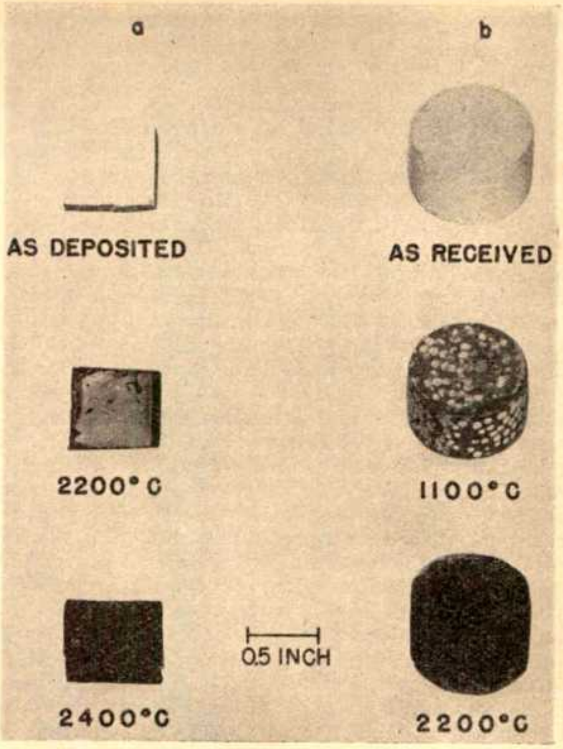

Fig. 1. Appearance of specimens after 30 min at various temperatures. *a*, Pyrolytic boron nitride; *b*, hot-pressed boron nitride

During the past months techniques have been developed for producing massive pieces of boron nitride by a pyrolytic process similar to that used to produce pyrolytic graphite. It had been hoped that the boron nitride thus produced would show an improvement in mechanical properties over the hot-pressed material similar to the improvement in pyrolytic graphite properties over pitch-coke graphite. Tensile properties of pyrolytic boron nitride parallel to the substrate direction have been measured from room temperature to 2,200° C and have been compared with hot-pressed boron nitride and with pyrolytic graphite.

The pyrolytic boron nitride tested was produced by High Temperature Materials Inc. using a process similar to that used for pyrolytic graphite. It was deposited at 1,900° C on a substrate of synthetic graphite by decomposition of a mixture of boron chloride and ammonia. The as-deposited density was 2·10 g/c.c.

The test method and equipment used were the same as used for testing graphite and have been previously described[1,2]. All specimens had a gauge section 0·50 in. long by 0·06 in. wide by 0·1 in. thick. The thickness direction was perpendicular to the basal planes. Since the pyrolytic boron nitride test specimen reacted with the graphite specimen grips and the carbon bearing atmosphere of the test furnace at 2,400° C (Fig. 1) test temperatures above 2,200° C were not used.

Table 1 summarizes the tensile data in the basal plane direction obtained for this one lot of pyrolytic boron nitride at a strain-rate of 2×10^{-4} sec^{-1}. Over the temperature-range studied the tensile strength is approximately 1/3–1/2 that of pyrolytic graphite. At room temperature, the tensile strength of pyrolytic boron nitride in the basal plane direction was not markedly different from the strength of hot-pressed boron nitride in the direction parallel to the grain[3,4]. Unlike the hot-pressed material, which showed a

Table 1. TENSILE PROPERTIES OF PYROLYTIC BORON NITRIDE IN THE BASAL PLANE DIRECTION

Block No.	Test temperature (° C)	Ultimate tensile strength (lb./in.^{2})	Recorded elongation (%)
01	Room	5,370	1·6
04	Room	5,260	2·0
05	Room	6,980	1·1
01	1,650	7,800	1·0
04	1,650	7,360	0·5
05	1,650	8,760	0·5
01	2,000	9,700	1·0
03	2,000	5,660	0·6
05	2,000	17,200	2·1
01	2,200	5,620	0·6
03	2,200	13,200	2·6
06	2,200	16,400	3·6

Strain rate $\sim 2 \times 10^{-4}$ sec^{-1}.

decrease in strength to approximately 600 lb./in.^{2} at 1,500° C and then an increase to approximately 4,000 lb./in.^{2} at 2,200° C[4], the pyrolytic material showed a continuous increase in strength with temperature reaching values greater than 16,000 lb./in.^{2} at 2,000 and 2,200° C. Elongation values over the range room temperature to 2,200° C were similar to those reported for pyrolytic graphite and hot-pressed boron nitride.

The reported[4] decrease in strength from room temperature to 1,500° C for hot-pressed boron nitride may be due to the boron oxide which was found to be present in the hot-pressed material[3]. The increase in strength above 1,500° C in the hot-pressed boron nitride may be associated with carbonization of the material. A comparison of the two types of boron nitride after 30 min at various temperatures in the testing furnace is shown in Fig. 1.

Since the pyrolytic boron nitride contains no detectable amount of boron oxide and since there is no appreciable reaction with the test furnace atmosphere up to 2,200° C, the explanation of the increase in strength with temperature may be the same as that proposed for pyrolytic graphite[5], that is, relief of internal stress concentrations with an increase in ductility with temperature.

At temperatures as low as 2,480° C, pyrolytic graphite undergoes a structural transformation[5] when it is deformed in a direction parallel to the basal planes. Since the sublimation temperature of boron nitride is approximately 600° C lower than that of graphite, it was thought that boron nitride might show a similar transformation at 2,200° C or below. Unit cell dimensions calculated from Debye–Scherrer X-ray diffraction patterns obtained from the gauge and butt sections of the tensile specimens indicated that no transformation had occurred. The unit cell height for pyrolytic boron nitride after heating to 2,200° C and after deformation at this temperature was found to be 6·86 ± 0·01 Å. This would indicate a turbostratic structure with no three-dimensional ordering. From the position of the two-dimensional (10) band, a unit cell width of 2·49 ± 0·01 Å was calculated. These dimensions were not different from the values obtained for the as-deposited material. Pease[6] has reported a unit cell height of 6·6612 ± 0·0005 Å and a width of 2·50399 ± 0·00005 Å for boron nitride recrystallized at 2,050° C for 2 h in a nitrogen atmosphere.

Further studies are at present in progress which are aimed at explaining why pyrolytic boron nitride apparently does not transform or recrystallize at 2,200° C.

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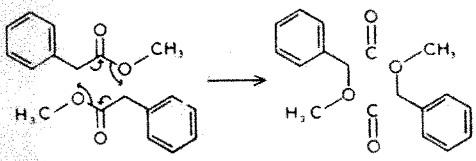
Thermal Decomposition of Methyl Phenylacetate

IN recent years, the mechanism of the thermal decomposition of esters has been thoroughly investigated, and at the present time extensive evidence has been presented to support a *cis* elimination mechanism[1]. Although this mechanism appears to be the major reaction pathway, the presence of secondary decomposition pathways has also been amply verified. Evidence for these mechanisms was reported by Hurd and Blunck[2] in their classic paper, and supporting evidence has been accumulated in the studies on the pyrolysis of methyl phenylacetate and related esters[3–7].

The thermal decomposition of methyl phenylacetate was first reported by Engler and Löw[3] to afford toluene, methanol, carbon monoxide and carbon dioxide. Later, Hurd and Blunck pyrolysed the ester at 625° C, and they reported the presence of benzaldehyde, toluene, formaldehyde, methane, and carbon monoxide as the major products. Since the earlier work did not disclose the existence of benzaldehyde in the decomposition mixture, the pyrolysis was repeated in an attempt to substantiate the presence of this compound. The pyrolysis was conducted at 675° C under Hurd's reaction conditions. The apparatus used for the decomposition consisted of an electrically-heated Vycor tube 28 cm long and 2 cm in diameter packed for a distance of about 20 cm with 'Pyrex' glass beads about 6 mm in diameter. The tube was heated to 675° C, and then 20 ml. of the ester was allowed to drop into the tube at a rate of about one drop per second. The pyrolysate was analysed directly by gas chromatography. At a column temperature of 195° C, 'Apiezon *L*' as the liquid phase, and a helium flow-rate of about 2 bubbles per second, three peaks were recorded which had retention times of 4, 8, and 23 min, respectively. These peaks corresponded to toluene (21 per cent), benzaldehyde (39 per cent), and methyl phenylacetate (40 per cent).

The composition of the products leads one to suggest the presence of an unsymmetrical ether intermediate, since the thermal decomposition of ethers such as benzyl methyl ether affords similar products[8]. In an attempt to isolate such an intermediate, a pyrolysis of the ester was attempted at 150° C for 96 h. The ester was remarkably stable under these conditions, and decomposition was not noted. The ester was then pyrolysed in a distilling apparatus at 210°–215° C for 96 h. The pyrolysis consisted of placing 20 ml. of the ester in a distilling apparatus equipped with a thermometer which extended into the liquid. The flask was then heated to a temperature of 210°–

215° C, and kept at this temperature for a period of 96 h. During this time a small amount of oil (302 mg) distilled from the reaction flask. This oil was analysed directly by gas chromatography. The chromatogram consisted of two peaks which had retention times of 12 and 23 min. These peaks corresponded to benzyl methyl ether (24 per cent) and methyl phenylacetate (76 per cent), respectively. The analysis was conducted at a column temperature of 195° C, 'Apiezon *L*' as the liquid phase, and a helium flow-rate of about 2 bubbles per sec. These results suggest that the ether may be a major intermediate in the thermal decomposition of this ester. The mechanism of the decomposition may involve a bimolecular elimination via a *quasi* six-membered intermediate:



At the present time, the extent of this secondary decomposition pathway in the pyrolysis of esters is being investigated.

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Chelates formed by Tin IV with Ethylenediamine Tetraacetic Acid

In a recent publication[1] the chelates formed by tin II with ethylenediamine tetraacetic (EDTA) acid were investigated by *p*H titration, spectrophotometry and polarography. However, some apparent discrepancies have been pointed out by Langer and Bogucki[2], who state that the reported 1 : 1 tin II–EDTA *p*H titration curve is similar to their results for the 1 : 1 tin IV–EDTA chelate, for which compound definite evidence exists for the formation of a hydroxo-chelate.

The properties of tin IV in low acid media are dominated by its tendency to hydrolysis[3] and one might expect that hydrolytic action in the tin IV–EDTA system would be much more marked than for tin II. With this point in mind the *p*H-titration curves for tin IV in the presence of various ratios of the ligand were determined (Fig. 1). Curve *C* for the 1 : 1 ratio shows an inflexion at $a = 3$, where a is the number of equivalents of alkali added per g-atom of metal ion. Thus the tin IV chelate readily undergoes hydrolysis and the species $\mathrm{Sn}Y(\mathrm{OH})^{-1}$, where Y^{-4} is the tetra-negative anion of EDTA, accounts for curve *C* below *p*H 5·0. This is confirmed by curve *D* where no evidence for a 1 : 2 chelate exists. Thus Langer and Bogucki's statement is not borne out by the facts, the tin IV chelate being much more readily hydrolysed than that of tin II. A further distinguishing feature is the pronounced plateau which occurs at about *p*H 7·7 in curve *C* and corresponds to the further release of two protons presumably as a result of hydrolysis of $\mathrm{Sn}Y(\mathrm{OH})_3^{-3}$.

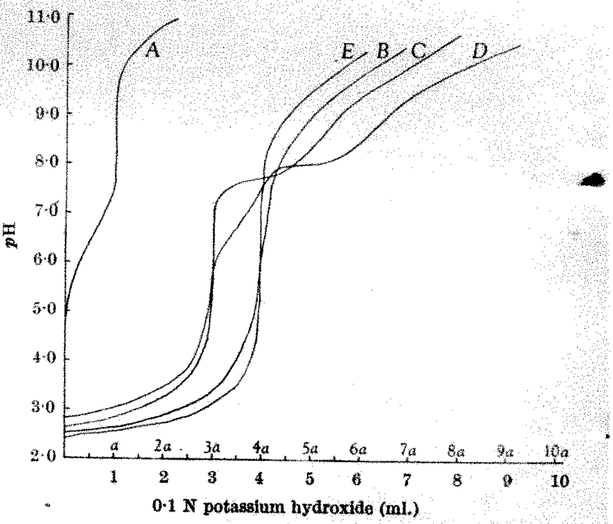

Fig. 1. *p*H titration of the tin IV–EDTA system. Ratio tin IV: H_2Y^{-2}: *A*, 0 : 1; *B*, 1 : 0·5; *C*, 1 : 1; *D*, 1 : 2; *E*, 1 : 0

Curve *B* represents the behaviour for the metal-ligand ratio of 1 : 0·5 with a single inflexion at $a = 4$. This appears to be evidence for a readily hydrolysed 2 : 1 chelate as follows:

$$\mathrm{Sn}^{4} + \tfrac{1}{2}\mathrm{H}_2Y + 3\mathrm{H}_2\mathrm{O} = \tfrac{1}{2}[(\mathrm{HO})_3\mathrm{Sn}Y\mathrm{Sn}(\mathrm{OH})_3] + 4\mathrm{H}^{+}$$

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BIOCHEMISTRY

Appearance of Parenterally Administered Proteins in Saliva

Immunochemical investigations have revealed that proteins which react with specific antisera to serum albumin and globulins constitute a substantial fraction of the salivary proteins[1,2]. Thus, barring the possibility of synthesis of proteins with similar immunological properties by salivary glands, albumin and globulins are secreted into the saliva from plasma.

To gain some idea of the permeability of the salivary glands to proteins we have studied the appearance of protein-bound iodine-131 in the whole saliva of man and rabbits after parenteral administration of human albumin labelled with iodine-131. The results provided a basis for administering unlabelled foreign proteins to rabbits; the identification of these proteins in saliva was carried out immunologically.

Man. 300–400 μc. of human albumin labelled with iodine-131 (Abbott Laboratories) were administered intravenously to an adult of each sex. Saliva was collected for 3 h in iced containers and was frozen overnight. The saliva was thawed and was freed of

Table 1. LABELLED PROTEIN IN SALIVA

	Dose				Radioactivity in saliva			
	Radioactivity		Protein					
Man	Age	Counts ($\times 10^{-7}$)	Carrier added mg	Total injected mg	Vol. of saliva ml.	Centrifuged saliva Counts ($\times 10^{-5}$)	Effluent from anion exchange Counts ($\times 10^{-3}$)	Impermeate Counts ($\times 10^{-3}$)
Female	35	58·9	0	200	75	14·3		7·3
Male	36	165	0	190	110	19·7	7·4	7·4
Rabbit								
1		3·39	10	10·3	110	1·57		3·2
2		4·66	0	8	120	2·32		4·8
3		4·05	30	31	123	2·03		29
4		3·00	25	30	100	3·12	6·8	6·5
5		8·74	9	30	58	2·92		1·5
6		8·74	9	30	92	7·79		14
7		9·15	196	250	70	4·87	2·7	3·2

All counts are corrected for background and interpolated to zero time (administration of dose). Samples were counted for 3 min. Observed counts were within the range of 85–24,000 counts above background.

cell debris and mucin strands by centrifugation in the cold at 18,000*g* for 1 h. Aliquots of the chilled supernatant saliva were removed for measurement of radioactivity in a well-type scintillation counter. Other aliquots were dialysed against cold running water until the radioactivity of the dialysis sac contents (impermeate) remained constant. Still other portions of the saliva were passed through an anion exchange column (Squibb Co. 'Rezikit') and the radioactivity of the effluent was measured.

Rabbits. Labelled Protein : 17–50 μc. of human albumin labelled with iodine-131 were administered to urethane-sedated, pilocarpine-treated[3] adult male rabbits weighing 4·1–4·6 kg. Approximately 100 ml. of saliva which dripped from the mouth over the course of 1·5 h were collected and treated as already described. The effect of varying the amount of radioactivity and the total protein content of the dose, which included added unlabelled carrier human albumin, was noted.

Most of the radioactivity which appeared in the saliva, presumably contributed by inorganic iodide, was dialysable. To confirm the completeness of separation of inorganic and nondialysable iodide, trichloroacetic acid was added to the impermeate. In all cases the precipitate contained all the radioactivity.

Alternatively, passage of centrifuged saliva over an anion exchange resin removed most of the radioactivity. The column effluent as well as a precipitate resulting from treatment of the effluent with trichloroacetic acid contained approximately the same number of counts as the impermeate of the previous experiment. The results of the experiments with labelled human albumin are listed in Table 1. The results show that individual variation exerts a much greater effect on total and non-dialysable radioactivity secreted into saliva than does the μc. of protein-bound iodine or protein content of the dose. The possibility that secreted iodide-131 of saliva may be adsorbed by salivary proteins seems to be excluded by the experiments of Ferguson, who administered Na^{131}I to rabbits and found that all the radioactivity that appeared in the saliva was dialysable[4].

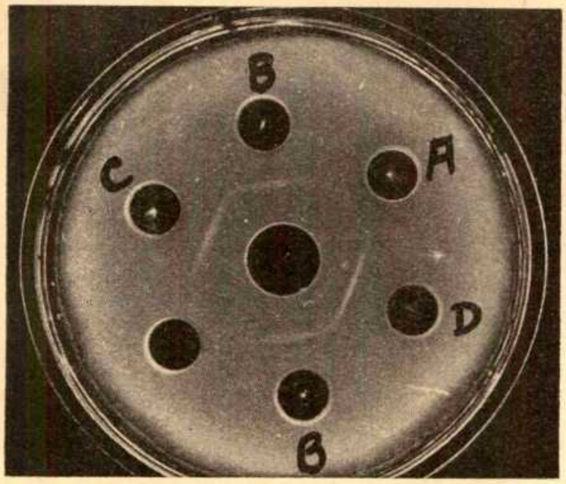

Fig. 1. Centre well, undiluted antiserum (rabbit anti-human albumin). Peripheral wells: *A*, control (25 times concentrated rabbit saliva before administration of human albumin); *B*, standard (1 μg human albumin added to 0·25 ml. of control, unconcentrated saliva *in vitro*); *C* and *D*, 30 times concentrated rabbit saliva after administration of human albumin

Unlabelled Proteins. 0·1–1·0 g amounts of a variety of unlabelled proteins were administered intravenously and 58–123 of saliva were collected as already described. After centrifugation, the saliva was concentrated to 1/25 to 1/50 its volume by vacuum dialysis against isotonic saline with a Schleicher and Schuell collodion sac at 2° (ref. 5).

The concentrated saliva was placed in a peripheral well of an agar diffusion plate. Undiluted antiserum was placed in the centre well. The plate was covered and set aside at room temperature. After 3 days the plates were examined by diffused fluorescent light in an otherwise dark room. Fig. 1 illustrates the type of reaction which appears as a zone of precipitation between the saliva from a rabbit receiving human albumin and specific antiserum. A reaction of identity to specific antiserum was obtained with saliva from a test animal and saliva from a control animal to which the human albumin had been added *in vitro*. Positive results similar to those shown in Fig. 1 were obtained for other protein—antiserum pairs including bovine serum albumin, ovalbumin, chicken and horse serum.

It is clear that parenterally administered proteins may appear in human and rabbit saliva, as demonstrated by radiochemical and immunological techniques. This is evident even with proteins foreign to the rabbit.

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Effect of Glucagon on Liver Glycolysis

Several of the known metabolic effects of glucagon are consistent with the possibility that it exerts an inhibitory effect on the Embden–Meyerhof pathway. For example, glucagon promotes ketogenesis[1], inhibits the synthesis of fatty acids[1], and lowers the concentration of pyruvate in the blood[2]. We now wish to report that glucagon influences the glycolytic process in rat liver slices. This effect is probably mediated by 3′,5′-adenosine monophosphate (3′,5′-AMP), the cyclic nucleotide which mediates the glycogenolytic action of glucagon and epinephrine, since both hormones, and the nucleotide itself, exhibit the same effect on pyruvate formation.

Liver slices obtained from male Wistar rats were incubated for 90 min in Krebs–Ringer bicarbonate buffer equilibrated with 5 per cent carbon dioxide and 95 per cent oxygen. In the experiments in which oxygen consumption was measured Krebs–Ringer phosphate buffer was used, with a gas phase of 100 per cent oxygen. Pyruvate and lactate were measured by the methods of Straub[3] and of Barker and Summerson[4] respectively. Oxygen consumption was measured by the direct manometric method. The results will be expressed in terms of micromoles of a metabolite formed or consumed, per 100 mg dry weight of liver per hour. This unit will be denoted by 'R', with a subscript to indicate the particular metabolite referred to.

Table 1 shows the marked reduction in the quantities of pyruvate and lactate in the medium after incubation of the slices in the presence of either of the three agents. It is interesting to note that the degree of inhibition was rather constant, irrespective of the rate of glycolysis in the control slices.

There are, in general, two possibilities to account for the finding of decreased amounts of pyruvate and lactate in the incubation medium. Either a decreased rate of formation or an increased rate of oxidation of pyruvate, or both, could be involved. The latter merits consideration particularly in view of the known calorigenic effect of glucagon in the intact animal[5]. In order to decide between these two possibilities, the experiments were repeated under anaerobic conditions (gas phase 95 per cent nitrogen and 5 per cent carbon dioxide).

The effect of the three agents on lactate production under anaerobiosis was not consistent (Table 2). The reasons for the inconsistency, which contrasts sharply with the situation under aerobic conditions, were not determined. Although the anaerobic experiments were perhaps not conclusive, the measure of the simultaneous rates of oxygen consumption and pyruvate production (Table 3) would appear to indicate that the decreased amounts of pyruvate in the incubation medium cannot be accounted for by an increased oxidation of the metabolite under the influence of glucagon, since 1 mole of lactate requires 3 moles of oxygen for complete oxidation. The small (not statistically significant) increase in oxygen consumption that was observed may have been due to the increased oxidation of fat which probably occurs in the presence of glucagon[1,6].

Table 1. Effect of Glucagon, Epinephrine and 3′,5′-AMP on Formation of Pyruvate and Lactate in Rat Liver Slices

Rat No.	Added substrate	Active agent	R_{pyruvate} (means ± $S.D.$)	R_{lactate} (means ± $S.D.$)	Degree of inhibition (per cent) Col. 4	Degree of inhibition (per cent) Col. 5
38 (fed)	Glucose 12·5 mM/l.	Glucagon 45 µg/ml.	0·48 ± 0·02 (4)*		25	
			0·36 ± 0·01 (4)			
70 (Fed)	None	—	1·09 ± 0·01 (4)	6·9 ± 0·6 (4)*		
		Glucagon 50 µg/ml.	0·87 ± 0·11 (4)	2·7 ± 0·5 (4)	20	60
47 (Fasted 24 h)	Glucose 12·5 mM/l.	—		1·7 ± 0·3 (4)		
		Glucagon 50 µg/ml.		1·0 ± 0·3 (4)		41
	Fructose 12·5 mM/l.	—		8·9 ± 0·5 (4)		
		Glucagon 50 µg/ml.		4·8 ± 0·5 (4)		46
	Sorbital 2·5 mM/l.	—		1·5 ± 0·3 (4)		
		Glucagon 40 µg/ml.		0·8 ± 0·1 (4)		47
96 (Fasted 24 h)	Sorbital 7·5 mM/l.	—		4·0 ± 0·2 (4)		
		Glucagon 40 µg/ml.		1·9 ± 0·1 (4)		52
	Sorbital 12·5 mM/l.	—		5·3 ± 0·5 (4)		
		Glucagon 40 µg/ml.		2·9 ± 0·1 (4)		45
40 (Fed)	Glucose 12·5 mM/l.	—		3·2 ± 0·01 (4)		
		Glucagon 50 µg/ml.		1·4 ± 0·02 (4)		54
		Epinephrine 60 µg/ml.		2·1 ± 0·02 (4)		34
		3′,5′-AMP 50 µg/ml.		2·7 ± 0·02 (4)		16
89 (Fed)	Glucose 12·5 mM/l.	—		6·6 ± 1·1 (5)		
		3′,5′-AMP 50 µg/ml.		3·9 ± 0·7 (4)		41

* No. of determinations.

Table 2. Effect of Glucagon, Epinephrine and 3′,5′-AMP on Lactate Production under Anaerobic Conditions

Rat No.	Added substrate	Active agent	R_{lactate} (means ± $S.D.$)	't' test
41 (Fed)	Glucose 12·5 mM/l.	—	22·3 ± 4·6 (4)*	
		Glucagon 25 µg/ml.	26·4 ± 3·3 (4)	$P > 0·05$
		Epinephrine 40 µg/ml.	16·9 ± 6·0 (4)	$P > 0·05$
		3′,5′-AMP 25 µg/ml.	11·2 ± 3·3 (4)	$P < 0·05$
42 (Fed)	Glucose 12·5 mM/l.	—	42·3 ± 1·9 (4)	
		Glucagon 25 µg/ml.	30·4 ± 4·8 (4)	$P < 0·01$
		3′,5′-AMP 25 µg/ml.	34·0 ± 2·9 (4)	$P < 0·05$
74 (Fasted 24 h)	Glucose 12·5 mM/l.	—	2·8 ± 0·9 (4)	
		Glucagon 50 µg/ml.	1·9 ± 0·6 (4)	$P > 0·05$
	Fructose 5 mM/l.	—	11·4 ± 1·0 (4)	
		Glucagon 50 µg/ml.	8·7 ± 1·1 (4)	$P < 0·05$
87 (Fed)	Glucose 12·5 mM/l.	—	28·6 ± 2·0 (5)	
		Glucagon 70 µg/ml.	26·3 ± 1·7 (6)	$P > 0·05$
		3′,5′-AMP 70 µg/ml.	26·0 ± 2·0 (6)	$P > 0·05$
90 (Fasted 24 h)	Fructose 2·5 mM/l.	—	4·4 ± 0·6 (6)	
		Epinephrine 66 µg/ml.	3·8 ± 0·6 (6)	$P > 0·05$
		3′,5′-AMP 66 µg/ml.	3·7 ± 0·4 (6)	$P < 0·05$

No. of determinations.

Table 3 EFFECT OF GLUCAGON ON THE SIMULTANEOUS RATES OF LACTATE FORMATION AND OXYGEN CONSUMPTION BY RAT LIVER SLICES

Rat No.	Glucagon concentration	R_{lactate} (means ± *S.D.*)	R_{O_2} (means ± *S.D.*)
94	—	6·4 ± 1·7	−18 4 ± 2 0 (6)*
	40 μg/ml.	3·1 ± 0 3	−21·5 ± 1 3 (5)
95	—	6·4 ± 0 3	−20·3 ± 1·0 (5)
	40 μg/ml.	3 3 ± 0·8	−22 4 ± 1·3 (6)

* No. determinations.

The observation that pyruvate production from glucose, fructose, sorbital, and from endogenous sources is decreased suggests that the initial phosphorylation of hexose units is not the step affected. It has not yet been determined whether the decreased formation of pyruvate is due to an inhibition of one or more of the reactions in the glycolytic sequence or whether it is a consequence of a diversion of an intermediate in the sequence into another pathway.

The foregoing results indicate that at least two metabolic effects occur in the liver as a result of the stimulation of 3′,5′-AMP production by glucagon, namely, glycogenolysis and decreased production of pyruvate. The observation that glucagon exerts a pronounced effect on the rate of pyruvate formation in the liver suggests that it may play an important part in the economy of the mammalian organism.

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Drug-induced Hæmolytic Anæmias and the Mechanism and Significance of Heinz Body Formation in Red Blood Cells

SASS-KORTSAK *et al.*[1] recently presented an interesting discussion of the mechanism of drug-induced hæmolytic anæmia and the mechanisms and significance of Heinz body formation in red blood cells. In introducing this subject they cited recent observations of Harley and Maurer. Allen and Jandl, and of their own group as demonstrating that oxidation of hæmoglobin may be an important part of the mechanism of drug-induced hæmolysis. They then stated that, in a review published prior to the contributions of these authors, I[2] had denied this possibility. This is an inadvertent misinterpretation of my published views, which I would like to have the opportunity to correct. I have always regarded the mechanism by which drugs cause hæmolysis to be oxidation of a red cell component, probably hæmoglobin, and have so stated repeatedly[3]. The point which I have questioned is whether methæmoglobin plays an essential part in this process.

In 1955, I wrote: "It may be that Heinz body-forming compounds act as oxidation-reduction 'carriers' oxidizing the red cell component"[3a]. In 1957, I demonstrated that hæmoglobin was altered by acetylphenylhydrazine to a form which oxidized GSH to GSSG, and that this form was not methæmoglobin[3b]. In my 1959 review[2], to which Sass-Kortsak *et al.* refer, I stated: "the administration of a hæmolytic drug causes oxidative damage to either hæmoglobin and/or the stroma of the sensitive cell. Heinz bodies are a visible manifestation of such damage". I would like to emphasize, therefore, that far from denying that oxidative damage to hæmoglobin was responsible for the changes observed in drug-induced hæmolysis, I actually have stated this view repeatedly over the past seven years[3]. Prior to this, the same possibility had been suggested by others[4]: this concept was not originated by me nor by the groups cited by Sass-Kortsak.

The confusion, apparently, has arisen from the part which methæmoglobin plays in the process of oxidative degradation of hæmoglobin. The position which I took in my 1959 review, based on the data available at that time, was that methæmoglobin was not a precursor of the degradation products which were formed on the administration of these drugs. The recent extension of the well-known fact that methæmoglobin production is associated with oxidative degradation of hæmoglobin cannot be considered evidence that methæmoglobin is actually the precursor of hæmoglobin degradation products. The association of methæmoglobin formation with degradation products has long been recognized[4] and could as easily be explained by the reaction pathways schematically presented in Fig. 1*A* as those which have been suggested by Harley and Maurer (Fig. 1*B*).

Since the publication of recent papers in which it was suggested that methæmoglobin was a precursor of degradation products, we have further investigated this problem. We have reasoned that if methæmoglobin is a direct precursor of degradation products of hæmoglobin, then manipulating the steady-state concentration of methæmoglobin should produce predictable effects on the rate of denaturation. Further, complexing methæmoglobin with cyanide might be expected to prevent its further degradation. Our *in vitro* findings, which are published in detail elsewhere[5], are essentially that: (1) decreasing the steady-state level of methæmoglobin by adding methylene blue to the incubating medium did not inhibit destruction of 'intact hæmoglobin'; (2) increasing the methæmoglobin concentration by pre-incubating red cells with sodium nitrite did not increase oxidative denaturation of hæmoglobin; (3) complexing methæmoglobin with cyanide decreased

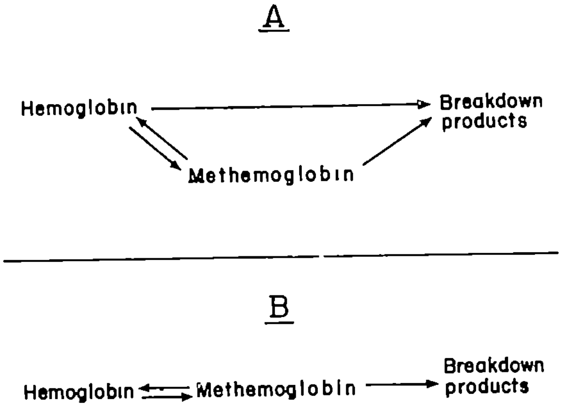

Fig 1. Two possible pathways of oxidative degradation of hæmoglobin. The pathways shown in *A* seem to me to be equally likely, or more so, than the reaction pathways shown under *B*

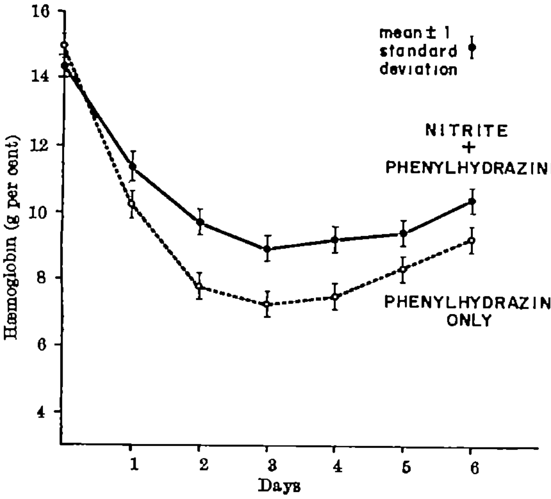

Fig. 2. The effect of methæmoglobinæmia on phenylhydrazine-induced hæmolysis in rats. Each curve represents the mean of the values obtained in six animals. Induction of methæmoglobinæmia with nitrite not only failed to increase hæmolysis, but also actually resulted in some degree of protection

the formation of the small quantities of insoluble hæmoglobin derivatives, but did not decrease the formation of quantitatively more important degradation products.

A further *in vivo* investigation also has some bearing on this matter. If methæmoglobin were a precursor of further hæmoglobin degradation products, one should expect that the induction of methæmoglobinæmia by sodium nitrite would potentiate the hæmolytic effect of a hæmolytic agent like phenylhydrazine. A group of 6 rats has been given 50 mg of sodium nitrite per kg body-weight subcutaneously. This quantity of sodium nitrite has been shown previously to induce methæmoglobinæmia of more than 3 h duration[6]. The peak-levels of methæmoglobin are achieved at the end of 60–90 min. In these studies, methæmoglobin values of 49–60 per cent were achieved after 60 min. 75 min after the injection of nitrite, the six nitrite-treated rats and six control rats which had not been pre-treated with nitrite were given 20 mg of phenylhydrazine hydrochloride subcutaneously.

The hæmoglobin values of these animals are presented in Fig. 2. Not only was there no enhancement of phenylhydrazine-induced hæmolysis by the injection of this methæmoglobin-forming agent, but also there was, if anything, actually protection against hæmolysis. Similar results were obtained in another experiment in which nitrite injection was started before the administration of phenylhydrazine, and an additional injection of nitrite was given 6 h after the first one. Thus, in spite of approximately 12 h of methæmoglobinæmia associated with the injection of phenylhydrazine, there was no enhancement of hæmolysis.

In order to duplicate more closely the *in vitro* studies which have been reported by Sass-Kortsak *et al.*[1], I have attempted to repeat these studies by the use of vitamin K_3. In rats, this compound was not hæmolytic, even when doses as large as 20 mg were given to each animal. It is possible that the results obtained in these studies with phenylhydrazine are not strictly applicable to the problem of hæmolytic activity of vitamin K_3; however, most investigators have viewed the mechanism of hæmolysis by the various oxidant drugs as probably being identical. I am also inclined toward this point of view.

While I do not regard these investigations as conclusive, neither do I find that any convincing evidence has been presented that methæmoglobin plays an important part in the oxidative degradation of the hæmoglobin. These findings are difficult to reconcile either with the view that methæmoglobin is a precursor of further hæmoglobin degradation products, or with the view[1] that methæmoglobin enhances hæmolysis by draining the red cells' supplies of TPNH.

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WE agree with Dr. Beutler and admit that one of our remarks in our original communication may be easily misinterpreted and may constitute an "inadvertent misinterpretation" of Dr. Beutler's published views.

Beutler further discusses the role of methæmoglobin in the process of oxidative denaturation of hæmoglobin and presents the results of experiments which do have an important bearing on this question. We agree with Beutler that the question whether the formation of methæmoglobin is an essential step preceding the oxidative denaturation of hæmoglobin is unsettled, and the results of his recent experiments with acetyl-phenylhydrazine constitute an argument against this possibility. Whether other oxidant drugs (for example, 'Menadione') have an effect similar to acetyl-phenylhydrazine remains to be determined.

In addition to the foregoing, we feel that the question whether oxidative denaturation of hæmoglobin (by whatever route) is the decisive step in the production of drug-induced red cell damage leading to hæmolysis *in vivo* is unsettled as well. In our original communication, we discussed the merits and the possibility of an alternative mechanism proposed by Ernster[1], fully realizing and making it clear that there is no proof of the validity of this hypothesis as well. Some of Beutler's recent experiments do cast in fact some measure of doubt on this possibility. The fact that nitrite-induced methæmoglobinæmia did not accelerate a hæmolytic crisis produced by acetyl-phenylhydrazine is a serious objection. However, there may be some doubt whether this observation can be applied to all other oxidant drugs. Moreover, the production of methæmoglobin by nitrite may be accompanied by the formation of nitroso-hæmoglobin and nitroso-methæmoglobin[2], which may then influence the system in an unpredictable manner.

Nevertheless, these objections are minor and may not be convincing, and, therefore, Beutler's findings are of great interest and importance and will, no doubt, stimulate further work towards the clarification of the mechanism of drug-induced hæmolysis.

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Eriochrome Black *T* as a Protein Dye

BINDING of eriochrome black *T* to protein was suspected during experimental ultramicro-titration of solutions containing serum proteins, when using eriochrome black *T* as indicator, because of difficulty in obtaining a value for the indicator blank titration.

The binding was confirmed by dyeing protein on cellulose acetate membrane. The use of eriochrome black *T* as a protein dye following electrophoresis of serum on cellulose acetate was therefore investigated. The dye bath solution which gave complete staining of the protein fractions after electrophoresis was 0·1 per cent w/v eriochrome black *T* in alcoholic glacial acetic acid (9 vols. + 1 vol.). This dye solution is stable for at least three months.

Electrophoresis of the serum was carried out on 3 × 1 in. strips of cellulose acetate, using the buffer solution recommended by Afonso[1], at 1·5 m.amp. per strip for 1 h. The strips were taken directly from the electrophoresis chamber and placed in the dye bath for 1 h. The excess dye was removed, to leave a clear background, by washing in a bath of 5 per cent v/v glacial acetic acid in 50 per cent alcohol. After a final wash in water the strips were dried between filter papers under light pressure. The stained dried strips were cleared with liquid paraffin and scanned with a modified EEL scanner using an *OGR* 1 broad spectrum filter. An example of the trace from a scanned strip is shown in Fig. 1.

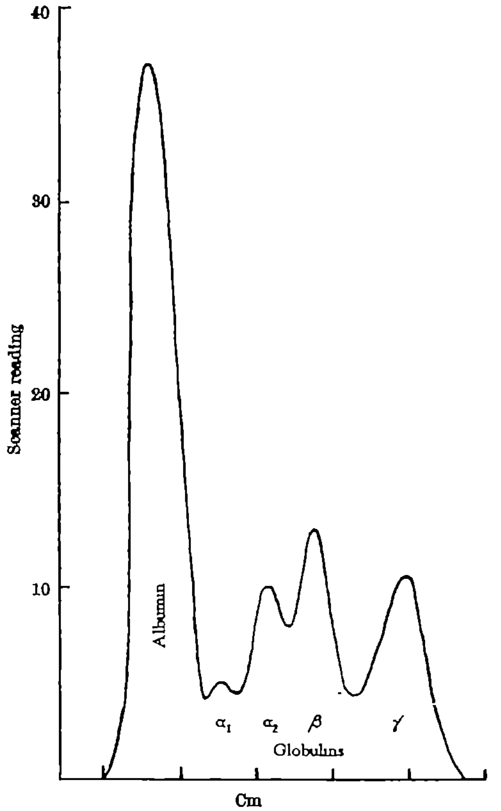

Fig. 1. Scanned curve after electrophoresis and staining of approximately 2 μl. of serum, total protein 6·4 g/100 ml., albumin 3·5 g/100 ml.

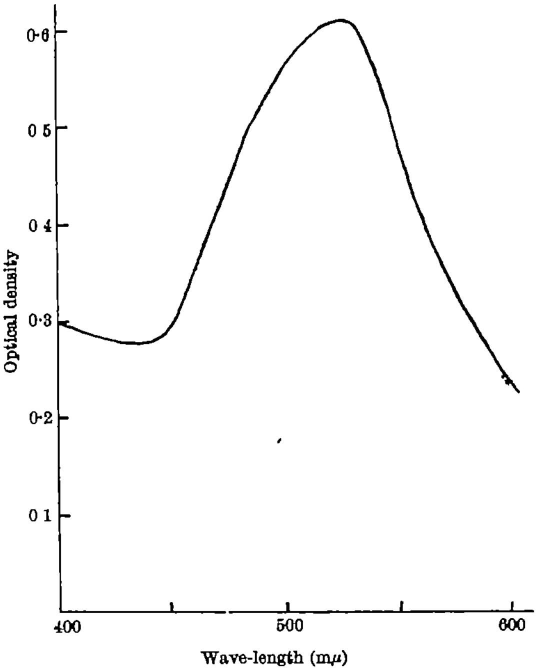

Fig. 2. Absorption spectrum of eriochrome black *T* stained protein on cellulose acetate dissolved in chloroform–alcohol

Spectrophotometric examination of the dyed protein fractions was carried out after placing the required section of the dry stained strip in 5 ml. of a mixture of 9 vols. chloroform and 1 vol. alcohol and allowing to stand, with occasional shaking, for 5–10 min. At the end of this time the cellulose acetate membrane had dissolved leaving the dyed protein in solution. Very large amounts of dyed protein occasionally delayed solution of the cellulose acetate, but the addition of 2 drops of 'Hyamine 2389' helped to give complete solution and the original colour of the dyed protein was restored by adding 2 drops of glacial acetic acid and mixing. The absorption spectrum of the dyed protein from cellulose acetate membrane dissolved in the chloroform–alcohol mixture is shown in Fig. 2. An absorption maximum was found at 525 mμ.

Eriochrome black *T* appeared to be a sensitive dye for protein so the dye bath, as used to stain electrophoretic strips, was used to stain the lines of precipitate obtained during immuno-double diffusion on cellulose acetate as described by Consden and Kohn[2]. The precipitated protein stained readily and it was found that post-staining the strip with nigrosine did not enhance the staining of faint lines.

During the initial experiments with the dye bath two methods of precipitating the protein on the cellulose acetate following electrophoresis were tried using trichloroacetic acid. The first was to use a separate fixing bath of 5 per cent w/v trichloroacetic acid in 95 per cent methanol before staining and the second was to add trichloroacetic acid to the dye bath to give a concentration of 5 per cent w/v. By both these methods it was found that the albumin band did not stain. The precipitation reaction of the trichloroacetic acid had therefore blocked the albumin dye binding sites to eriochrome black *T*. This is direct experimental evidence of a difference in the dye binding of albumin and globulins which agrees with the suggestion by various authors of using a factor to correct for different dye binding capacities of albumin and globulins with other dyes.

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Human Casein and its Caseino-glycopeptide

When rennin is allowed to react with caseins prepared from milk of different ruminant species at neutral *p*H and in the presence of calcium, para-casein precipitates and the supernatant contains a glycopeptide, called caseino-glycopeptide[1]: it does not dialyse, and remains soluble in 12 per cent trichloroacetic acid (TCA). We prepared and analysed recently the caseino-glycopeptides from cow, goat and sheep caseins, which are analogous but not identical substances[1]. In the case of cow's casein the glycopeptide was obtained from κ-casein by rennin digestion in the absence of calcium[1]. κ-casein has been analysed[2] and seems to be the specific substrate for rennin. In this paper we want to report the amino-acid composition of human casein and to comment on some results concerning its digestion by rennin.

Human casein has been prepared by several methods[3,4]. We used the following procedure: pooled human milk was defatted by centrifugation and dialysed against water at 2° in the presence of 5 parts per thousand of toluene The casein was precipitated by acidification with hydrochloric acid to *p*H 4·6. If the dialysis is omitted the precipitation does not occur regularly. The casein was washed with water, alcohol and ether: during this treatment, 35 per cent of the initial weight is lost and the nitrogen content increases from 8·6 to 14·8 per cent.

Human casein has been analysed: the amino-acids have been determined after total hydrolysis (6 N hydrochloric acid; 110°; 18 h) with a Technicon auto-analyser following the method of Piez and Morris[5], tryptophan according to Spies and Chambers[6], the amino-sugars according to Rondle and Morgan[7] and *N*-acetylneuraminic acid (Nana) according to Warren[8]. The results are given in Table 1.

Table 1. Composition of Human Casein and of Human Caseino-Glycopeptide

Amino-acid or sugar	Casein (g/100 g casein)	Caseino-glycopeptide (molar ratio)
Asp	7·5	3
Thr	4·35	6
Ser	4·8	4
Glu	18·3	5
Pro	10·9	5
Gly	1·8	3
Ala	2·8	5
Cys	0·95	0
Val	4·85	3
Met	0·7	0
Ileu	4·4	3–4
Leu	10·8	1
Tyr	5·1	0
Phe	3·8	0
Try	2·4	0
Lys	8·85	3
His	2·15	0
Arg	2·05	0
Galactose	2·55	+ see text
Galactosamine	2·20	+ see text
Sialic acid	0·37	+ see text

The amino-acid analysis presents important differences with the results reported a great number of years ago by Plimmer[9], Beach *et al.*[10] and Williamson[11], especially concerning Gly, Met, Asp and Lys. Our sugar and amino-sugar content is higher and the Nana content lower than those reported by Johansson and Svennerholm[12]. Human casein has more Cys and less basic amino-acids than cow's casein.

Carboxypeptidase liberates after 2 h at *p*H 7·8 and 37° the following amino-acids from human casein: Val (0·25 μM/mg casein), Leu (0·12), Thr (0·08) and Ala (0·04). The amino-acids liberated from κ-casein (cow) were Val, Thr, Ala and Ser[1]. By Sanger's method it was possible to characterize some *N*-terminal Arg.

At *p*H 6·8, human casein, even in the presence of calcium, precipitates only with difficulty during the digestion with rennin (0 05 unit/ml. 1–2 per cent protein solution). At *p*H 4·6, human casein precipitates, but in the presence of rennin a soluble substance(s) becomes present in the supernatant.

By analogy with the previously examined caseino-glycopeptides[1], we tried to prepare the human caseino-glycopeptide in the same manner: rennin digestion at *p*H 6·8, precipitation by 12 per cent TCA, dialysis of the supernatant and lyophilization of the clear, not dialysable fraction containing the caseino-glycopeptide. In the absence of rennin the supernatant obtained after treatment of human casein with 12 per cent TCA presents some nitrogen-containing substances. After dialysis and hydrolysis of the non-dialysable fraction, its amino-acid content has been determined. It is very low (0·6 per cent). In the presence of rennin, the supernatant obtained after treatment of human casein with 12 per cent TCA presents a higher nitrogen content. But this increase is not regularly obtained and in the course of five experiments we observed it only twice. This fact may be explained by the presence in some lots of human casein of substances preventing the rennin action. Keller[13] also reported the formation of a nitrogen-rich substance during the rennin digestion.

The substance formed after rennin digestion is called human caseino-glycopeptide. Its amino-acid and sugar content is given in Table 1. The molar ratios of the different amino-acids are very closely related to those reported for the caseino-glycopeptides of cow, goat and sheep[1]. In this connexion are especially noted the characteristic high content in Thr and Ileu and the lower ones in Ser and Leu (Thr/Ser = 5/3; Ileu/Leu = 3/1): it should be realized that human casein contains nearly the same proportion of Thr and Ser and more Leu than Ileu as is also found in cow's casein. As with all previously examined caseino-glycopeptides, the human one contains only traces of aromatic and sulphur-containing amino-acids which may belong to some traces of remaining casein, no histidine or arginine and a great quantity of sugars, namely, 8·37 per cent galactose, 6·36 per cent galactosamine and 1·26 per cent *N*-acetylneuraminic acid Carboxypeptidase liberates here again Val. Contrary to the other caseino-glycopeptides, we have found a high content of Gly.

In this communication we have shown that after rennin digestion of human casein which has been analysed, a substance appears which is soluble in 12 per cent TCA and not dialysable: it seems to be very closely related to the previously studied caseino-glycopeptides. But it has not been determined until now if human casein contains a component similar to the κ-fraction of cow's casein. As human casein is not so sensitive to rennin as cow's casein, Imada *et al.*[14] suggested that such a fraction may fail, but the presence of our caseino-glycopeptide fraction is not in favour of such an interpretation.

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Fractionation of Nitrogenous Compounds from Corn Leaves

INCREASED resolution of many components of complex biological mixtures has been accomplished by the use of substituted cellulose compounds in chromatography. Calf lens proteins have been fractionated into ten components on DEAE (diethylamino-ethylcellulose) by Spector[1]. Wheat glutin was fractionated on carboxymethyl-cellulose columns into four components by Woychick *et al.*[2]. DEAE columns were used to purify β-glucosidase from yeast by Duerkson and Halvorson[3]. Changes in the elution pattern of proteins of white potato tubers infected with *Phytophthora infestans* were found when extracts were chromatographed on DEAE[4].

Extracts of soluble components of healthy leaf tissues were made from 2 inbred differential corn lines, *B*38 and *B*14[5], at the 3–5 leaf stage. The extracts were prepared by grinding the frozen leaf tissue at 4° C in 2–3 v/w of ice-cold 0·05 M phosphate buffer *p*H 6 8. Following grinding, the cell wall materials, along with other debris, were removed by centrifugation at 5,000*g* for 15 min. Chloroplasts were removed by centrifugation at 15,000 r p.m. (19,600*g*) for 1 h. The resultant supernatant was clear and light yellow in colour. The proteins in the supernatant were stabilized by adding sucrose to a final concentration of 12·5 per cent w/v. Polyhydroxy compounds, for example sucrose, were reported by Heitfuss *et al.*[6] to stabilize plant proteins. The fractionations were performed on these extracts without further purification. Protein nitrogen values were obtained using the method of Warburg and Christian[7] or by Lowry's modification[8] of the folin–phenol method. A quantitative determination was made by determining the amount of protein in the whole extract and subtracting the amount remaining in the supernatant after treatment with 10 per cent trichloroacetic acid.

Electrophoresis of these extracts on paper indicated only 2 or 3 bands when stained with bromphenol blue, nigrosin or buffalo blue black. The most rapidly moving band was broad and diffuse followed by 1 or

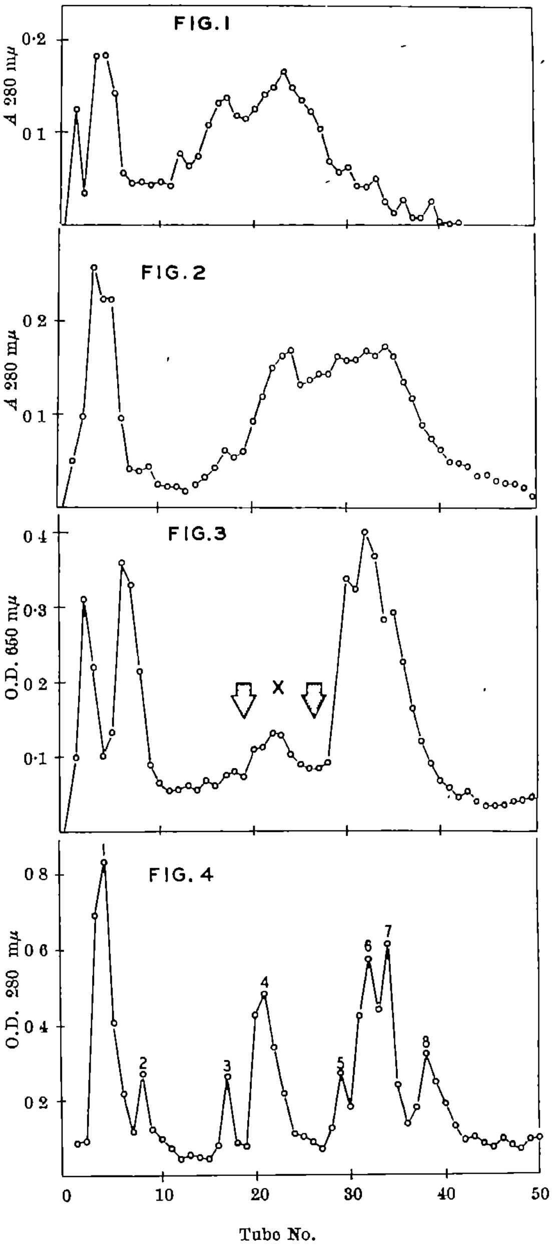

Fig. 1. Elution patterns of the inbred corn line *B*-14 using a limit buffer 2·0 M sodium chloride. Fig. 2. Elution pattern of the same material as in Fig. 1 using 1·0 M sodium chloride as the limit to solution. Fig. 3. Elution pattern of inbred corn line *B*-38 using 0·5 M sodium chloride as the limit solution. Nitrogenous compounds were detected in Figs. 1, 2 and 4 by the absorbance at 280 mμ and 260 mμ. The compounds reported in Fig. 3 were detected by the folin–phenol method; the optical density at 650 mμ represents the peak used for detection. Fig. 4 represents the elution pattern of *B*-14 0 5 M sodium chloride as the limit solution. Eight rather sharp peaks are discernible in this system

2 narrow sharp bands. Agar-gel electrophoresis revealed 4 discrete bands. A broad band migrated most rapidly toward the anode followed by three sharp bands. To facilitate the further separation of the nitrogenous material, fractionation by means of DEAE column chromatography was utilized.

Columns were prepared from DEAE (Eastman organic chemicals lot No. 1056) in 1 × 30 cm 'Pyrex' chromatography tubes fitted with a sintered glass disk. Columns were packed to a height of 20 cm in the manner described by Spector[1].

A gradient system consisting of two cylinders of equal volume connected by a siphon and stirred by a magnetic system in the delivery side was used successfully to obtain reproducible linear gradients. Various combinations of starting and limit solutions were tried. Gradients constructed with phosphate buffer at 0·005 M or less as the starting solution and sodium chloride at different concentrations as the limit solution were used in this investigation. Figs. 1, 2, and 4 indicate the elution patterns utilizing 2·0, 1·0, and 0·5 M sodium chloride as the limit solution.

The extracts were placed on a nearly dry column and layered with phosphate buffer. When the sample had penetrated below the surface of the packed DEAE, the gradient system was connected.

A gradient involving 400 ml. total volume required 23–25 h to pass through the column. 6-ml. fractions were collected.

The patterns in Figs. 1, 2 and 3 indicate an increasing separation of components in extracts of *B*–14 grown at 28° C. The first two peaks in each pattern shift very little in each change of limit buffer until 0·5 M sodium chloride is used (Figs. 3 and 4). The area covered by the majority of compounds included in the tubes Nos. 10–40 shows increased definition as the concentration of limit solution is lowered. A total of 8 principal peaks or ridges of peaks are detectable by the use of this method, numbered 1–8 in Fig. 4. The increase in number of components located by this method is at least 100 per cent over electrophoresis on either agar or paper.

Application of DEAE chromatography with the increased resolution of nitrogenous compounds indicates biochemical differences in inbred lines *B*14 and *B*38 (Figs. 3 and 4).

Fig. 3 shows the elution pattern for inbred corn line *B*38 grown at 20° C. A difference in the elution pattern in the region between the arrows (*X*) in Fig. 3 was found in plants grown at 28° C. In this region the material fractionated from plants grown at 28° C did not show a peak. Apparently an influence of temperature on the biochemical composition of *B*38 is detected by the sensitivity of DEAE chromatography.

Attempts to isolate and characterize the material from region (*X*), Fig. 3, have been unsuccessful. *In vacuo* drying has not been successful in concentrating the material. The compounds found in area *X*, Fig. 3, are labile to dialysis with a half-life of 24 h at 4° C.

Future research will be directed toward identifying and characterizing the components in this area (*X*) as influenced by temperature and to a further definitive separation of these components.

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Citric Acid in Tissues of Normal and Dystrophic Animals

THE exact role of citric acid in the body is not yet fully understood. However, citric acid in body fluids seems to adjust itself to calcium-levels; when calcium is increased, citric acid is increased too[1].

In 1937, Fenn and Goettsch[2] found in dystrophic rabbits on a vitamin E-deficient diet elevations in calcium content of muscle as much as 3–5 times normal. These findings led us to the speculation that citric acid may also be increased, and we found this to be so.

First we investigated rabbits on a vitamin E-free diet[3] with and without α-tocopherol supplement. (We thank Dr. I. Weinstock and his staff for providing these animals.) The animals were decapitated and blood was collected directly into centrifuge tubes. Fresh tissue samples of muscle, liver and kidney were weighed and immediately put into tubes containing trichloroacetic acid. The tubes were heated in a water bath of about 80° C with frequent stirring until the tissue disintegrated to a fine powder; finally 1 ml. of 100 per cent trichloroacetic acid was added. The tubes were cooled and the volume was adjusted to 10 ml. After centrifuging an aliquot was taken for analysis[4,5]. As shown in Fig. 1, citric acid was greatly increased in muscle, less so in liver and very little in kidney. There was no increase in serum. While the concentrations of citric acid in muscle and liver of the control animals were almost the same, these levels were much higher in kidney tissue.

We investigated also another species, namely, mice with hereditary muscular dystrophy. The 129/*Re-dydy* dystrophic animals and the new *C*57 *BL*/6*J-dy* type described by Russell *et al.*[6] were obtained from the Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine. The results are shown in Fig. 2. In spite of the gross differences between the citric acid concentrations of normal mouse muscle and normal rabbit muscle, the increases in dystrophic muscles are of the same order of magnitude in both species.

The fact that the increase of citric acid is greatest in muscle may point to its specificity in this abnormal state. Though its role is not completely clarified, citric acid may act as a chelating substance in the interaction of minerals and trace elements. We believe this is the first time that citric acid has been investigated in dystrophic muscle. Studies of human muscle are in progress.

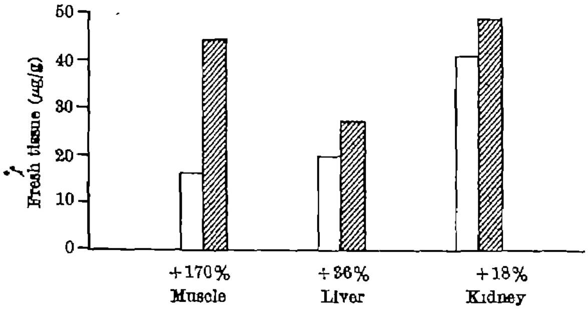

Fig. 1. Arithmetic mean of nine normal and six vitamin E-deficient rabbits (♀). White, normal; hatched, vitamin E

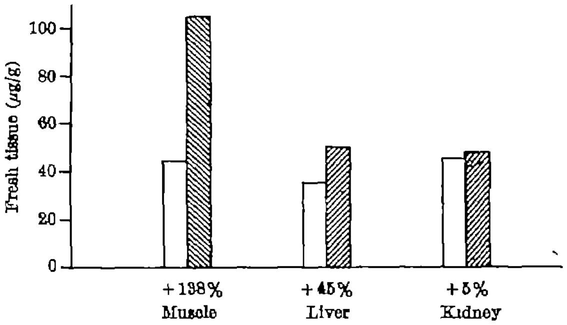

Fig. 2. Arithmetic mean of 20 dystrophic mice and their normal litter mates (♀, ♂). White, normal; hatched, dystrophic

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Biochemical Changes in Pigeon Breast Muscle Mitochondria following Nerve Section

NUMEROUS chemical and biochemical investigations have been performed on muscle in the course of denervation atrophy, but very few observations have been made on isolated mitochondria. Aloisi *et al.*[1], in a preliminary investigation on denervated pigeon breast muscle, observed a diminished yield of the mitochondrial fraction and a lowered oxidative ability of the mitochondria towards α-oxoglutarate 15–18 days after nerve section.

Our work was carried out on pigeons denervated from a few hours up to 14 days to see whether any biochemical lesion occurred in muscle prior to the appearance of morphological atrophy of the tissue.

Table 1. CHANGES IN PIGEON BREAST MUSCLE MITOCHONDRIA THREE DAYS AFTER NERVE SECTION

		Normal	Denervated	Change (%)
Pyruvate	$Q_{O_2}^{pr}$	81·0	60·5	−25
	P : O	2·59	2·32	−10
	R.C.I.	2·6	2·0	−36
α-Oxoglutarate	$Q_{O_2}^{pr}$	66·5	55·3	−17
	P : O	2·72	2·97	+9
	R.C.I.	3·2	2·2	−48
ATPase (μmoles P/mg pr./h)				
Not activated		3·75	1·72	−54
DNP-activated		8·28	3·37	−59
Mg^{++}-activated		13·40	11·65	−13
Cytochrome oxidase				
Mitochondria (μl. O_2/mg pr./h)		3,081	2,494	−17
Homogenate (μl. O_2/g fresh tissue/h)		833	464	−44
←Mitochondrial yield (mg pr./g fresh tissue)		3·25	3·85	—

R.C.I., respiratory control index.

The wing nerve plexus of one side was severed under ether anæsthesia. Mitochondria were isolated from the pectoralis major muscle and assayed for a number of variables expressing their functional abilities related to energy metabolism. The mitochondria of the contralateral muscle were used as controls. The isolation and testing methods were essentially the same as used by Azzone *et al.*[2]; cytochrome oxidase activity was measured following the method of Schneider and Potter[3].

No significant changes of the measured variables were observed in the first three days following nerve section. After this interval some modification became apparent in isolated mitochondria (Table 1); their oxidative ability towards pyruvate and α-oxoglutarate decreased significantly and the same happened for cytochrome oxidase activity. These changes progressed with time as illustrated in Figs. 1*A*, *B*. The addition of coenzyme *A*, thiamine pyrophosphate, and diphosphopyridine nucleotide to the mitochondrial preparations did not raise their oxygen uptake. The adenosine triphosphatase (ATPase) activity of the mitochondria was concomitantly lowered. Both the ATPase activity without added activators and the 2,4-dinitrophenol (DNP)-activated one were affected; however, the activation by DNP, as expressed by the ratio DNP-activated ATPase/non-activated ATPase, was not significantly modified in mitochondria from denervated muscle (Fig. 1*C*). The addition of magnesium caused an increase of ATPase activity of the same order in normal and 'denervated' mitochondria; this behaviour supports the suggestion[3,4] that magnesium-activated ATPase activity of muscle mitochondrial preparations is in fact due to some contaminating structures, possibly fragments of sarcoplasmic reticulum.

The coupling of electron transport with phosphorylation was barely affected three days after denervation. However, the modification was limited to the respiratory control index and to the phosphorus : oxygen ratio for pyruvate, which resulted slightly lowered. Thus, from the fourth day onward after nerve section the isolated mitochondria appeared as 'loosely coupled'.

With succinate as a substrate five days after nerve section a diminution of about 50 per cent of the phosphorus : oxygen ratio was observed; this change remained constant during the following days. We have circumstantial evidence that this decrease of the phosphorus : oxygen ratio was not due to uncoupling in the usual sense, but to a loss of ATP from the denervated muscle mitochondria, in accordance with

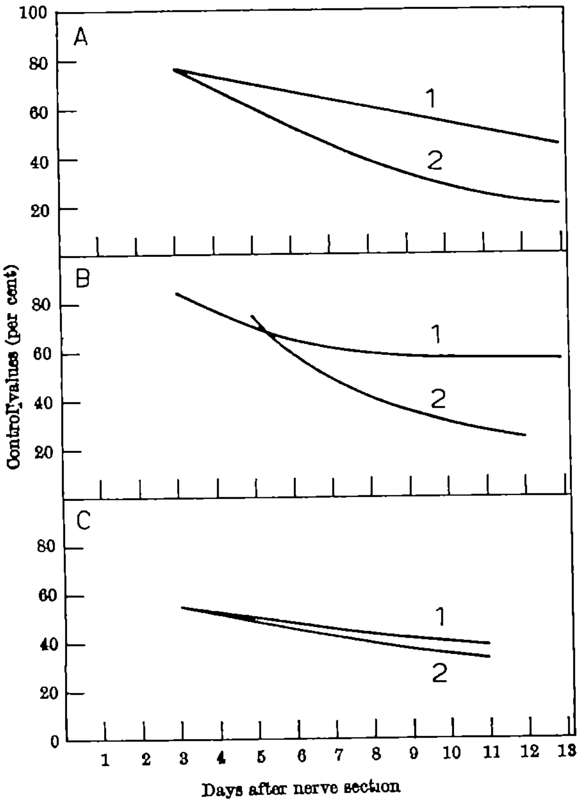

Fig. 1. Effect of denervation on pigeon breast muscle mitochondria activities

*A*1, pyruvate oxidation (control mean $Q^{\text{pr}}_{O_2}$ = 66·4); *A*2, α-oxoglutarate oxidation (control mean $Q^{\text{pr}}_{O_2}$ = 70 9). *B*1, cytochrome oxidase activity (control mean $Q^{\text{pr}}_{O_2}$ = 2004); *B*2, mitochondrial yield (control value = 2·99 mg pr./g fresh tissue). *C*1, non-activated ATPase (control mean value = 8·70 μmoles phosphorus/mg pr./h); *C*2, DNP-activated ATPase (control mean value = 6·20 μmoles phosphorus/mg pr./h)

some recent views advanced[5] on the role of ATP in the functional link of succinate with the respiratory chain.

Also cytochrome oxidase activity of both the whole homogenate and isolated mitochondria began to diminish three days after nerve section and soon reached values around 50 per cent of normal ones. The mitochondrial yield remained normal up to the fifth day, then it dropped rapidly to reach levels around 25 per cent of the control (Fig. 1*B*).

The parallel decrease of the rate of respiration with pyruvate and α-oxoglutarate as substrates and of non-activated and DNP-activated ATPase activity, together with the results of the cytochrome oxidase assay, seem to indicate that as a result of nerve section muscle mitochondria undergo depletion of their enzyme contents followed by a diminution of the mitochondrial complement of the fibre.

These biochemical regressive changes of the main energy-producing structures of the cell precede histological atrophy of the muscle and most probably contribute to its pathogenesis; however, the relatively long interval between nerve section and the onset of the first detectable biochemical changes in mitochondria, together with the kinetics and the sequence of the modifications observed, strongly suggest that other functional sites of the cell are affected by denervation before mitochondria.

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PHYSIOLOGY

Rate of Oxygen Uptake of Erythrocytes of Vitamin E-deficient Rats

Vitamin E-deficient animals are much more sensitive to oxygen under high pressure than are normal animals[1]. In particular, the erythrocytes of the deficient animals, on exposure to oxygen under high pressure, rapidly undergo a remarkable degree of hæmolysis *in vivo*, which is prevented by even very small doses of tocopherol[2,3]. It is known[4,5] that conditions which increase the rate of oxygen consumption of the whole animal increase its sensitivity to oxygen under high pressure, and there is evidence[6] that vitamin E deficiency falls into this category. It seemed important, therefore, as a first step in the study of this type of hæmolysis, to find out if vitamin E deficiency was associated with any change in the rate of oxygen consumption of the erythrocytes.

Sixteen rats, comprising 2 litters, each containing 4 of either sex, were allotted at random to 2 groups, one of which was given a diet deficient in vitamin E and the other the same diet with the addition of α-tocopherol acetate (10 mg/week). The composition of the diet, and the procedure adopted in administering the fat-soluble vitamins, have been fully described in earlier papers[2,3]. At the age of 16 weeks the animals were lightly anæsthetized with ether, a few drops of tail blood were taken for the dialuric acid test of Rose and György[7] and the animals were then bled from the femoral artery into heparinized containers. Passage through sintered glass filters with gentle suction removed approximately 90 per cent of the leucocytes. The blood was then centrifuged and the erythrocytes washed in 0·9 per cent sodium chloride solution and suspended in a volume of Krebs's buffer solution such as to give final hæmatocrit values of approximately 66 per cent. The rate of oxygen uptake of 1 ml. samples, after shaking for 30 min to ensure complete saturation of hæmoglobin with oxygen, was then determined over 2 h in 5-ml. Warburg flasks at 37° C. After this time, some degree of hæmolysis was obvious in the samples from deficient rats and was accompanied by a falling off in the rate of oxygen consumption.

The dialuric acid test, presence of uterine pigment and loss of testicular weight showed that none of the 'controls' and all the 'deficient' rats were in fact vitamin E deficient.

There was a clear difference in the rates of oxygen uptake of the red cells from the 2 groups, that of the cells from the deficient animals being twice as great as that of the controls—0·090 ± 0·009 μl./mg dry wt./h compared with 0.045 ± 0.006 µl./mg dry wt./h ($P < 0.0001$).

There seems no doubt, then, that vitamin E deficiency is associated with an increased rate of oxygen uptake by the erythrocytes. Vitamin E-deficient red cells do in fact hæmolyse *in vitro* under a variety of conditions, due it is suggested to uninhibited peroxide formation[8], a process which may occur independently on exposure to oxygen under high pressure[9]. It is also known that at least some types of hæmolysis, for example, that due to methylene blue, are associated with an increase in erythrocyte oxygen consumption[10]. The problem of relating these facts to hæmolysis occurring on exposure to oxygen under pressure remains.

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Adipose Tissue Lipoprotein Lipase Activity in Rats of Two Different Ages

THE physiological part played by the clearing factor or lipoprotein lipase has not been fully established in man[1]. The parenteral administration of heparin to normal human adults after a fatty meal and overnight fasting has been shown to cause the appearance of this enzyme in the plasma[2,3].

Heparin acts by mobilizing the enzyme from the tissues where it is normally present and effecting its rapid attachment to the substrate. The adipose tissue of various animals is a common source of this enzyme and that this may apply to man as well has been suggested by the experiments of Renold and Marble[4]. Other workers, however[5,6], failed to find any lipoprotein lipase activity in human adipose tissue.

In an attempt to explain such an absence of enzyme from the human adipose tissue Angervall speculated[5] that the lipase might be present only in growing subjects but not in adults, since he found appreciable quantities of the enzyme in the adipose tissue of chickens, and only negligible amounts in that of hens. No account, however, was given of attempts to confirm this hypothesis in other species.

I tested human breast adipose tissue taken from middle-aged women on whom mastectomy was performed for carcinoma of the breast. The method used is described here. No lipoprotein lipase activity was found on the human adipose tissue so tested.

The human adipose tissue, however, tested by Angervall[5], Engelberg[6] and by me was taken during surgery and therefore from fasting subjects, and as fasting decreases considerably the lipoprotein lipase activity of the adipose tissue of rats[7,8] it is conceivable that the absence of any such activity in the human adipose tissue was due, at least partly, to the fact that the subjects whose adipose tissue was tested were fasting.

On the other hand, the striking differences that Angervall[5] found between chickens and hens offer another possible explanation.

Since the effect of age on the adipose tissue lipoprotein lipase activity has not been tested on mammals I decided to determine this activity in the adipose tissue of rats at two different ages.

Three experiments were performed, in which 10 young (4–5 weeks old) and 9 old (more than 5 months old) male Wistar rats, fed on a standard diet, were killed by decapitation, and fragments of the anterior part of their epididymal fat bodies were assayed for lipoprotein lipase. The method described by Cherkes and Gordon[7] was utilized, modified to the extent that an activated emulsion of cottonseed oil ('Infonutrol') was used as a substrate and that the incubation of the tissue was carried out for 40 min in a Krebs–Ringer bicarbonate buffer. The concentration of heparin sodium in the medium was 3·4 mg (384 units heparin sodium B.P.) per litre of medium. In preliminary experiments we found that up to this level a ten-fold increase in the concentration of heparin caused a two-fold increase in the detachment of the enzyme from the tissue. With a further rise of heparin, however, there was no more increase in the enzyme detachment from the tissue nor was there any decrease.

The free fatty acids evolved were determined using a modification of Dole's method[9]: 2 ml. of the heptane phase were pipetted into conical tubes to which 2 ml. of nile blue indicator[10] were added. The results were expressed in units of lipoprotein lipase per gram of wet adipose tissue[6,7], one unit representing the amount of lipase which would release one µequiv. free fatty acid/h.

The average enzyme activity so defined was found to be $19{\cdot}36 \pm 8{\cdot}25$ (mean $\pm$ $S.D.$) units for young and $2{\cdot}92$ (mean $\pm$ $S.D.$) units for old rats ($t = 5{\cdot}3$, $P < 0{\cdot}0001$).

It seems, therefore, that the finding of Angervall is not confined to chickens, but may be a more general phenomenon of the growing process during which a reduction or redistribution of the adipose tissue enzyme may occur and which may involve man as well. Whether this phenomenon is related to the amount of circulating growth hormone and whether the properties of this hormone as regards fat metabolism are due to this possible relationship is an interesting speculation.

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Salicylate and Increased Vascular Permeability

We have previously found that high doses of systemically injected salicylate (600 mg/kg) reduce the œdema subsequent to injury in the rat[1] and that in the rat similar systemic doses of salicylate cause a non-specific suppression of the increased vascular permeability following intradermal injections of histamine, 5-hydroxytryptamine (5HT), 'globulin permeability factor' and bradykinin.

In an attempt to detect a more specific action of salicylate which might explain some of the drug's anti-inflammatory effects, salicylate was injected intradermally with various substances that increase capillary permeability. It was found that salicylate in local concentrations of 2·5 mg/ml. caused total suppression of increased vascular permeability as indicated by the accumulation of circulating protein-bound trypan blue[2] at intradermal injection sites following injections of bovine pancreatic kallikrein (Bayer) in concentrations of 1–10 units/ml. It also caused total suppression of the similar action of a sample of human salivary kallikrein (about 5 units/ml.). At 1·0 mg/ml. salicylate had little effect on the leakage of trypan blue due to intradermal injection of these samples of kallikrein. Locally injected salicylate (2·5 mg/ml.) caused a 90 per cent suppression of the dye leakage following intradermal injection of 'globulin permeability factor' prepared by ether/ethanol fractionation of rat serum[3] (Table 1).

Table 1. Effect of Local Concentrations of Salicylate on the Increased Capillary Permeability in the Rat induced by Different Permeability Factors

Concentration of local salicylate (mg)	Permeability factor (P.F.)	Accumulation of trypan blue (μg) (increased permeability)
—	Kallikrein	6·3
2·5	Kallikrein	0
1·0	Kallikrein	6·1
—	Bradykinin	3·5
2·5	Bradykinin	3·8
6·0	Bradykinin	3·0
—	Globulin P.F.	6·1
2·5	Globulin P.F.	1·1
—	P.F. dil.	3·2
2·5	P.F. dil.	3·1
—	Substance *P*	6·0
2·5	Substance *P*	5·9
—	Histamine	5·6
2·5	Histamine	5·4
—	5 HT	6·2
2·5	5 HT	3·2

Salicylate, however, was without demonstrable effect on the increased vascular permeability caused by intradermal injections of synthetic bradykinin (10–100 µg/ml., Sandoz, Ltd.) partially purified ox blood bradykinin (50 µg/ml.) dilute permeability factor prepared by diluting rat serum 1 : 50 – 1 : 100 and standing 1 h before testing or 1 : 10 – 1 : 20 and leaving 14 min before testing[4]. Similarly, salicylate was without effect on the permeability factor formed in rats' plasma after the addition of turpentine[5]. The increased capillary permeability caused by intradermal injections of substance *P* (1–10 units/ml.) was also unaffected by salicylate in local concentrations of 2·5 mg/ml. However, local salicylate in these concentrations caused a 50 per cent suppression of the dye accumulation induced by intradermal injections of 5-hydroxytryptamine (5–20 µg/ml.).

In the guinea pig it was found that salicylate in a local concentration of 2·5 mg/ml. caused a total suppression of the leakage of circulating trypan blue due to the intradermal injection of 1 unit/ml. kallikrein, and a 50 per cent suppression of that due to 2 units/ml of kallikrein. As in the rat these doses of salicylate failed to modify the vascular response due to intradermal bradykinin.

Recently, Northover and Subramanian[6] showed that salicylate and phenylbutazone were effective in preventing the formation of kallidin from guinea pig and ox serum kallidinogen by the action of human salivary kallikrein and guinea pig serum kallikrein. These findings are consistent with the results of the present investigation. Bhoola, Calle and Schacter[7] have previously remarked on the similarity of serum 'globulin permeability factor' to the enzyme kallikrein. The suppression by local salicylate of the vascular effects of these factors is another point of resemblance. The failure of salicylate to inhibit what may be the respective active end products of these two substances, that is, kinins and dilute permeability factor, may also be significant in this regard.

It thus appears there is some support for the view[8] that part of the anti-inflammatory effect of salicylate may be due to the suppression of kallikrein-like enzyme systems which form permeability factors during inflammation. The results suggest also that, at least in the rat, systemic administration of salicylate may have effects which partially mask the more specific actions of the drug, demonstrable when salicylate is injected intradermally with substances which increase vascular permeability.

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Uniformity of the Contractile Response of Ventricular Muscle in High-Calcium Tyrode's Solution

Among several conditions related to the sequence and rate of stimulation which affect the contractile response of cardiac muscle, prematurity of an excitation, interposed in a regular sequence, results in a beat of reduced tension or shortening. The weakness of the premature beat is unrelated to factors of electrical excitability, time-course or amplitude of the transmembrane action potential or pre-contractile length of the muscle[1,2]. The possibility that developed tension or shortening may vary with the transient level of intracellular calcium (or calcium-containing 'activator') has been experimentally supported[3,4]. Unlike the various potentiating effects, such as the ascending limb of staircase, post-extrasystolic potentiation and post-stimulation potentiation, however, the weakness of a premature beat does not clearly follow from such a mechanism without additional assumptions which would predict a low intracellular calcium in the time-interval immediately following a contraction. The residual effect of any contraction on the succeeding one has thus been seen as consisting of separate positive (potentiation) and negative (restitution) components with different time courses of decay[5].

The present communication directs attention to the easily achievable variability of the negative inotropic effect and to the fact that an environment sufficiently high in calcium concentration abolishes all such effects, positive or negative. The resulting contractile response is fixed in a manner ordinarily uncharacteristic of heart muscle, being independent of all variations in the sequence and rate of contraction.

Cat papillary muscles or trabeculæ carneæ from the right ventricle were used. An experimental arrangement previously described[6] permitted sustained and moderately uniform electrotonic alteration of the transmembrane potential over a 0·25-mm length of the muscle. Tension development was recorded from this length of muscle and the transmembrane potential time course was recorded push–pull from the end farther from the polarizing electrodes. Composition of the Tyrode's solution was that conventionally used[7], except where indicated.

The developed tension in a very premature beat appears to vary with the phosphate content of the Tyrode medium, amounting to about 10 per cent of that of the driven beat in high (3·2 mM) phosphate and often exceeding 50 per cent of the driven beat tension in low (0·32 mM) phosphate Tyrode. Fig. 1 illustrates another factor, the time-course of the transmembrane potential, which influences the strength of contraction in the period following a driven beat. Two successive exposures are superimposed. The two inside curves are the electrical (depolarization upwards) and mechanical (tension downwards) records of a driven beat (rate 0·4/sec) followed by a premature beat, the action potential upstroke of which falls on the previous repolarization limb. The outer curves demonstrate a fact, previously reported[6], that tension (lowest trace) is maintained at near-peak levels during sustained membrane depolarization (highest trace). At a time when a premature beat delivers only 65 per cent of the driven beat tension, sustained depolarization is associated with a tension amounting to 85 per cent of the initial peak value. The weakness of the premature beat thus appears to depend on some factor associated with either the electrical repolarization process or with relaxation itself.

In a medium of composition equivalent to that of '12-fold calcium' Tyrode (6-fold calcium, 75 per cent sodium, see ref. 8), weakness of a premature beat as well as postextrasystolic potentiation are entirely abolished, as is shown in Fig. 2. The traces at the upper left show a driven beat followed by a stimulus so premature as to evoke only a non-propagated electrical response. When the second stimulus is delivered a bit later (upper right), the premature contraction is not at all reduced and fuses with the driven one. The lower traces show premature responses at progressively greater time intervals after the driven beats. All contractile responses have magnitudes within 5 per cent of the mean. In addition, the traces at the lower left represent events immediately following those on the upper right (driven rate 0·4/sec) and there is no postextrasystolic potentiation following a beat of extreme prematurity.

The contractile response in '12-fold calcium' Tyrode medium also did not vary with rate of stimulation, being fixed at the level shown in Fig. 2 at rates of 0·4/sec, 1/sec, and 6/sec. At the last rate, a contractile time-course close to the maintained tension with prolonged depolarization is obtained. This is shown in Fig. 3, where the electrical and mechanical events associated with continued flow of depolarizing

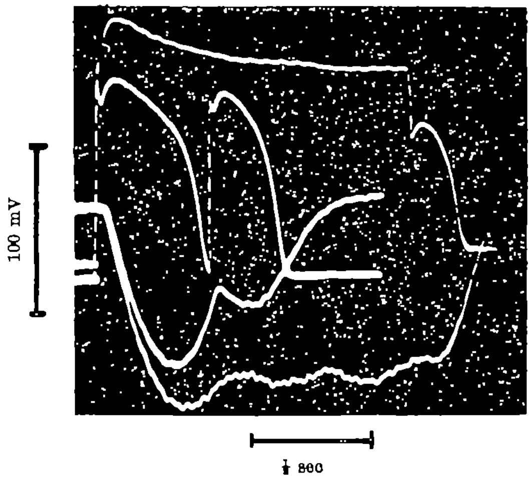

Fig. 1. Superimposed records of. a premature beat following a driven one (inner traces); an electrotonically induced contracture (outer traces). Transmembrane action potentials upward and isometric contractions downward. Action potential upstrokes and initial return from sustained depolarization are drawn in as dashed lines. Tension is of the order of 200 mg. Driven rate 0·4/sec. Normal Tyrode medium

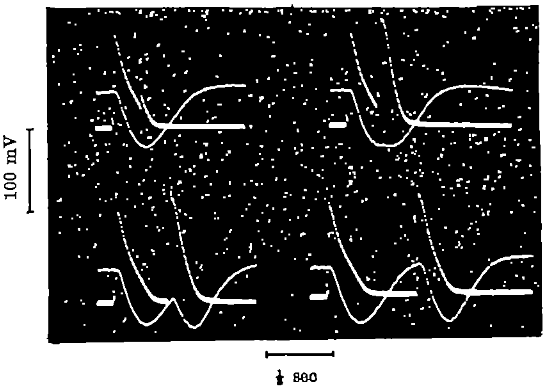

Fig. 2. Four successive driven beats, each followed by a premature stimulus (in the order left to right, lower traces after upper traces). Transmembrane potentials and isometric tensions as in Fig. 1. Peak tension is about 600 mg. Driven rate 0·4/sec. '12-fold calcium' Tyrode medium

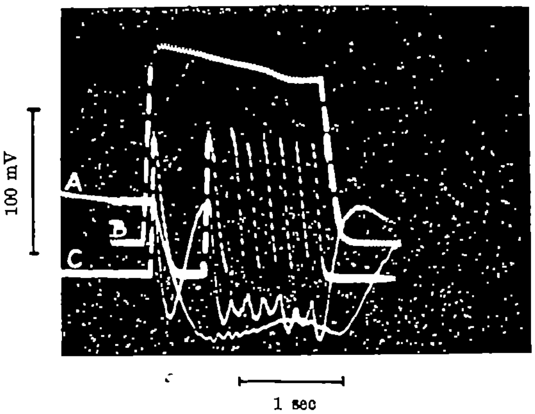

Fig. 3. Superimposed exposures. Upward-going traces display sustained depolarization (*B*) and the last of a series of action potentials at 0·6/sec, followed by a train at 6/sec (*C*). Downward-going traces (*A*) represent the tension time-courses associated with *B* (smooth contracture) and with *C* (incompletely fused contractions). The depolarization and repolarization limbs in *B* and the first two action potential upstrokes in *C* are drawn in as dashed lines. '12-fold calcium' Tyrode medium

current and with rapid repetitive stimulation are superimposed. With the abolition of the negative inotropic effect of a contraction on the succeeding one (restitution), a current contracture due to sustained depolarization is approached by the contractile response evoked by a fast train of action potentials. It may be that the restitution phenomenon is brought about by the release of a substance, governed by repolarization or relaxation, which competitively inhibits subsequent calcium entry into the cell.

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PHARMACOLOGY

Quasi-Cholinergic Action of Acetylating Agents

THE process by which acetylcholine causes muscle to contract presumably includes an action of acetylcholine on a definite site. The exact nature of this site, whether it is a small molecule, or a protein or enzyme, or a membrane, is still unknown. Although it is still inexactly defined, this substance or structure has acquired the presumably synonymous names 'cholinergic receptor' and 'acetylcholine receptor'[1,2]. This communication offers evidence that the receptor structure of smooth muscle includes a chemical grouping capable of being acetylated, and that this acetylation process is a part of the chain of events that leads to contraction.

The knowledge that *N*-acetoxytrimethylammonium bromide has an action resembling that of acetylcholine[3] and also that this substance can acetylate several sulphydryl compounds, including glutathione, coenzyme A, and mercaptoethanol, led us to test the effect of dilutions of acetic anhydride on conventional guinea pig ileum preparations[4]. It was found that small concentrations of acetic anhydride caused muscular contraction. Because acetic anhydride reacts with water with a considerable speed, its concentration at the time of contact with the ileum cannot be stated exactly, but the dilutions were made in an ice bath and were used within 1 min of mixing. Under these conditions, contractions were regularly observed at concentrations of 0·17 mg/ml. Measurements of *p*H at the end of each experiment showed only small changes: from 7·5 to 6·5, or in some longer experiments to 6·0. In the presence of atropine, the classical acetylcholine blocking agent, addition of acetic anhydride did not cause contraction. Hexamethonium did not prevent the contraction, a fact that suggests that the action is not upon the ganglia. More extensive experiments were performed with isopropenyl acetate, a more tractable acetylating agent[5]. This substance likewise stimulated the ileum, and was not sufficiently hydrolysed during the experiments to alter the *p*H of the medium. This substance likewise did not cause contraction when atropine was present. It will be recalled that Burgen, Burke and Desbarats-Schonbaum[6] showed that isopropenyl acetate is hydrolysed by cholinesterase faster than is acetylcholine. Tests showed that ileum that had been stimulated by isopropenyl acetate was still responsive to subsequent addition of acetylcholine, *N*-acetoxytrimethylammonium, or to additional portions of isopropenyl acetate. Results with acetylimidazole prepared according to Boyer[5] were, in general, similar. Here again we observed stimulation by the substance, and prevention of stimulation by atropine. Stimulation by acetylimidazole did not prevent subsequent stimulation by acetylcholine.

Table 1. QUASI-CHOLINERGIC ACTION OF ACETYLATING AGENTS

Substance	Stimulatory concentration (mg/ml.)	Concentration of atropine inhibiting (mg/ml.)
Acetic anhydride	0·17	0 00017
Isopropenyl acetate	0·42	0 00017
Acetyl imidazole	0·067	0 00017
N-Acetoxytrimethylammonium bromide	0·001	0 00005
Acetylcholine bromide	0·00002	0·00013

The results are summarized in Table 1. As four of these substances are acetylating agents capable of reacting with sulphydryl groups, the results hint that the receptor site may include a sulphydryl group. This must for the present remain conjectural, since other groups (for example, hydroxy, amino, imidazol) capable of acetylation cannot be excluded.

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Enhancement of the Intestinal Absorption of Glucose by Small Doses of Cetrimide and Sodium Lauryl Sulphate

AFTER the growth-promoting activity of aureomycin was discovered in chicks and piglets, some surface-active germicides were tried as feed-additives, and Ely[1] reported a parallel effect with synthetic detergents such as lauryl ethylene oxide. Stern and McGinnis[2] obtained equal growth responses on the addition of vitamin B_{12}, aureomycin or detergent to Ely's basal diets, so that Ely's results were attributed to supplementation of a dietary deficiency in this vitamin, and not to primary growth stimulation. However, claims that related compounds, such as trimethylhexadecyl-ammonium stearate, possessed primary growth stimulating activity persisted. In attempting to clarify the problem during the present investigation, both trimethyl-hexadecylammonium stearate and its corresponding bromide ester, cetrimide, were tested on three-week old and adult albino and *C3H* mice by incorporating the drugs in diet 41 in concentrations ranging from 0·0025 to 0·1 per cent, but no favourable effect on growth was obtained[3,4]. With higher concentrations (0·1–1 per cent), loss of weight was marked, and post-mortem appearances did not differ from those of inanition produced by simple food reduction[5].

In intestinal perfusion experiments, carried out *in vivo* on mice, rats and rabbits, the cationic quaternary ammonium germicides such as cetrimide showed striking inhibitory effects on the absorption of nutrients[6,7]. The anionic sodium lauryl sulphate had weaker inhibitory activity. Furthermore, both potentiated greatly the inhibitory effects of phloridzin on glucose and sodium butyrate absorption[8,9].

When the investigation of the effect of cetrimide by the intestinal perfusion method was extended to low concentrations, the substance showed an enhancing effect on the absorption of glucose, but not of sodium butyrate or methionine. The percentage absorption increased from 35·0 ± 1·0 in the control to 51·3 ± 4·5 at a concentration of 0·0005 per cent of cetrimide ($P < 10^{-5}$). This represents a rise of 46·6 per cent, and is probably close to the peak of the stimulant effect (Table 1 and Fig. 1). Inhibitory activity appeared at higher concentrations. At 0·001 per cent, the percentage absorption was 47·8 ± 2·1, which still differed appreciably from the control ($P < 10^{-10}$), but at a concentration of 0·002 per cent the stimulant effect was almost completely nullified. The number of experiments at each dose-level is given in Table 1.

Sodium lauryl sulphate showed similar stimulating activity at a concentration of 0·001 per cent. The percentage absorption was 51·4 ± 2·8, representing a rise over the control of 46·9 per cent, with *P* value $< 10^{-10}$. Higher concentrations reversed this effect, and significant inhibition was established at 0·1 per cent.

The enhancement of absorption at low concentrations of these drugs is unlikely to be a non-specific effect due to surface-activity of the compounds, for it is absent in the case of sodium butyrate and methionine. An even more pertinent observation is that these same stimulating concentrations of the two compounds produce striking inhibition, with absorption percentages for glucose of 7·0 and 16·5 per cent with cetrimide and sodium lauryl sulphate respectively (4 experiments each), when combined with a concentration of 0·001 per cent of phloridzin. When phloridzin was used by itself at this concentration it had scarcely any effect, giving a percentage absorption of 34·4 per cent for the mean of eight experiments.

Whether these findings provide an alternative explanation for Ely's results is doubtful, for no stimulant effect has been obtained by putting mice on diets containing small concentrations of cetrimide, the stearate ester or sodium lauryl sulphate. The possibility that the stimulant effect is nullified by some food component must, however, be considered. The foregoing findings may prove of value in clinical malabsorption.

Table 1

Drug concentration (%)	Percentage absorption of glucose from perfusion fluid. No. of experiments in parentheses Cetrimide	 Sodium lauryl sulphate
0·0	35·0 ± 1·0 (37)	35·0 ± 1·0 (37)
0·00025	40·9 ± 4·3 (6)	
0·0005	51·3 ± 4·5 (6)	
0·001	47·8 ± 2·1 (20)	51·4 ± 2·8 (11)
0·002	36·1 ± 1·7 (31)	
0·01	25·2 ± 2·0 (14)	34·9 ± 1·9 (6)
0·1	3·7 ± 1·5 (4)	20·8 ± 1·3 (6)

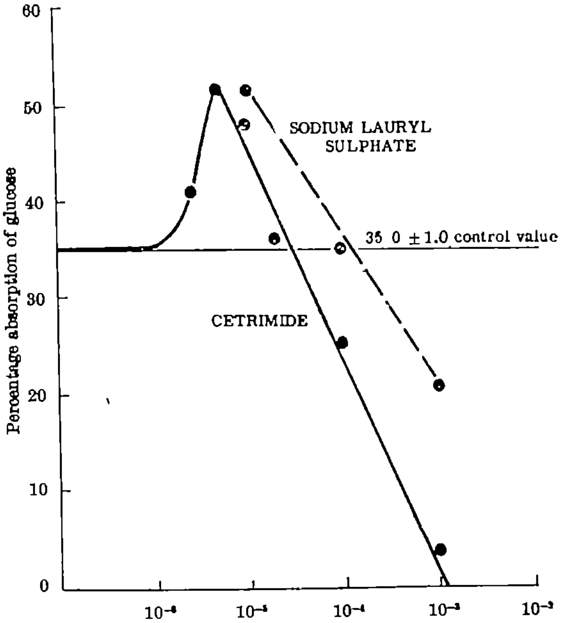

Fig. 1 Percentage absorption of glucose—out of a total of 50 mg in 25 ml. fluid—in ½ h intestinal perfusion experiments in mice. Low concentrations of cetrimide and sodium lauryl sulphate had a stimulant effect and high concentrations an inhibitory effect as compared with the control

The enhancement of glucose absorption in perfusion experiments with cetrimide and sodium lauryl sulphate is also of theoretical interest in view of the rate theory of drug action put forward by Paton[10], according to which antagonists would be expected to possess stimulant activities at low concentrations. The circumstances for which this theory holds remain to be elucidated, but it is interesting that acetylcholine antagonists studied by Paton in his drug-receptor investigations included trimethylalkylammonium compounds.

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'Cross-Protection' Method as a Means of Differentiating Receptors

In 1954 Furchgott[1] described a method for determining whether two drugs act on a common receptor. One of his experiments may be used to illustrate this. A spiral strip of rabbit aorta was exposed to 3 × 10^{-6} dibenamine for 20 min either with no other drug in the bath or with 10^{-4} adrenaline present. The tissue was washed after the 20-min exposure and sensitivity to noradrenaline was tested. This was zero when dibenamine had been given alone, but still 20 per cent of normal when adrenaline had been present. It was presumed that the adrenaline occupied an appreciable fraction of the receptors and thereby protected them from combination with dibenamine and consequent blockade. "From results such as these it is concluded that epinephrine, norepinephrine and isopropylarterenol react with the same set of contraction receptors in the aortic smooth muscle."

This method has since re-appeared periodically as a tool for demonstrating that two drugs act on the same receptor. Pharmacologically, however, the basis of the method is not sound. The purpose of this communication is to point this out and demonstrate why. Two pharmacological concepts are involved here. (1) There is no *a priori* reason (as Furchgott himself points out) to suppose that any drug combines with only one site. Usually this appears to be the case in practice since the concentration is kept low so as to produce a pharmacologically effective drug-receptor combination only at the most sensitive site(s). But in the cross-protection method a large dose of the protecting agent is used so it is to be expected that an appreciable occupancy of many sites will result. (2) There is considerable evidence that stimulant drugs can stimulate a tissue markedly when only a small proportion of the receptors involved are free. (Whether this be pictured as 'spare-receptors' or 'spare-receptor capacity'[2] is immaterial here.) Consideration of these two concepts shows that the original conclusion is not unique. As an example let us consider a given receptor pool. We may picture a drug which (in the usual pharmacological concentrations) combines negligibly with it. However, at the much higher doses generally used for protection, this drug might occupy and protect say 5 per cent of receptors. This number of receptors would probably be quite sufficient for the production of a strong effect by a second drug (which is characteristically a strong agonist). Thus two drugs would appear to 'react with the same set of receptors', but the interaction would be of little or no pharmacological or physiological significance.

The converse conclusion, that is, that absence of protection is evidence that the two receptors are different, is also not rigorous. This is especially true if the stimulant drugs behave as Paton[2] has suggested, that is, that their drug-receptor interaction is associated with a high turnover-rate. The blocking agent could then have access to all the receptors even during a relatively short exposure and the degree of protection could be negligible.

The method of 'cross-protection' seems therefore to be rather less useful than is generally recognized.

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PATHOLOGY

Effect of Thiouracil on the Development of Mammary Tumours in Rats induced with 9,10-Dimethyl-1,2-Benzanthracene

THE induction of mammary tumours in rats by the oral feeding of a chemical carcinogen has been found to depend on the hormonal state of the animal[1-3]. This communication deals with the attempted production of such mammary tumours by the oral administration of 9,10-dimethyl-1,2-benzanthracene (DMBA) in rats made hypothyroid with propylthiouracil (PTU).

Table 1. SUMMARY OF METHODS AND GROUPS

No. of rats	PTU	DMBA
30	5 mg daily for 95 days	10 mg once/week. Reduced to 5 mg after 4 weeks. Stopped at 95 days
80	None	As above
80	5 mg daily for 95 days	None

Table 2. MAMMARY GLAND TUMOUR INCIDENCE IN RATS GIVEN DMBA AND DMBA + PTU

Days after first administration of DMBA		60	80	100
Rats with tumours	DMBA	10/24	16/22	20/20
	PTU + DMBA	1/22	4/21	7/14
P*		0·003421	0·000503	0·000637

* P was calculated by the method of Yates and Fisher (see *Statistical Methods for Research Workers*, Fisher, R. A., thirteenth ed., 96 (1958)).

Non-inbred rats descended from stock imported from Sprague–Dawley of Madison, Wisconsin, and commercially available in Britain were bought at 40 days of age. After a formal randomization process they were divided into groups for treatment as shown in Table 1.

10 mg DMBA, dissolved in 0·5 ml. corn oil, was given by gastric tube once weekly, the first dose at 50 days of age. 5 mg PTU, suspended in 0·5 ml. of sterilized arachis oil, was given by mouth daily from 50 days of age.

Of the group given PTU + DMBA mammary gland tumours were found in 1 of 22 rats alive at 60 days, 4 of 21 rats at 80 days and in 7 of the surviving 14 rats at 100 days (Table 2). Corresponding figures for DMBA alone were 10 rats with tumours of 24 alive after 60 days, 16 of 22 rats at 80 days and 20 rats with tumours of 20 after 100 days. The difference in the numerical incidence of mammary tumours between rats having DMBA + PTU and rats having DMBA alone is highly significant statistically ($P < 0·01$). Mammary gland tumours were not seen in any rat given only PTU. The mortality of the rats given PTU + DMBA was somewhat higher than that of the rats given only DMBA.

Jull and Huggins[2] showed that after DMBA, fewer rats developed mammary tumours in thyroidectomized animals than in intact controls. Our results indicate that hypothyroidism chemically induced by PTU is capable of producing the same effect. Jull and Huggins have suggested that this might be the result of diminished caloric intake.

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Uptake of Proteins by Malignant Cells

A RECENT immunohistochemical investigation of *p*-dimethylaminoazobenzene-induced hepatomas in rats[1] has supported the view that plasma proteins can be taken up by cancer cells without prior extracellular hydrolysis[2]. However, hepatic parenchymal cells after only minor injury are known to be permeable to serum proteins[3]. We have therefore studied by direct and indirect fluorescent tracing methods[4] the uptake of serum proteins in tumours arising in other tissues.

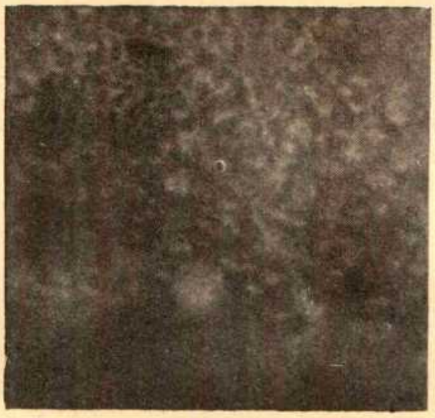

Fig. 1. Frozen section of solid Ehrlich carcinoma taken 3 h after intravenous injection of rabbit serum conjugated with *RB* 200. The fluorescent protein, sometimes distinguishable as droplets, is present in the cytoplasm of the malignant cells above but not in the narrow zone of normal subcutaneous tissue below. (× *c* 80)

The tumours used were primary benzpyrene-induced fibrosarcomas in adult (240 g) Wistar rats, and Ehrlich ascites tumour, Landschutz tumour, *S* 37, and *T* 2146 maintained in the ascites form by serial transplantation in Swiss albino mice (25 g) bred in a closed colony. The ascites tumours were also used in the corresponding solid form obtained by subcutaneous transplantation.

For direct tracing, whole normal rabbit serum conjugated with lissamine rhodamine *B* (*RB* 200) was injected at a concentration of 58 mg protein/ml.: in the rats 2 ml. doses were injected through the jugular vein and in the mice 0·4 ml. through the tail vein. For immunofluorescence tracing, a γ-globulin fraction (separated from normal human serum by passage through DEAE-cellulose using 0·0175 M phosphate buffer at *p*H 6·3) was injected at the same dose and by the same routes.

Animals were killed 1–3 h after the injection, and tissue blocks both from tumour-bearing and normal animals were taken immediately and snap frozen at − 76° C. On the same day, solid tumours and samples of liver, spleen, kidneys, lungs, gonads, adrenals, lymph nodes, muscle and subcutaneous tissue were studied by sectioning at 5μ in a cryostat at −20° C. The ascites tumours were examined as air-dried smears which were treated in the same way as the sections.

In the direct tracing experiments, the sections were fixed in 95 per cent ethanol for 30 sec, rinsed in phosphate-buffered saline (*p*H 7·1) and mounted in buffered glycerol. In the immunofluorescence experiments, the unlabelled human globulin was located in ethanol-fixed sections treated for 30 min with goat anti-human-globulin conjugated with *RB* 200. Tissues from normal and from tumour-bearing animals not injected with human γ-globulin served as controls, and specificity of fluorescent staining was assessed by treating parallel sections with labelled goat anti-rabbit-globulin, which failed to stain, and by the demonstration of blocking of staining in preparations pre-treated with unlabelled anti-human-globulin.

Examination of preparations by fluorescence microscopy showed that the only normal tissues to take up exogenous serum proteins were the Kupffer cells of the liver, and to a lesser extent the phagocytes of spleen and an occasional littoral cell in the adrenal medulla; none was found in the parenchymal cells of any organ. In contrast, the ascites tumour cells as well as the cells of the solid neoplasms showed in the cytoplasm a substantial amount of protein conjugate in the direct tracing experiments (Fig. 1) and of the γ-globulin in the immunofluorescence experiments. There was no detectable conjugate or γ-globulin in the necrotic areas or the stromal cells of the solid tumours. In the cytoplasm of ascites tumour cells the administered proteins were distributed diffusely whereas in the solid tumour they often appeared as droplets. The serum proteins were not found in the nucleus of any cell.

Thus it would appear that the property of direct protein uptake reported in hepatoma cells is shared by malignant cells of other primary and transplanted tumours. This process is distinct from the known capacity of tumour cells and all rapidly growing tissues to sequestrate amino-acids from the common metabolic pool[5], though both phenomena fit Mider's apt description of tumours as nitrogen traps[6]. Whether the protein uptake is attributable to increased permeability of tumour cell membrane[7] or to a more active process remains to be determined.

We thank Prof. A. Haddow, Chester Beatty Research Institute, London, for supplying the Wistar rats with benzpyrene-induced fibrosarcomas. This work was supported by grants from the Medical Research Council and the Scottish Hospital Endowments Research Trust.

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Familial Hypothalamic Diabetes Insipidus in Rats

WE are at present raising a strain of rats with hypothalamic, vasopressin-sensitive diabetes insipidus. This disease arose as an apparently spontaneous mutation along with a mutation for albinism in some of the affected animals, from a pure strain of Long-Evans hooded rats being bred for an unrelated research problem not involving radioactivity. The original litter of 17 included 4 albino females with diabetes insipidus, one albino male without diabetes insipidus, and one hooded male and one hooded female each with diabetes insipidus.

Response of one of the diseased female rats to injections of vasopressin tannate in oil ('Pitressin Tannate in Oil', Parke, Davis and Co.) is illustrated in Fig. 1. Each point represents a 24-h collection period, either in the untreated state or from one to five days after a single injection of 0·5 pressor unit. The graph is a composite of several experiments and represents the response to progressively diminishing blood titres of vasopressin. During the period of these tests the mean weight of this rat was 260 g, and the mean water intake in the untreated state was 105 g water/100 g

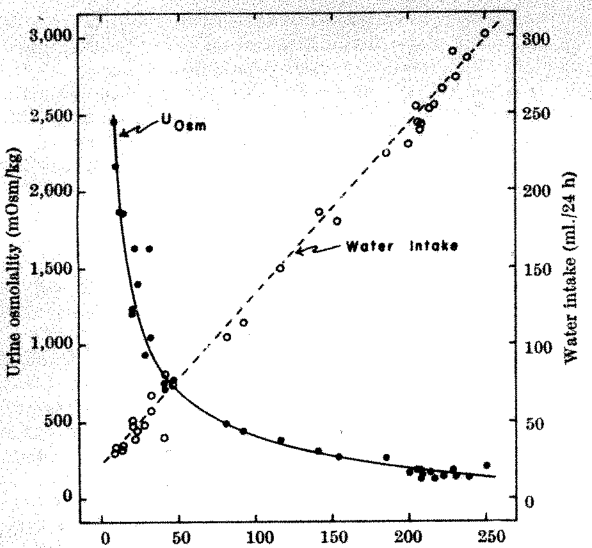

Fig. 1. Urine flow, urine osmolality and tap water intake of a single rat with familial hypothalamic diabetes insipidus, both untreated and after subcutaneous injections of vasopressin tannate in oil

body-weight/24 h, as compared with 14 g water/100 g body-weight/24 h of control female rats. While 6 h of dehydration in this rat caused reduction in urine flow of about 50 per cent, urine osmolalities did not rise above 250 mOsm/kg. These periods of dehydration were accompanied by a mean weight loss of 19 g or 7·5 per cent of body-weight. The urine was negative for reducing substances, and the rats had a normal non-fasting blood glucose concentration of 103 mg per cent. The pars nervosa of the pituitary gland of this rat showed a reduction in neurosecretory material to about one-third of normal, when stained by the aldehyde fuchsin method. Other physiological, anatomical and pathological studies are under way.

Although the disease has been carried into the *F*2 generation, the mode of inheritance is not yet fully defined. There is a high degree of semi-sterility, especially in the males, and a high incidence of still-births and runts. Thus far all the diseased animals in the *F*1 and *F*2 generations are hooded; of these 9 are males and 12 are females. In contrast to the disease in man[1,2], the diabetes insipidus in our rats is inherited neither as a simple dominant nor as a sex-linked trait. Albinism without co-existent diabetes insipidus also has been carried into the *F*2 generation, and is inherited as an autosomal recessive.

So far as we can determine, familial hypothalamic diabetes insipidus has not been reported in a common laboratory animal. In the mice described by Silverstein *et al.*[3] excessive thirst appears to be the primary defect, whereas in our animals the water deprivation test and histological picture indicate that some defect in production of anti-diuretic hormone is primary.

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IMMUNOLOGY

Prevention of Delayed Foreign Marrow Reaction in Lethally Irradiated Mice by Early Administration of Methotrexate

A MORTAL wasting disease occurs in lethally irradiated mice which have been engrafted with allogeneic marrow that is capable of reaction against host tissue antigens. The disease appears late, usually several weeks after exposure to radiation. Two components have been described: an early injury to the host, particularly to the lymphoid areas, by immunologically competent lymphoid cells contained in the marrow injection; and a delay in the restoration of the host's lymphoid centres which increases mortality by leaving the host vulnerable to infection and intercurrent disease over a prolonged period[1].

One puzzle has been why the immunologically competent cells of the foreign marrow are not 'paralysed' in the presence of excess host tissue antigen. If time is required and if the critical injury to the host occurs in the first few days, before tolerance or paralysis develops, it might be expected that the restraining action of methotrexate on immunological response[2] and foreign marrow reaction[3] would be most efficacious if administered promptly.

Table 1. EFFECT OF VARIED SCHEDULES AND DOSES OF METHOTREXATE ADMINISTRATION ON MORTALITY FROM FOREIGN MARROW REACTION IN IRRADIATED *BDF*$_1$ (*C57B*1/6*J* × *DBA*/2*J*) MICE INJECTED WITH PARENTAL *C*57 BLACK MARROW

Group	Treatment	Survivors on post-irradiation days		
		10	30	90
1	850 r. (250 kV, X-ray)	6/10	0/10	0/10
2	850 r. + *BDF*$_1$ marrow	10/10	10/10	10/10
3	850 r. + *C57B*1 marrow	9/10	6/10	1/10
4	850 r. + *C57B*1 marrow. Methotrexate 1·5 mg/kg intraperitoneally on post-radiation day 1, 3, 6 and 8	10/10	10/10	10/10
5	850 r. + *C57B*1 marrow. Methotrexate 1·5 mg/kg intraperitoneally three times weekly for 5 weeks beginning on day 1	9/10	7/10	5/10
6	850 r. + *C57B*1 marrow. Methotrexate 0·25 mg/kg intraperitoneally three times weekly for 5 weeks beginning on day 1	10/10	10/10	9/10
7	850 r. + *C57B*1 marrow. Methotrexate 1·5 mg/kg intraperitoneally three times weekly for two weeks beginning on day 14	9/10	2/10	1/10

* Irradiated mice were randomly divided into experimental groups as listed. Each mouse received 16–24 × 10^6 nucleated marrow cells intravenously on the day of radiation. Experiments comprising Groups 1, 2, 3 and 4; Groups 1, 2, 3 and 5; and Groups 1, 2, 3 and 7 were done on three occasions with results similar to those presented. Long-term survivors of Group 4 had hæmoglobin with the electrophoretic mobility of *C*57 black.

As shown in Table 1 this is the case. When methotrexate is administered beginning on the day following the infusion of marrow (Group 4), no mortal wasting disease, no loss of weight beyond that seen in controls given isogeneic marrow, occurs in irradiated *BDF*$_1$ mice injected with parental *C*57 black marrow. When the injections of drug are prolonged, loss of weight and death from drug toxicity are seen (Group 5); unless a lower dose is used (Group 6). When the injections are started late (day 14) the effectiveness of the treatment is less and an appreciable mortality occurs (Group 7). It is striking that the animals treated early and briefly with drug never become ill and wasting is prevented; whereas with those treated late, survival is a matter of recovery from a period of serious wasting.

The observations suggest that if the reaction of immunologically competent cells in the marrow infusion can be restrained during a short interval, either these cells pass through their life-span and die without injuring the host, or they and their progeny acquire tolerance to their environment without first having injured it and themselves by reaction.

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Immunological Investigation of a Christmas Factor Inhibitor by Means of Boyden's Technique

THE question whether the circulating anticoagulants are antibodies is still unsettled. An attempt was made to examine the Christmas factor inhibitor (factor IX inhibitor, or P.T.C. [plasma thromboplastin component] inhibitor) using an immunological method. The method of passive hæmagglutination, or Boyden's technique[1], was chosen because it is one of the most sensitive immunological techniques.

The antigen used was the P.P.B. (prothrombin, proconventin, antihæmophilic factor B) or the C.S.B. (convertin, Stuart factor antihæmophilic factor B) fraction prepared at the Coagulation Laboratory of the National Blood Transfusion Centre in Paris, according to a method improved by Didisheim, Loeb, Blatrix and Soulier[2].

The P.P.B. fraction is prepared from human plasma. It contains: prothrombin, proconvertin, the Stuart factor and the Christmas factor. These factors are extracted by adsorption on tricalcium phosphate. After washing they are eluted out by trisodium citrate at *p*H 8. Two successive ethanol precipitations allow a more extensive purification.

The C.S.B. fraction is prepared by the same method from human serum where prothrombin is consumed. It therefore contains only convertin, the Stuart factor and the Christmas factor.

These two fractions were used interchangeably in this investigation with identical results.

74 sera were tested against this antigen (Table 1).

Table 1. RESULTS OF BOYDEN'S TEST ON 74 SUBJECTS. ERYTHROCYTES TREATED WITH TANNIC ACID AND COATED WITH THE C.S.B. FRACTION

No. of cases	Diagnosis	Test for anticoagulant	Boyden's test
3	Christmas disease	Positive: Christmas factor inhibitor	Positive*
10	Christmas disease	Negative	Negative
4	AHG deficiency	Positive: against AHG:	Negative
13	AHG deficiency	Negative	Negative
1	Hæmorrhagic syndrome with acquired anti-coagulant	Positive: against AHG	Negative
1	Stuart factor deficiency	Negative	Negative
3	Thrombocytopænic purpura	Positive: against thromboplastin	Negative
1	Pan hæmocytopænia	Positive: against thromboplastin	Positive†
1	Myeloma	Positive: against thrombin	Negative
14	Multiple transfused patients with various blood disorders	Negative	Negative
23	Normal subject	Negative	Negative
Total 74			

* Titres were, respectively: 1 : 320; ⩾ 1 : 1,280; ⩾ 1 : 160.
† Titre, 1 : 20.

(1) 3 sera obtained from patients with Christmas disease (factor IX deficiency) and circulating anticoagulant active against Christmas factor.

(2) 71 control sera: Christmas disease without circulating anticoagulant (10 cases), antihæmophilic globulin (AHG) deficiency with (4 cases) and without (13 cases) circulating anticoagulants (AHG inhibitor), various hæmorrhagic disorders with circulating anti-coagulants active against AHG, exogenous thromboplastin, and thrombin (7 cases), multiple transfused patients (14 cases), normal subjects (23 subjects).

Boyden's test was strongly positive with the 3 first sera and negative with 70 of the 71 control sera. One test was unexpectedly weakly positive. It concerned a panhæmocytopænic patient. The serum contained a circulating anticoagulant of the antithromboplastic type. The serum was kept frozen for two years before being used. The tests made in 1959 showed an antithromboplastin activity. The inhibition tests of the AHG and the Christmas factor had not been performed at that time. They were negative in 1961 with the preserved serum. These results constitute, therefore, a discrepancy between the coagulation tests and the immunological test. Various hypotheses may be given to explain this discrepancy:

(1) The lack of specificity of the immunological reaction appears improbable since all the other tests gave satisfactory results.

(2) The C.S.B. fraction used as antigen could contain traces of thromboplastin. This hypothesis is contradicted by theoretical impossibility and by the fact that other anticoagulants of the antithromboplastin type gave negative results.

(3) The serum from this patient could contain a Christmas factor inhibitor, not detected by the coagulation tests, which may be less sensitive than the immunological tests.

This unexplained case does not seem to invalidate the technique used here, which showed itself in all other cases specific and reproducible.

A neutralization test of the Christmas factor inhibitor by the C.S.B. fraction was carried out. Both the anticoagulant activity and the hæmagglutination activity were inhibited by the C.S.B. The inhibition of the agglutination is analogous to the neutralization of an antibody by its antigen.

The serum obtained from a patient with Christmas disease, and containing an anticoagulant, was fractionated. The anticoagulant activity was tested by the thrombo-elastographic (TEG) method. The anti-coagulant activity was measured by the lengthening of the interval '*r*' on the TEG of normal plasma rich in platelets after contact with the various fractions from serum containing the anticoagulant. This lengthening was compared with that induced by the analogous fractions obtained from a normal serum. These fractions were simultaneously tested by Boyden's technique. Both the hæmagglutination activity and the anticoagulant activity are located in the γ-globulin fraction. The α-β-globulin fraction shows a weak activity, due perhaps to an incomplete separation of the fractions.

These results support the hypothesis that the anticoagulant and the agglutinating substance are identical.

Very substantial arguments may therefore be brought forth in favour of the hypothesis which states that this Christmas factor inhibitor is an antibody:

(1) Boyden's reaction is positive when one uses the Christmas factor as the antigen.

(2) The serum-like substance taking part in Boyden's reaction as well as in the coagulation test is a γ-globulin.

(3) It is neutralized by the Christmas factor in the same manner as that in which an antibody is neutralized by its antigen.

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BIOLOGY

Conjugate Spermatozoa of the North American Opossum

IN 1887 Emil Selenka[1] published a monograph describing the reproductive processes, and the embryology, of the North American opossum (*Didelphys marsupialis* L.). One of the many observations made on a colony of these animals, which Selenka maintained in Erlangen, Germany, was that the semen, recovered from the vagina of females shortly after coitus, contained many spermatozoa which swam in closely coupled pairs. Selenka referred to these paired cells as "copulating" spermatozoa, and the drawings which he published showed clearly that these paired spermatozoa are associated in a specifically oriented manner. In 1902, von Korph[2] showed that the paired spermatozoa occurred in the epididymis, but not in the testes. This observation was confirmed later by Retzius[3], Jordan[4], and finally by Duesberg[5] in 1920. Since that time the phenomenon has been briefly referred to only by Wilson[6], in 1928, who recommended the phrase 'conjugate spermatozoa' instead of 'copulating spermatozoa', and by McGrady[7] in 1938. In the past quarter of a century the phenomenon has been ignored, and is a notable omission from contemporary reviews on spermatozoa.

The purpose of this communication is to emphasize the fact, not appreciated hitherto, that conjugate spermatozoa are a normal occurrence in *D. marsupialis*, and to direct attention to a phenomenon which has many features of interest to cell biologists.

Since the beginning of January 1961 we have collected genital tracts from many male opossums live-trapped in south-eastern Pennsylvania.

The testes and epididymides of most of these individuals were fixed in Bouin's fixative shortly after death, but in a few cases spermatozoa were expressed from the epididymis and examined in the living state. To date, 59 male opossums containing epididymal sperm have been examined, and double spermatozoa have been observed in 55 of these individuals. One of the four specimens not containing conjugated spermatozoa was dead several hours before autopsy, and therefore the spermatozoa may have separated during this time; however, we noticed nothing unusual about the other three specimens.

Fig. 1 is a high-power photograph of a section of the contents of the epididymis, showing the heads of several conjugated spermatozoa. In the majority of specimens it is easy to find cross-sections of epididymides, containing considerable numbers of these paired cells. However, it is impossible at the present time to estimate the proportion of spermatozoa that are linked in pairs, since any mechanical manipulation of the epididymis is likely to separate them.

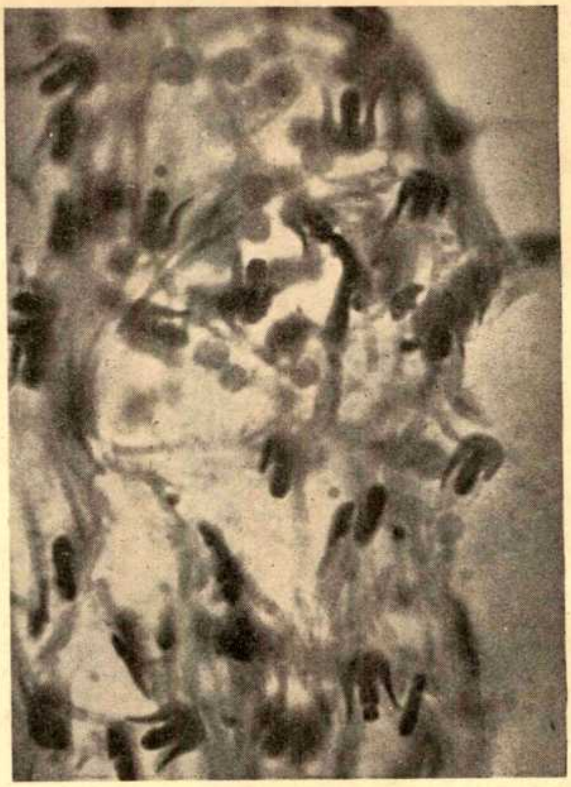

Fig. 1. Section of the contents of the epididymis of an opossum, showing several conjugated spermatozoa. (× *c*. 2,330)

The single spermatozoon of *Didelphys* is illustrated in Fig. 2. Its predominant peculiarity is the hook-shaped head, with one thickened portion and one longer more-pointed portion. The mid-piece of the cell is attached by a very fine filament to the centre of the concavity formed by the long and short portions. Fig. 3 shows two conjugated spermatozoa. All pairs unite by the exact juxtaposition of the shorter portions of the two heads, so that the combined bicellular structure is bilaterally symmetrical about the line of junction. We have examined many hundreds of these pairs of epididymal spermatozoa, and all of them are exactly aligned with this highly specific orientation.

Several of the early workers noted that these conjugate spermatozoa do not occur in the testes. We can fully confirm this fact, in that no conjugate spermatozoa have been seen in any sections of the testes. However, in smears prepared from two testes after tryptic digestion occasional pairs have been seen. It is possible that these may have been derived from the rete testis. Thus it seems that, in the North American opossum, the mature spermatozoa are liberated in the seminiferous tubules as single cells, and that somewhere between these tubules and the epididymis many of them link up in conjugated pairs. How this highly oriented linkage, which is quite distinct morphologically from head agglutination, comes about is a challenge for those cell biologists interested in problems of cell adhesion.

In six cases we have expressed the fresh spermatozoa from the epididymis into a physiological saline

(PBS). This causes a typical activation phenomenon in which all the cells swim violently. However, even under these conditions the conjugate spermatozoa remain paired, swim very powerfully, and describe a linear course of movement. This is in striking contrast to the circular course described by the single cells. Thus the motion of the double and single epididymal cells which we have observed is very similar to the motion of the double and single cells recovered from the vagina of female opossums by Selenka[1]. The mechanics of the motion of the conjugated pairs of spermatozoa has not yet been examined, and seems to pose a fascinating problem to those interested in the kinetics of movement by flagellated cells.

The linkage between the paired cells is strong, but does not involve cellular fusion. This can be easily observed in cells under adverse conditions. We have observed many pairs where one flagellum becomes anchored to the microscope slide. The remaining free flagellum may beat violently, but never strongly enough to free its head from the immobilized partner. Nevertheless, as the cells become exhausted the union between the two heads loosens, until the short portions of the heads of each cell are able to rock on each other. This phenomenon, which proves that cell membranes do not fuse, eventually results in separation of the conjugated cells just before the motility of both cells ceases.

The purpose of conjugated spermatozoa is still obscure. Selenka's early observations show that the pairs enter the female opossum at ejaculation. Whether the conjugation is essential for the ascent of the spermatozoa up the curious lateral vaginal canals of the female opossum is still unknown. What does seem clear is that more positive movement is carried out by the conjugated pairs than by single spermatozoa. If conjugation is a necessity for bringing spermatozoa and ova together, the events immediately prior to fertilization and fertilization itself may be of unusual interest.

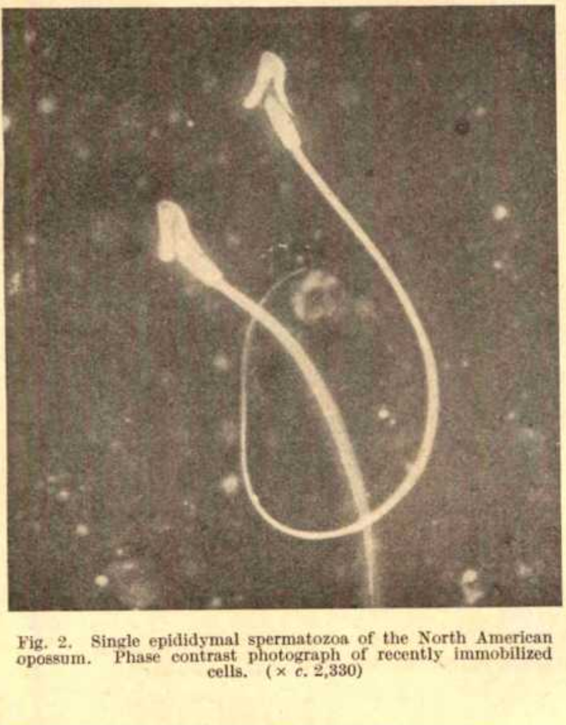

Fig. 2. Single epididymal spermatozoa of the North American opossum. Phase contrast photograph of recently immobilized cells. (× c. 2,330)

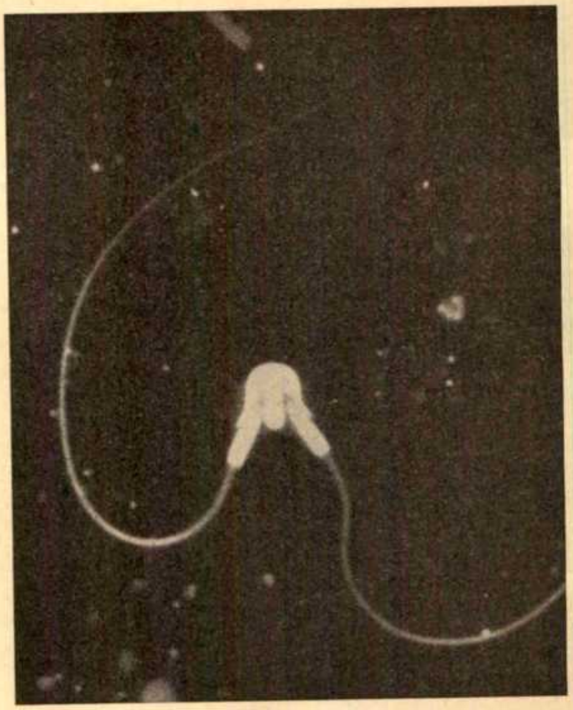

Fig. 3. Conjugated pair of spermatozoa obtained from the epididymis of the North American opossum. Phase contrast photograph of recently immobilized cells. (× c. 2,330)

The uniqueness, or otherwise, of conjugation between pairs of spermatozoa is of particular interest. To the best of our knowledge no adequate study has been made on the spermatozoa of Central and South American opossums, or on those of the Australasian marsupials other than that on the brush-tail possum (*Phalangista vulpiana*) by von Korph[2], who states that conjugate spermatozoa do not occur.

The phenomenon may not be unique, since very similar conjugate spermatozoa have been described in the water beetle (*Dytiscus marginalis* L.)[8] and the gastropod mollusc (*Turritella terebra* L.)[9]. Further investigations are required in the latter two cases, for it seems that at least in *Dytiscus* more than two cells can be involved in the linkage.

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Structure of the Sucker of *Echeneis*

A CENTURY ago Gunther[1] stated thus: "There is scarcely a fish of the existence of which the ancients have been equally certain and which has so much occupied their imagination—from a power thought to be inherent in the creature to counteract the strongest physical agencies—as the *Echeneis* of the Greeks or the *Remora* of the Latins". This was followed in 1918 by Gudger[2], who called it "The most interesting of the marine fishes not merely from the popular but even more from the scientific point of view". He, however, could not reconcile himself to the view that the fish could exert enormous pressure, and accepting Ekman's "deadwater theory" said "Another myth of the ancients is dissipated in thin air". Norman[3] describes the sucker as being a highly specialized structure but goes no further. I have directed attention[4] to the presence of a pear-shaped body, the pyriboss, and a chamber in front of it, and the possibility that the plates are lowered rather than raised adhering. Mention is made of the pyriboss as "a curious round ossification" and the chamber as "a notch at the posterior extremity" by Storms[5].

Fig. 1, I, shows the following parts of the pumping mechanism: (1) The basal plate of the hind region specially enlarged, flattened and directed backwards called the basiplatt. (2) A very small oval or polygonal plate in the middle line immediately behind the basiplatt called the basilid because it is a covering placed over an opening. (3) Then comes an arch which is partly overlapped by the two pieces mentioned already. (4) The front end of the basiarch is prolonged into a process running below the basilid and capable of tipping it open when required. (5) The hind prongs of the arch are partly covered over by the pyriboss. The basiarch has two acute projections pointing forwards from the hind end which have a very important function to perform in pumping. (6) The pyriboss itself has an opening in front, a pair of grooves to accommodate the hind tips of the arch and a second pair of grooves which though unaccommodated must have something to do with the pumping.

Fig. 1, II, shows all the parts when they are separated and further details became visible. In a dead specimen the skin covering the hind region has a double set of muscles symmetrically placed which pass downwards, pass through the gap between the basiarch and the pyriboss and are attached to the basiarch on its underside. The nerve supply to these muscles is clearly visible. Contractions of these muscles will lower the plates and increase the volume of the chamber. The pyriboss is flat dorsally and rounded ventrally. It is attached by elastic tissue passing forward between the vertical muscles just described to the basiarch. There are also strong muscles inserted in tubular grooves in the pyriboss in front and the hind part of the marginal flap behind. These by their contractions can separate the pyriboss from its attachments to the prongs of the basiarch and the elastic tissue in front will now draw it forward at a lower plane. The result will be that it will now occupy the gap in the basiarch and the acute prominences in it will fit into the hind pair of grooves which were not in use before. This is shown in Fig. 1, III. After taking up the new position the pyriboss moves up and down as though hinged. It can open only downwards as the lower surface of the body is broader and so cannot allow it to open the other way.

In Fig. 1, IV, the basilid, which is capable of being raised from behind, is shown attached by two sets of strong muscles which are inserted into openings in the lid and frayed out into grooves in relation with them. The other ends of the muscles pass through the opening to the underside of the basiplatt. They help to keep the lid tightly closed. There are also two other sets of paired muscles which are attached in front below the hind margin of the lids and behind to the groove between the basiplatt and the basiarch. This arrangement means it is a valve which opens upwards and so any fluid can be admitted from the chamber below the basal plates but nothing can get in. It must, however, be noted that the prominence in front of the arch can tip the lid open if it comes in contact with it. This is rendered more probable by a deep cleft in the front margin of the lid intended to accommodate the tip.

A full understanding of the working can be obtained only by observing live specimens in action. This will be undertaken, but the construction of the various parts leaves no doubt that any explanation of the sucking mechanism without taking the parts now explained into consideration must of necessity be defective. A new orientation of our views on the

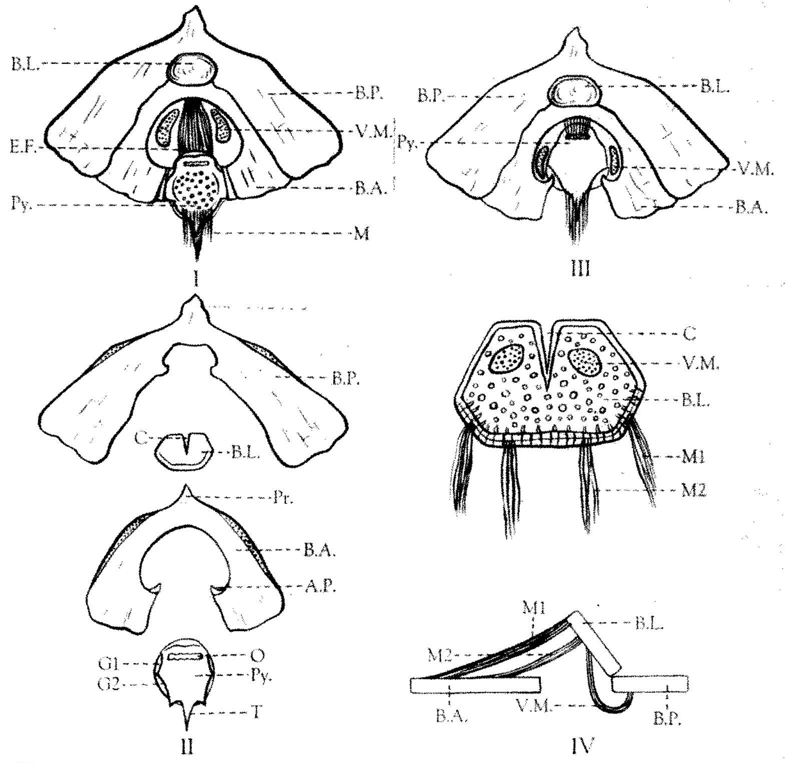

Fig. 1. I, Parts of the hind region of the sucker; II, the parts isolated; III, probable position of the pyriboss while at work; IV, the basilid (dorsal and diagrammatic side-view). *A.P.*, Acute process; *B.A.*, basiarch; *B.L.*, basilid; *B.P.*, basiplatt; *C*, cleft; *E.F.*, elastic fibres; *G* 1 and 2, grooves; *M*, M_1 and M_2, muscles; *O*, opening in the pyriboss; *Pr.*, process to tip open basilid; *Py.*, pyriboss; *T*, tail; *V.M.*, vertical muscles

working of the sucker is inevitable. The possibility of the sucker being a hydraulic structure cannot be ignored.

I thank Alhaj Moulana, S. A. W. Bukhari Sahib, principal, New College, Madras 14, and the management, for permitting me, after my retirement, to continue this work in the College laboratory.

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Determination of Certain Phosphate Compounds in Plant Extracts

Except when isotopes have been used, the investigation of the metabolism of the sugar phosphates and related compounds in animal and plant tissues has long been restricted by a lack of specific methods of determination[1]. Recently, however, specific enzymatic techniques have been developed for the estimation of both sugar phosphates and glycolytic intermediates in extracts from animal and plant tissues[2–7]. The enzymatic methods present greater difficulties with plant than with animal extracts, partly because the contents of phosphate compounds are smaller, and partly because the presence of pigments and inhibitors interferes with the assays. But successful results have now been obtained with extracts of potato tubers, strawberry leaves and fruits of banana, tomato and apple.

Using the technique of Isherwood and Barrett[8], the tissue is extracted in trichloroacetic acid and this acid is removed. In some cases (for example, potato) glucose and fructose-6-phosphates can then be determined in the neutralized extract without further treatment. In general, however, after concentration *in vacuo* below 30° C, the extract is cleared with activated charcoal (Harington; 0·4–2·5 g charcoal/10 g plant tissue; two or more treatments as required) to remove pigments and inhibitors. Glucose and fructose-6-phosphates, fructose diphosphate, dihydroxyacetonephosphate, 3-phosphoglycerate and phosphoenolpyruvate were then determined by following the changes in optical density due to reduction of oxidized or oxidation of reduced pyridine nucleotides; these changes were measured either with a spectrophotometer or, for other components, such as fructose diphosphate, the content of which was usually low, a fluorimeter (ref. 9; an E.I.L. fluorimeter was used; three other makes of fluorimeter proved unsatisfactory for the purpose). With potato and banana, but not with strawberry leaf extracts, a further purification treatment was necessary to remove interfering material before using the fluorimeter.

Table 1 gives the contents of various phosphate compounds in certain plant tissues. In bananas a correlation was observed between the changes in the rate of respiration during ripening and the content of fructose diphosphate[10].

Table 1. Content of Certain Phosphate Compounds in Some Higher Plant Tissues as µmoles/100 g Fresh Weight

	Potato tuber (mature)	Strawberry leaf	Banana fruit (green)	Tomato fruit (green)	Apple fruit (immature)
Glucose-6-phosphate	7·0–16·0	16·0–19·0	7·0	8·0	8·0
Fructose-6-phosphate	1·0–3·0	2·0–4·0	1·5	1·5	1·0
Fructose-1,6-diphosphate	0·02–0·04	0·3–0·4	0·05	0·03	0·11–0·17
Dihydroxyacetonephosphate	0·1–0·2	0·5–0·6	0·6–0·7	—	0·2–0·4
3-Phosphoglycerate	2·0–3·0	—	—	—	0·8–1·4
Phosphoenolpyruvate	1·0–1·7	2·5–3·0	1·0	1·5–2·0	0·5–1·0

We thank Dr. L. W. Mapson, Dr. P. J. Randle, Dr. E. A. Newsholme and Dr. P. J. Syrett for advice.

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Dependence of Length of Leaf Lamina on Length of Leaf Sheath in the Rice Plant

In 1960, I reported that water-logging reduced the size of the leaf lamina in upland rice variety Kindinga and that no such reduction occurred in swamp rice variety Dima, when grown under the same soil conditions. If the length of a leaf lamina depends on the length of its leaf sheath a reduction in the length of the latter will also lead to a consequent reduction in the length of the former. If this proposition holds true, the reduction in size of leaf lamina in upland rice variety due to water-logging can, therefore, be attributed to its similar effect on the length of the leaf sheath especially as the width of leaf lamina is not increased by this condition.

For this investigation twenty-four plants of upland rice variety, Agbede, and swamp rice variety, *BG*.79, were raised in pots, each pot containing one plant. Half this number of plants in each variety were grown under water-logged condition and the other half under 'dry' soil (about 75 per cent saturation) condition. Twenty-five leaves of different sizes and ages were selected at random from each group of plants in each variety and the lengths of their leaf laminæ and leaf sheaths measured. From the results obtained the regression line for each variety was drawn.

The lengths of the leaf sheath of the 5th, 6th, 7th, 8th, and 9th leaf of the main shoot of plants grown in water-logged and 'dry' soil conditions in each variety were also measured. Figs. 1 and 2 show that there is a linear relationship between the length of leaf sheath and that of leaf lamina in both upland and swamp

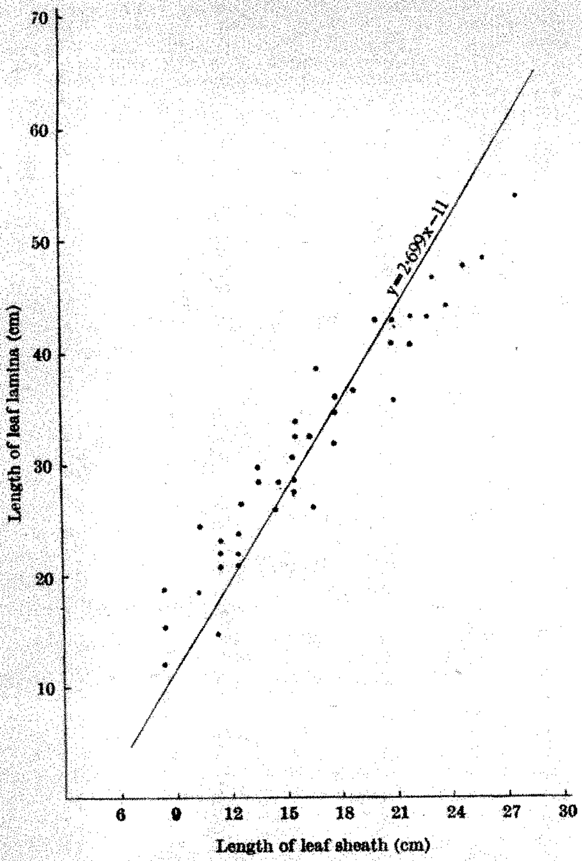

Fig. 1. Regression of length of leaf lamina (y) and of leaf sheath (x) in upland rice variety Agbede (γ = 1·0)

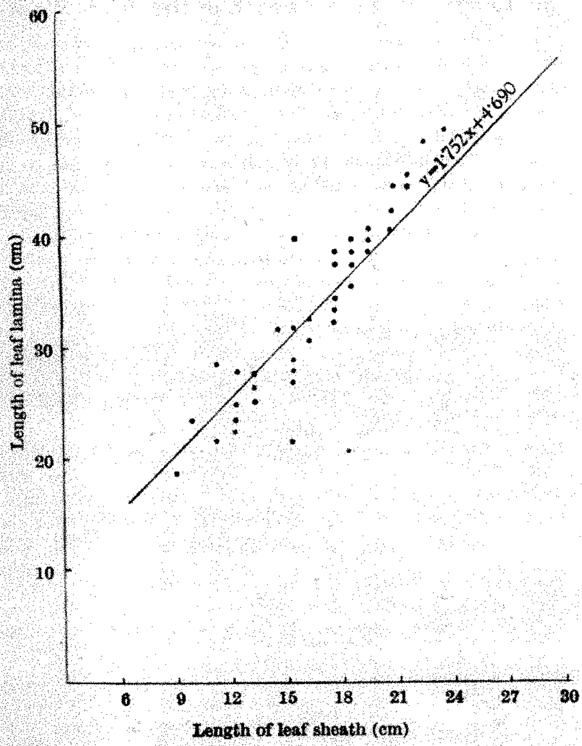

Fig. 2. Regression of length of leaf lamina (y) against length of leaf sheath (x) in swamp rice variety *BG*.79 (γ = 0·98)

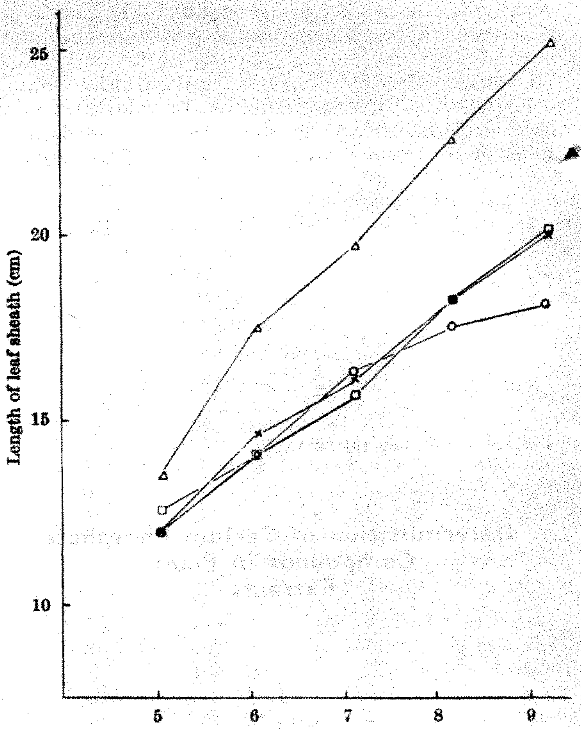

Fig. 3. Effect of soil conditions on length of leaf sheath in swamp and upland varieties of rice. (△) Dry-soil plants and (⊙) water-logged plants of upland variety. (×) Dry-soil plants and (□) water-logged plants of swamp variety

rice varieties used in this study. The correlation coefficients are 1·0 and 0·98 for upland and swamp rice variety respectively, showing, therefore, that there is a complete and a highly positive association between the length of leaf lamina and length of leaf sheath in upland and swamp rice variety respectively.

Fig. 3 shows that there is little or no difference in the length of leaf sheath of swamp rice grown under water-logged and 'dry' soil conditions, but in the upland rice variety water-logging reduced considerably and significantly the length of the leaf sheath.

As it has been established in this work that there is a linear relationship between the length of leaf sheath and that of the leaf lamina in both varieties of rice, a reduction in the length of the leaf sheath in upland rice variety that was brought about by water-logged condition would be expected to lead to a consequent decrease in the length of leaf lamina and therefore to a decrease in its size. In the swamp rice variety no such reduction in the length of leaf sheath of plants grown under water-logged conditions occurred; consequently there should be no difference in the length of leaf lamina and therefore in the size of leaf lamina of dry and water-logged plants.

The presence and absence of such difference in upland and swamp rice varieties respectively between the size of leaf lamina of 'dry' and water-logged plants reported by me[1] can be explained by the findings reported in this communication. It is difficult, however, to explain, at the present time, the cause of the reduction in the length of leaf sheath of upland rice variety grown under water-logged soil.

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Effect of Halogenated Benzoic Acids on *Elodea densa*

A WIDE variety of plants have been treated with halogenated benzoic acids since the effect of these compounds as plant growth regulators was first reported by Zimmerman and Hitchcock[1]. A search of the literature has revealed no information on the effects of such compounds on aquatic plants. Accordingly, an experiment was set up to discover the effect of halogenated benzoic acids on *Elodea densa*.

Sprigs of *E. densa* were collected from a local pond. Terminal shoots 25 cm long were examined with a low-power (×40) dissecting microscope. All shoots showing buds or roots were discarded. Each remaining shoot was placed in a 3·1. glass jar containing a litre of tap water and 250 g of washed sand. The appropriate iodo-, bromo-, or chloro-benzoic acids substituted in the *ortho*-, *meta*-, or *para*-position were dissolved in 1 c.c. of ethanol and added to the jars containing *Elodea*. The final concentrations of the halogenated benzoic acids were 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} M. One c.c. of ethanol was added to each control jar. All solutions were replaced every seven days. The experiment was conducted in a greenhouse and replicated three times over a four-month period. While the absolute responses to the acids varied from replication to replication, the relative responses to each acid were the same in each replication.

Data on total length and number of buds and roots were taken at weekly intervals for four weeks. Table 1 gives the total data for one replication. All shoots, regardless of treatment, had three or four buds. The chloro-substituted benzoic acids had little effect on number of roots. At the highest concentration iodo- and bromo-benzoic acids substituted in either the *ortho*- or *para*-positions inhibited the eruption of roots; at 1×10^{-5} M the same compounds had little effect on root number while at the lowest concentration they led to a greater number of roots. *m*-Iodobenzoic acid had little effect on the number of roots. The two highest concentrations of *m*-bromobenzoic acid had little effect on root number while the lowest concentration increased the number of roots.

The *m*-iodo- and *m*-bromo-substituted acids had little effect on shoot elongation. In general, increasing the concentrations of the *ortho*- and *para*-iodo- and bromo-benzoic acids and all chlorobenzoic acids led to inhibition of shoot elongation. There appeared to be little correlation between shoot elongation and root emergence. A compound, that is, *p*-chlorobenzoic acid, could affect elongation and not affect root emergence, or conversely, a compound, that is, *m*-bromobenzoic acid, could affect root emergence but have little effect on elongation. Where a compound did affect both root emergence and stem elongation, that is, *p*-bromobenzoic acid, the magnitude of the effects were quite different.

It would appear that halogenated benzoic acids can affect both stem elongation and differentiation in *E. densa*. Microscopic examination of sections through the nodal regions of shoots similar to those used in this experiment showed complete absence of bud and root primordia. It would seem, therefore, that in *Elodea* halogenated benzoic acids have no effect on initiation of bud primordia, enhance the differentiation of root primordia and in addition stimulate elongation of the stem. The effects of the acids on root primordia initiation and stem elongation appeared to be separate and not a result of an interaction. That is, the acids do not appear to affect elongation first and then elongation results in root differentiation and eruption. If shoots 10 or 15 cm long were treated with the acids, the results so far as elongation was concerned were identical to that of the 25 cm shoots. None of the two shorter shoot-lengths showed either buds or roots at the end of four weeks. Once again it would appear that the benzoic acids were affecting elongation without affecting primordia differentiation.

Table 1. EFFECT OF VARIOUS HALOGENATED BENZOIC ACIDS ON STEM ELONGATION AND NUMBER OF ROOTS IN *Elodea densa* AFTER FOUR WEEKS OF TREATMENT. PRIOR TO TREATMENT THERE WERE NO ROOTS AND STEM LENGTH WAS 25 CM

M Concentration	*Ortho* No. of roots	*Ortho* Length of stem	*Meta* No. of roots	*Meta* Length of stem	*Para* No. of roots	*Para* Length of stem
			Iodo-substituted			
1×10^{-4}	0	21	5	29	0	25
1×10^{-5}	6	32	8	28	8	30
1×10^{-6}	15	35	5	28	17	37
			Bromo-substituted			
1×10^{-4}	1	25	7	28	1	25
1×10^{-5}	5	25	8	28	5	26
1×10^{-6}	12	35	13	28	12	35
			Chloro-substituted			
1×10^{-4}	7	28	6	29	6	28
1×10^{-5}	6	34	5	31	7	32
1×10^{-6}	7	36	7	46	7	37

	No. of roots	Length of stem (cm)
Controls	6, 6, 7	28, 29, 29

Van Overbeek[2] found 50γ equivalents of indolyl-3-acetic acid (IAA) per kilogram fresh weight of *Elodea*. This is a relatively high concentration. Niedergang-Kamien and Skoog[3] have reported that 2,3,5-tri-iodobenzoic acid interfered with the polar movement of IAA. It is possible, therefore, that the benzoic acids here were interfering with the IAA metabolism. It is interesting that the benzoic acids had no effect on the formation of bud primordia but had definite effects on the formation of root primordia and on stem elongation. In all three processes, IAA has been reported to play an important part. Since both treated and control shoots formed roots the benzoic acids did not initiate a new process but enhanced an already occurring process.

The *ortho*- and *para*-substituted halogenated benzoic acids show more reactivity in terrestrial plants than the *meta*-substituted compounds, which show little activity. The same position effects were seen in *Elodea*. The low activity of the chloro-substituted compounds on *Elodea* is at variance with the high activity of these compounds on terrestrial plants.

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Branched Lint Hairs in Cotton, *Gossypium hirsutum*

DURING investigations into the development of lint hairs in Upland cotton (*Gossypium hirsutum* L.) branched hairs were frequently observed in slides examined microscopically. Up to 9 per cent of the hairs on a slide were found to be branched and occasionally two or more branches were observed on a single hair. Branched hairs were found to occur in a wide range of cotton types within the species. The branches were blunt, tubular structures with a large

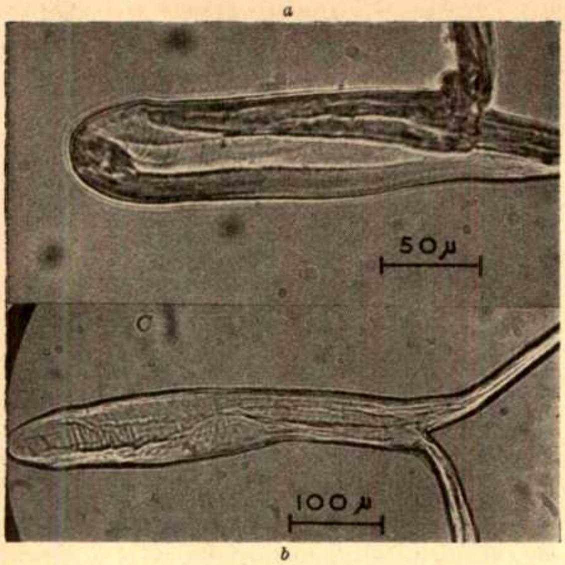

Fig. 1. Photomicrographs of lint hair branches in *Gossypium hirsutum*. The large central lumen is visible in *a*; in *b* the fibrillar nature of the cellulose thickening can be distinguished near the distal end of the branch

central lumen continuous with the lumen of the remainder of the hair cell (Fig. 1*a*). They varied in length from 50µ to more than 1,200µ and were approximately 50µ in diameter.

It is suggested that the branches are formed following the occurrence of folds in the lint hairs during an early stage in their development and before secondary cellulose deposition is initiated. The walls on the inside of the fold fuse, and a large proportion of the fused wall material is resorbed by the cytoplasm of the cell to leave a single, central lumen in the folded region of the hair. Secondary thickening later takes place throughout the walls of the hair including those of the branch.

A number of observed facts support this explanation: a branch is usually twice as wide as the remainder of the hair cell; the unbranched parts of the hair cell always meet at an acute angle at the base of the branch; it is possible to distinguish a definite fibrillar structure extending across the whole wall of the branch, indicating that it has been thickened as a single unit (Fig. 1*b*). The lint hairs are always folded to some extent in the developing boll and the complete doubling necessary for the formation of a branch in the way suggested could easily occur.

The walls of growing plant cells are extremely labile and can be digested by the cell in certain circumstances[1]. In the Cladophoraceae, for example, the walls of all but the basal cells may be pierced by holes which are digested shortly before the liberation of swarmers[2]. There is no reason to suppose that the septum formed by the fusion of the inside walls of the fold in the lint hairs of cotton could not be digested and resorbed in a similar way.

I thank Prof. R. D. Preston for comments on the above explanation of the development of branched lint hairs; and Dr. D. A. Perry, who took the photomicrographs.

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MICROBIOLOGY

Mutants of *Salmonella* with Unusually Low Toxicity for Mice

IT has been previously reported from our laboratory[1-3] that galactose-sensitive mutants of *Salmonella enteritidis* and *Salmonella typhimurium*, which are defective in uridine diphospho-galactose-4-epimerase (epimerase), contain only glucose in their cell wall, entirely lacking galactose, rhamnose, mannose and tyvelose (or abequose), which are regarded as the usual constituents of the cell wall sugars of wild-type cells. These cell wall polysaccharides are known to constitute a part of the endotoxins of bacteria[4,5], and it seemed of interest to examine what effect the alteration in the composition of polysaccharides might have on toxicity of bacteria.

The properties of the strains used in the investigation reported here are summarized in Table 1, together with the principal results. As seen in Table 1, while the cell wall of the wild-type strains contained glucose, galactose, mannose, rhamnose and tyvelose (or abequose), a rough mutant ($11Rx$) of *S. enteritidis* had only glucose and galactose in its cell wall, which confirmed the observation of Westphal's group[6]. It was also found that the cell wall of epimerase-less mutants contained only glucose.

In the first series of the experiments, the toxicity of heat-killed cells was examined in regard to their lethal effects on mice. Heat-killed cells were prepared by heating saline suspension of an 18-h agar culture of each strain at 58° C for 60 min. *dd* strain mice weighing about 16 g were injected intraperitoneally with 0·3 ml. of various dilutions of the heat-killed cell suspension, and deaths in each group of five mice were recorded during a period of 72 h. As shown in Table 1, mutant $11Rx$ was somewhat less toxic than its wild-type. Furthermore, the heat-killed cells of epimerase-less mutants of both *S. enteritidis* and *S. typhimurium* were found to have extremely low toxicity; the LD_{50} appeared to be much larger than 16 mg. The strains of *Salmonella* having such low toxicity have never been experienced before in our laboratory.

It is generally believed that most, if not all, of the toxicity of heat-killed bacteria is due to the lipopolysaccharide (endotoxin) they contain. Therefore, it seemed of interest to compare the amount of endotoxin extractable from the cell, and also to compare the toxicity of extracted endotoxin from the various strains mentioned here. Lipopolysaccharide was obtained from each strain by the phenol–water procedure[7], which is believed to give nearly quantitative yields of the material and then purified by ultracentrifugation. As shown in Table 1, the yield of lipopolysaccharide was poor in the strain of low toxicity. At least in the case of $11\text{–}1\text{–}M$, there is evidence that the low yield is not due to the difficulty of extraction[8]. When the lethal toxicity for mice of lipopolysaccharide was determined in the same way as for heat-killed cells, no remarkable difference in the toxicity was found among the lipopolysaccharides from various strains. Consequently, at least in the case of mutant $11Rx$, the low toxicity of heat-killed cells may be explained by the low content of endotoxin, but the toxicity of heat-killed cells of epimeraseless mutants is too low to be explained by the content of lipopolysaccharide alone. It appears that the mode of existence of lipopolysaccharide, especially its association with the other constituents of the cell

Table 1. RELATION BETWEEN SUGAR COMPONENT OF CELL WALL AND TOXICITY OF VARIOUS STRAINS OF *Salmonella enteritidis* AND *S. typhimurium*

Species	Strain	Derived from	Defective in	Sugar component of cell wall*						Toxicity (LD_{50}) of heat-killed cells i.p. mice (wet weight)	Yield of lipopolysaccharide by Westphal's method	Toxicity (LD_{50}) of lipopolysaccharide i.p. mice (dry weight)
				Glu.	Gal.	Man.	Rha.	Tyv.	Abe.			
Salmonella enteritidis	No. 11 (*S*-type)	—	—	+	+	+	+	+	–	1 08 mg	4·8 and 3·3 per cent	203 γ
	11*Rx* (*R*-type)	No. 11	—	+	+	–	–	–	–	2 46 mg	1·1 and 0·7 per cent	281 γ
	11–1–*M* (galactose sensitive)	No. 11	Epimerase	+	–	–	–	–	–	>16 mg (1/5)†	0·7 per cent	194 γ
	11–1–*TW* (galactose resistant)	11–1–*M*	Epimerase galactokinase	+	–	–	–	–	–	>16 mg (2/5)†	0·8 and 0·4 per cent	374 γ
Salmonella typhimurium	LT_2 (*S*-type)	—	—	+	+	+	+	–	+	2 35 mg	4 0 per cent	124 γ
	LT_2–M_1 (galactose sensitive)	LT_2	Epimerase	+	–	–	–	–	–	>16 mg (2/5)†	0 2 per cent	283 γ
	LT_2–T_1 (galactose resistant)	LT_2–M_1	Epimerase galactokinase	+	–	–	–	–	–	>16 mg (1/5)†	0·5 per cent	105 γ

* These analyses were carried out as previously described (ref, 2).

Hexosamines and heptoses (probably occurring in phosphorylated form) were removed during the deionization procedure, and thus were not demonstrated.

† Fractions = $\frac{\text{total number of dead mice}}{\text{total number of mice tested}}$ (by administration of 16 mg of heat-killed cells).

Abbreviations: Glu., glucose; Gal., galactose; Man., mannose; Rha., rhamnose; Tyv., tyvelose; Abe., abequose.

wall, may play an important part in the manifestation of the toxicity of heat-killed cells. It has been shown that the live cells of epimerase-less mutants are markedly less virulent for mice than those of wild-type[9], and this fact might also be related to the results obtained in this investigation.

The finding that lipopolysaccharide from epimerase-less mutants, in spite of the absence of various sugars other than glucose, showed the same degree of toxicity as that of the wild-type, confirmed the conclusion of Westphal and Lüderitz[5] that the polysaccharide part of endotoxin was not directly related to the toxicity.

I thank Prof. O. Westphal, Prof. D. Ushiba, and Dr. K. Saito for suggestions and discussions, and also to Drs. T. Fukasawa and H. Nikaido for advice and for carrying out the analysis of cell wall and purification of the lipopolysaccharides.

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Plant Protein Agar, a Medium for the Isolation and Estimation of Proteolytic Organisms in Grassland Soils

GELATIN, casein and occasionally albumen are used for isolation and counting of proteolytic soil organisms. Among the principal substrates for such organisms in grassland soils will be the dead tillers, the sloughed off roots and, after ploughing, the stubble and root mat of the sward. Since plant protein will be present in plant residues, its choice as a medium for proteolytic soil organisms seems in this case more logical than that of either gelatin, casein or albumen, proteins of animal origin. Waksman and Starkey[1] have examined the decomposition of vegetable proteins by some soil organisms.

The plant protein used in these experiments was prepared by a method of Tilley and Wilson (personal communication). Juice expressed from fresh minced lucerne was heated to 55° C and centrifuged to remove chloroplastic material. Sulphuric acid (2 N) was added to precipitate the cytoplasmic protein, which was washed with hot water, alcohol and acetone. It was allowed to dry in air to yield a white powder of about 14 per cent nitrogen content. The dried powder was ground in demineralized water (25 g/500 ml.) in a ball mill for 5 h to give a uniform suspension. This was sterilized by steaming on three consecutive days. Since the suspension coagulates on heating, shaking at intervals during sterilization, especially on the first day, is essential.

The protein–agar medium was prepared by adding plant protein suspension to mineral salts agar containing 0·01 per cent Oxoid yeast extract, to give a final concentration of 8 per cent protein suspension. The *p*H was adjusted to 7 by the addition of sodium carbonate solution before the addition of the protein suspension.

This medium has been used by Grossbard and Hall[2,3] primarily for counting proteolytic bacteria, fungi and Streptomycetaceae in soils of various plots forming part of an experiment on the effects of leys on

Table 1. RELATIVE PROTEOLYTIC AND CELLULOLYTIC ACTIVITIES OF SOME FUNGI AND STREPTOMYCETACEAE

Organism	Cellulolysis (10 days incubation) Area of lysis (mm²)	Cellulolysis (10 days incubation) Activity index	Proteolysis (6 days incubation) Area of lysis (mm²)	Proteolysis (6 days incubation) Activity index
Fungi:				
Botryotrichum (*Chaetomium*) *piluliferum*	1,991·0	10	498·6	8
Pellicularia filamentosa	2,882 0	11	2,718 0	11
Trichoderma viride	2,504·0	11	2,307·0	11
Unidentified—*F*.1	117·9	6	0·0	0
F.2	454·0	8	172·5	7
F.4	131·0	7	0·0	0
I.94	412·2	8	257·0	8
I.98	192·4	7	98·1	6
Streptomycetaceae: All unidentified—				
A.1	54 6	5	102·4	6
A.2	113·2	6	96·7	6
I.95	3·2	1	19·2	4
I.96	70·5	6	219·9	7
I.97	131 3	7	230·7	7
I.105	85·1	6	23 2	4

Activity index scale based on $\log_2$ (area of lysis in mm²) soil fertility. The plate dilution method was used. Organisms were rated as proteolytic when, after 6 days of incubation at 25° C, the plates showed clear (sometimes transparent) zones around or underneath the microbial colony. The Streptomycetaceae gave well-defined areas of lysis the diameter of which was usually greater than that of the colony. The Streptomycetaceae were counted as a representative family of the Actinomycetales because of the ease of identification by their aerial mycelium under the low-power microscope.

The relative proteolytic activities of various organisms were expressed in terms of the area of lysis in mm^2. On this an activity index was based[4]. A similar index was obtained for cellulolytic organisms cultured on mineral agar with cotton cellulose as the sole source of carbon[5]. These indices were used for a broad comparison of the proteolytic and cellulolytic activities of specific soil organisms. They serve as a criterion of the ability of these organisms to decompose plant residues (Table 1).

Plant protein agar has the advantage over gelatin agar in deriving from important forms of protein found in plant residues. Moreover, the zones of lysis can be read off directly from an untreated isolation plate. If gelatin agar is used the plates must first be flooded with $HgCl_2$ and HCl for the zones of lysis to become apparent. Consequently the organisms are killed and cannot later be isolated.

We thank Dr. William Davies for his help and Dr. J. M. A. Tilley and Mr. R. F. Wilson for preparation of plant protein.

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Detection of Marine Alginolytic Bacteria

IN the examination of the hydrolases of algal polysaccharides in marine bacteria, a simple method to detect alginase is desirable[1]. A difficulty arises in the preparation of media because of the precipitation of alginate by the divalent ions in sea-water. The methods previously described were reviewed by Thjøtta and Kåss[2]. The disadvantages of these methods are that the measurement of decrease in viscosity of an alginate broth requires a special apparatus[2], and the alginate agar medium of Waksman *et al.*[3] cannot be used for marine strains which liquefy agar. The preferred method is one based on the liquefaction of an alginate gel. A simple method for the formation of this gel in a sea-water medium is described here. The alginate gel is formed by the slow diffusion of divalent ions from a sea-water agar medium into a solution of sodium alginate. This method is similar to the double-layer technique for the preparation of a pectate gel[4].

A modified Hugh and Leifson medium[5] was chosen as the base medium and consisted of sea-water, 100 ml.; dipotassium hydrogen phosphate, 0·03 g; trypticase (Baltimore Biological Lab.), 0·25 g; and agar (Difco, 'Bacto-agar'), 1·5 g, but any sea-water agar medium may be used.

Table 1. HYDROLYSIS OF ALGINATE AND AGAR

	Alginate	Agar*	Alginate/agar†
Ps. carrageenovora	Liquefied	−	−
Ps. atlantica	Pit	+	+
Strain 7	−	+	

+ Clear area around colony.
* Gram's iodine.
† Clear area around colony indicates use of alginate in alginate/agar medium of Waksman *et al.* (ref. 3).

The upper layer contained sodium alginate (Hallmark, 'Manucol *SS/LM*, (η)' 11·6)[6] 2·5 g; sodium chloride 3·0 g; dipotassium hydrogen phosphate 0·03 g; and distilled water 100 ml. A mixture of the dry sodium chloride and alginate was added slowly to a solution of the dipotassium hydrogen phosphate while stirring vigorously. This medium had a pH of 7·0 after autoclaving for 15 min at 121° C. A layer (0·5 cm deep) of the sterile solution of sodium alginate was poured over 15–20 ml. of the agar base in a Petri dish.

The strength of the alginate gel depends on the molecular weight and concentration of the sodium alginate, the concentration of divalent ions in the sea-water, and the rate of ion exchange which leads to the formation of the alginate gel. For the purposes of this test a gel equivalent to that obtained with 1 per cent agar (Difco 'Bacto-agar') was desired. Under the conditions described here, a suitable gel was obtained 1 h after the alginate was poured over the agar base layer.

Precautions were taken to remove the moisture which was produced by the syneresis of the alginate gel. The agar base was dried initially for 24–48 h at 37° C and an absorbent disk was placed in the glass Petri plate cover. If necessary the alginate surface was dried with the aid of a bunsen flame.

Hydrolysis of the alginate was indicated by complete liquefaction of the gel or the formation of a depression around the colony. This pit was easily demonstrated if the alginate layer was floated off the surface of the agar base layer, with a 5 per cent solution of calcium chloride. After the alginate layer had been removed, the agar was flooded with a solution of iodine prepared according to Gram, to determine whether it had been hydrolysed by the bacteria.

A loopful from a 24 h culture of *Pseudomonas atlantica*, *Ps. carrageenovora* and a number of unidentified marine bacteria was placed on the surface of the dry alginate gel. The plates were incubated at 25° C. The results in Table 1 show that the alginate was liquefied by *Ps. carrageenovora*. This strain hydrolysed κ-carrageenin but not agarose[7]. A pit was formed in the alginate gel with *Ps. atlantica*.

In the double layer medium a depression was observed in the alginate around the colony of strain 7. However, when the alginate layer was floated off the agar base layer, it was seen that the alginate was not hydrolysed. This pit was due to the sinking of the intact alginate gel into a deep hole produced in the agar by an agarase. Strain 7 was an unidentified marine bacterium which liquefied agar.

The double-layer method was compared with Waksman's alginate agar medium[3] prepared according to Skerman[8]. A clear area around the colony was obtained with *Ps. atlantica* but not with *Ps. carrageenovora*, the strain which actively liquefied the alginate gel (Table 1).

High concentrations of calcium ions in the base layer increased the strength of the alginate gel, caused excessive syneresis, and inhibited the alginase of *Ps. carrageenovora* (Table 2).

This method can also be used for detecting the hydrolysis of alginate by soil bacteria. For this

Table 2. EFFECT OF CALCIUM ON ALGINATE GEL

Calcium chloride in sea-water agar base (%)	Syneresis	Gel strength	Activity of alginase *Ps. carrageenovora*
0	–	–+	Liquefaction
0·1	–	+	Liquefaction
0·25	–+	+	Pit 25 mm
0·5	+	++	Pit 25 mm
1·0	++	+++	Pit 10 mm

purpose calcium is added to a soil extract agar medium and the sodium alginate is dissolved in an aqueous solution of dipotassium hydrogen phosphate.

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Aminopterin as an Inducing Agent in Bacteriocinogenic Bacteria

INDUCTION of lysogenic bacteria was first produced by the use of ultra-violet light[1]. It was shown later that other radiations[2], nitrogen mustard, ethylene-imines, epoxides and organic peroxides[3] can also induce lysogenic bacteria to produce phage. The process of induction is not yet clear. Several lines of evidence indicate that the primary lesion initiated by inducing agents could be an alteration in the nucleic acid economy of the host, an effect of unbalanced growth[4].

In 1952[5] it was demonstrated that exposure of bacteriocinogenic bacteria to ultra-violet light induces the production of bacteriocin. The fact that phage production and bacteriocin formation are induced by the same agents, and many other similarities[6] suggest that the mechanism of induction might be analogous in both systems. Since bacteriophage production appears to be the more complex of the two systems, it may be easier to study the triggering effect of induction in the case of bacteriocins.

The experiments described here were performed using strain *P* of *Pasteurella pestis*, which produces bacteriocin, when irradiated with ultra-violet light[7,8] or when treated with mytomycin *C*[9]. Cultures of exponentially grown cells start to produce bacteriocin almost immediately after irradiation[7], that is, too early for the resumption of DNA synthesis to occur. If DNA synthesis is, indeed, not needed for bacteriocin production (in contrast to phage production), the system will permit the demonstration of triggering the induction even by a substance which will stop synthesis of DNA completely.

Aminopterin (California Corporation for Biochemical Research), when given in a dose of 200 γ/ml., induces the *P* strain of *P. pestis* to produce bacteriocin. In a typical experiment, 200 γ/ml. of aminopterin was added to a sample of an exponentially grown culture of *Pasteurella pestis* in synthetic medium[10]. Another sample was irradiated with ultra-violet light. The tubes containing these samples and the control were immersed in a water bath at 37° C, for 3 h. Chloroform was then added to each culture, to kill the bacteria, and bacteriocin (pesticin) in the medium titrated after decantation, as described previously[7]. The titre of the control was less than 1×10^2 arbitrary units/ml., the titre of the irradiated culture 1×10^4 and that of the aminopterin-treated culture 5×10^3 arbitrary units/ml.

Attempts to reverse induction of aminopterin by thymine, thymidine, folic acid or folinic acid have failed so far, both when these metabolites were added to the bacteria 1 h before or immediately after induction.

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GENETICS

Apparent Genetic Recombination in a Blue-Green Alga

THE blue-green algae (Myxophyceae) have generally been thought to be devoid of sexuality[1,2], although no genetical evidence for or against this supposition seems to have been reported so far. This communication describes experimental results obtained with a unicellular blue-green alga, generally known as *Anacystis nidulans* (Myers' strain), which seem to indicate the occurrence of sexual or parasexual phenomena in this organism.

From the wild-stock, strains of the alga which are, *inter alia*, about ten times as resistant to penicillin and streptomycin as the sensitive (parent) strain were derived by a few sub-cultures in basal medium (slightly modified Medium *C* of Kratz and Myers[3], diluted with equal parts of water) supplemented with gradually increasing concentrations of the respective antibiotic. These strains seem stable; a streptomycin-resistant strain has been passed through 40 sub-cultures in the absence of streptomycin without having lost its resistance.

Each of the resistant strains was tested for cross-resistance to the other antibiotic and found not to be cross-resistant. They were also inoculated—separately and jointly—into media which contained both the antibiotics. No growth was found to have occurred in any case even after six weeks.

Inocula from streptomycin-resistant and penicillin-resistant strains were then transferred together into the basal medium containing no antibiotics. At various times, inocula were withdrawn from the mixture and transferred to culture flasks containing both the antibiotics. Out of a total of 36 such flasks (in different experiments) growth occurred in 10. Two typical growth-curves are shown in Fig. 1. The duration of the lag-phase was found to vary from 8–13 days. Since neither of the strains can by itself grow in flasks containing both streptomycin and penicillin, in contrast to inocula from the basal mix of the two strains which can, it is highly probable that growth in the latter case is due to the appearance of

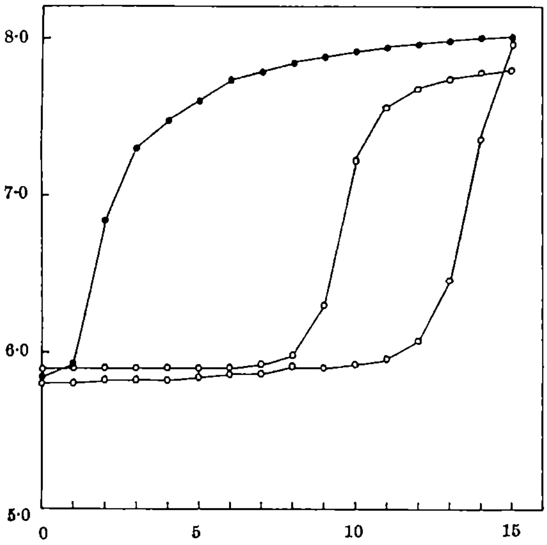

Fig. 1. ○, Growth curves (in $\log_{10}$ units) of 2-week-old inoculum from basal mix of penicillin- and streptomycin-resistant strains in medium containing both antibiotics; ●, growth curve of a double-resistant strain (resistant to penicillin and streptomycin) in medium containing chloramphenicol only

recombinant cells that are resistant to both the antibiotics. The other possible class of recombinant, namely, the double-sensitive, obviously could not be detected because of its inability to grow in the selective medium. It has so far proved impossible to grow clones from single cells of *Anacystis* on solid media so that it cannot yet be asserted that recombination has occurred within individual cells. However, the fact that no growth occurs when inocula of two resistant strains that have not previously grown together are introduced into an antibiotic mixture appears to rule out the possibility that some other mechanism is concerned. This conclusion is further supported by the fact that growth of inocula from the basal mix in medium containing both antibiotics occurred in only some, not all, flasks—an observation that is entirely in keeping with expectation if we attribute growth in these flasks to have been due to the chance inclusion and random distribution of rare recombinant cells among some of the otherwise identical inocula. On the other hand, if some enzymatic or 'antibioticase' mechanisms were concerned, uniform growth would be expected in all flasks.

Tentative estimates indicate that the double-resistant recombinants appear at a frequency of 1 in about 10^8 cells of the parental population—a figure which agrees closely with that reported originally by Lederberg and Tatum[4] (namely, 1 in about 10^6–10^7) for *Escherichia coli K*–12, although mutant donor strains of this bacterium showing a much higher degree of fertility have since been isolated[5,6].

Single-step attempts to isolate a triple-resistant strain of *Anacystis nidulans* by inoculating from basal mix of three strains resistant respectively to streptomycin, penicillin and chloramphenicol into medium supplemented with the three antibiotics were unsuccessful. However, the double-resistant strain, isolated as a product of the cross between streptomycin- and penicillin-resistant strains (both of which are sensitive to chloramphenicol), was found to be able to grow in chloramphenicol medium (Fig. 1). Further work on these and other strains of the alga and also genetic transformation studies are in progress and will be published elsewhere.

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CYTOLOGY

Chromosome Breakage in *Vicia faba* by Monochloramine

THE investigations reported here were prompted by the finding that our distilled water was producing chromosome breakage when used for soaking *Vicia faba* seeds. Monochloramine is used in the disinfection of the Atlanta, Georgia, water system and distils over quite readily. The activity of monochloramine as a bactericidal and virucidal agent has been examined by others[1], but the specific cytological effects apparently have not, and it is not included in lists of the chloramines[2] which have been tested by various screening agencies. Organic chloramines, which include the nitrogen mustard group, have found extensive application where the use of cytotoxic agents are required.

In these investigations a comparison was made between hypochlorous acid, monochloramine (NH_2Cl) and a nitrogen mustard as concerns their capacity for inducing chromosome breakage in the root meristems of *Vicia faba*. The hypochlorous acid was obtained through the addition of a commercial preparation ('Clorox') and the nitrogen mustard was commercially available mechlorethamine hydrochloride (Mustargen, Merck). Monochloramine was produced by steam distillation of ammonia hydroxide plus hypochlorous acid into an aqueous solution buffered at *p*H 9 with 10^{-2} molar sodium borate. A slight excess of hypochlorous acid was used to avoid the presence of excess ammonia in the distillate. The reaction proceeds as follows:

$$NH_3 + OCl^- \rightarrow NH_2Cl + OH^-$$

Monochloramine concentration was determined in the spectrophotometer, having a specific absorbance at 240 mμ[3]. A volumetric analysis (Palin method) was also used[4]. The concentration of hypochlorous acid was determined by the potassium iodide method[5]. Dosing concentrations were obtained by dilution; in the case of the monochloramine all solutions were 10^{-2} M sodium borate. Imbibed seeds of *Vicia faba* were soaked in the various solutions for a period of 60 min, washed in dilute sodium thiosulphate solution, and then allowed to germinate in the dark at 19° C until the radicals were about 2 cm long. At this time the first cell divisions occur and propionic-carmine stained preparations were made. Table 1 presents the results of these experiments. Those

Table 1

Treatment	Concentration (M)	No. of anaphases scored	No. abnormal	Abnormal (%)
OCl^-, 60 min	10^{-2}	518	27	5·2
NH_2Cl, 60 min	10^{-4}	582	139	24·0
Mechlorethamine HCl (Mustargen, Merck), 60 min	10^{-6}	631	183	29·0
Control 10^{-2} M $Na_2B_4O_7$	—	600	7	1·0

Table 2

Compound	Relative effectiveness
OCl^-	1
NH_2Cl	500
$CH_3N(CH_2CH_2Cl)_2 \cdot HCl$	60,000

anaphase figures which contained bridges and/or fragments were considered abnormal.

A 60-min exposure to 10^{-4} molar monochloramine produced 24 per cent abnormal anaphases while a 10^{-6} molar concentration of nitrogen mustard produced 29 per cent abnormal anaphases. A concentration of 10^{-2} molar hypochlorous acid was required to produce 5·2 per cent abnormal anaphases. The relative effectiveness of the three compounds in producing abnormal anaphases is presented in Table 2.

Nitrogen mustard and monochloramine at these concentrations did not produce visible external browning of the plant embryos, whereas the hypochlorous acid caused extensive surface effects. It would appear that monochloramine resembles the organic chloramines (nitrogen mustards) in its capacity to produce chromosome breakage at concentrations which apparently produce little evidence of tissue damage.

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SOIL SCIENCE

Formation of Gibbsite as a Primary Weathering Product of Acid Igneous Rocks

ONLY detailed sampling of early weathering stages combined with suitable analytical techniques can reveal the presence of gibbsite as a primary weathering product in certain weathering crusts of acid igneous rocks. It is therefore instructive to examine the findings of some previous workers in relation to the methods they used.

Harrison[1] found that acid igneous rocks give rise directly to kaolinitic earth without the intermediate formation of gibbsite, and Humbert[2] found no evidence to support the presence of gibbsite in weathered granitic material. Harrison sampled the weathering stages in detail but in examining thin sections under the microscope small amounts of gibbsite might have escaped detection. Humbert analysed a late weathering stage and may therefore have missed gibbsite present only at an earlier stage. Leneuf[3] sampled weathering stages of acid igneous rocks in detail and subjected samples of silt and clay to X-ray and differential thermal analysis. He concluded that the occurrence of gibbsite in the early stages of weathering of granite is well defined but only transitory. This conclusion is supported by the study of a weathered granite in Southern Rhodesia.

Table 1. SEMI-QUANTITATIVE ESTIMATES OF GIBBSITE AND KAOLINITE IN CLAY EXTRACTED FROM A GRANITIC WEATHERING CRUST NEAR SALISBURY, SOUTHERN RHODESIA

Sample No.	Gibbsite (%)	Kaolinite (%)
A	< 5	100
B	35	65
C	25	5
D	10	< 5

The site examined is a broadly curved hill-top near Salisbury at an elevation of about 5,140 ft. on the Gondwanaland surface. The climate is sub-tropical and divides naturally into a wet season and a dry season. A mean annual rainfall of 33 in. and a mean annual temperature of 19° C have been recorded at the Salisbury Observatory. A leached, pallid soil 18 in. deep is underlain by an argillaceous weathered crust 12 ft. thick, over a 3 in. layer of red arenaceous material, over grey, weathered granite of undetermined thickness. A description of samples of the weathered granite is given in Fig. 1 together with the differential thermal curves. The temperature recorded is that of the inert material. The endothermic peak of gibbsite occurs around 350° C and the kaolinite peak at about 600° C. The endothermic

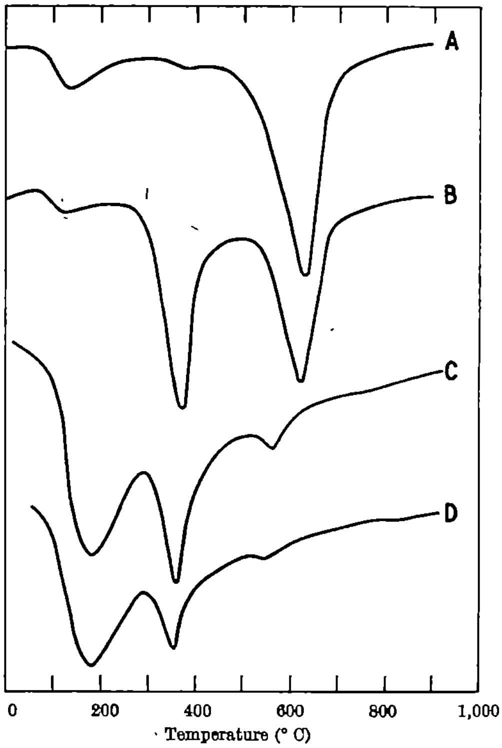

Fig. 1. Differential thermal curves for clay from a granitic weathering crust near Salisbury, Southern Rhodesia. *A*, 12 ft. 10 in. depth, red argillaceous material with abundant weathered minerals and clayskins (19 per cent clay); *B*, 13 ft. 6 in. depth, red arenaceous material, consisting largely of weatherable minerals (7 per cent clay); *C*, 13 ft. 9 in. depth, grey weathered granite that can easily be broken in the hand (0 3 per cent clay); *D*, 14 ft. 11 in. depth, grey weathered granite that can be broken with difficulty or not at all in the hand (0 3 per cent clay)

peak in the region of 180° C is probably due to amorphous material such as alumina gels, but allophane may also be present. The presence of gibbsite in samples *B*, *C* and *D* and kaolinite in all samples was confirmed by X-ray analysis. Semi-quantitative estimates of gibbsite and kaolinite (Table 1) were obtained from Fig. 1 by reference to a differential thermal trace made by standard kaolinite after allowing for the amount of water lost on heating gibbsite relative to that lost on heating kaolinite.

I thank Mr. G. Brown, Dr. R. Greene-Kelly and the late Dr. A. Muir for allowing me to use X-ray and differential thermal equipment at Rothamsted Experimental Station.

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Density of Clay-Water Systems

A RECENT paper by Deeds and Van Olphen[1] gives density data for the bentonite–water system which is not in agreement with the results of Anderson and Low[2]. Since the pressure chamber method designed by the latter authors gave promise of being extremely accurate, it seems necessary to re-examine their results.

Anderson's method makes use of the slope of the curve of water content versus volume of the clay–water system. This gives directly the partial specific volume of the water phase, so that changes of slope are interpreted as changes of specific volume. If we plot the deviations of Anderson's points[3] from a straight line, changes of slope can be magnified 100 or more times, allowing a visual estimation of their significance and reliability. When this is done, it is observed that each curve can be considered as a small number of straight line segments, with discontinuities of slope and water content occurring near some of the points at which the mercury capillary was refilled. A sample curve is shown in Fig. 1, and curves of partial density obtained in this way averaged over duplicate sets of results are shown in Fig. 2. (I can supply lists of deviations from straight lines of slope 0·990 for the eight tables of Anderson's thesis. These lists omit the 7 per cent of points containing major misprints. The lines were fitted at water contents between 3·5 and 4·5 gm/gm.)

A general decrease in density is indicated with decreasing water content, but the reliability of the results is obviously low, and detailed comparisons of the four curves are valueless. In addition, the density at high water contents does not agree well with the known density of water, suggesting that the calibration of the capillary or microburette may have been slightly in error.

It is difficult to suggest any sources of inaccuracy in the pressure chamber method which were not carefully investigated by Anderson and Low, and it is especially difficult to understand why refilling the mercury capillary should cause discontinuity of slope as it apparently does.

However, further evidence of inconsistency is obtained if we apply the equation $V = w\bar{v}_w + m\bar{v}_m$[3] to the results. We may use this equation in the form

$$V + \delta = w\bar{v}_w + mv_m + m\left(\frac{\partial V_w}{\partial m}\right)_w$$

where V is the experimentally measured total volume; δ a volume correction constant for each run; w and m are the weights of water and clay respectively in the sample; $\bar{v}_w$ is the partial specific volume of water; v_m the (constant) specific volume of the clay; V_w is the volume of water in the sample.

If the density of the water decreases near the clay surface in the manner of Anderson and Low, the final term must be a small positive quantity which increases with decreasing water content. δ can be evaluated at high water contents, and the other quantities are known. (If the water density is still varying at the highest water contents available, δ will include a small term due to the derivative $\left(\frac{\partial V_w}{\partial m}\right)_w$. This does not affect the argument.)

For every set of experimental results the last term in the equation is negative, and it usually becomes more negative with decreasing water content. Both these results are contrary to expectation; they are not significantly affected by assuming different values of clay or water density within wide limits.

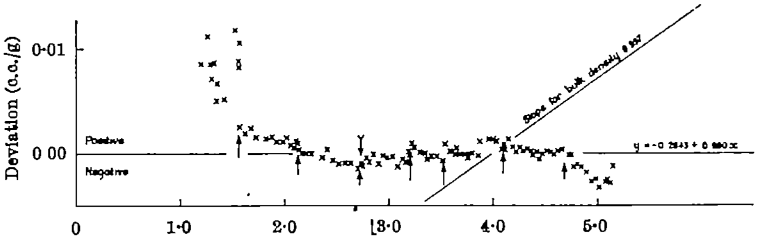

Fig. 1. Deviations of Anderson's Table 9 from a straight line of slope 0·990. Arrows are refilling points. Lithium bentonite at 25° C.

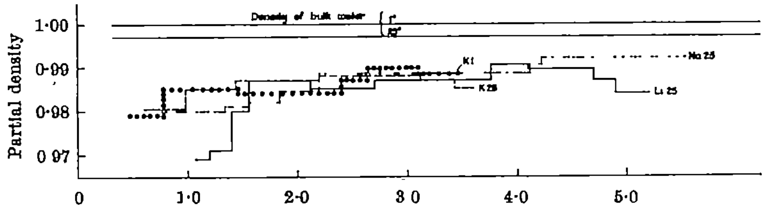

Fig. 2. Average density values from Anderson's data. Lithium, sodium and potassium bentonite at 25° C., potassium bentonite at 1° C.

Until such anomalies are cleared up it must be concluded that variation of the water density at distances greater than a few molecular layers from the clay surface has not yet been demonstrated.

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[1] Deeds, C. T., and Van Olphen, H., *Adv. Chem. Ser.*, 33, 332 (1961).

[2] Anderson, D. M., and Low, P. F., *Soil Sci. Soc. Amer. Proc.*, 22, 99 (1958).

[3] Anderson, D. M., thesis, Purdue Univ. (1958).

FORTHCOMING EVENTS

Monday, December 17

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p m.—Mr. R. H. Hughes: "Hong Kong—Far Eastern Meeting Point".

Institution of Electrical Engineers, Electronics Division (at Savoy Place, London, W.C.2), at 5.30 p m —Prof. H. E. M. Barlow, F.R.S.: "A Proposed New Method of Measuring Microwave Power and Impedance using Hall Effect in a Semiconductor"; "High-Frequency Radiation Pressure and Hall Effect in Semiconductors"; "A Microwave Electrostatic Wattmeter".

Institute of Metal Finishing (at the Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. J. Carder: "Satin Nickel + Chromium Plating".

Monday, December 17—Tuesday, December 18

Association of Applied Biologists (in the Physics Department, Imperial College of Science and Technology, London, S.W.7), at 2.30 p.m. on Monday and 10.30 a.m. on Tuesday—General Meeting.

Tuesday, December 18

Institution of Electrical Engineers, Science and General Division P G.S.8 (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Television in the Service of Technical Education" opened by Mr. J. Scupham.

Institution of Mechanical Engineers, Internal Combustion Engines Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Are the Technical and Quality Standards in the Component Suppliers' Industry Good Enough ?".

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. F. C. Harper. "Wear Testing of Flooring Materials".

Wednesday, December 19

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. R. H. Clarke: Pressure Oscillations and Fall-out Downdraughts"; Mr. J. S. Sawyer. "Gravity Waves in the Atmosphere as a Three-Dimensional Problem".

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Symposium on "Vibration Testing".

Institution of Electrical Engineers, Power Division (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. A. Davis, Mr D. J. W. Richards and Mr. R. A. Scriven: "Investigation of Conductor Oscillation on the 275kV Crossing over the Rivers Severn and Wye".

Institution of Mechanical Engineers, Nuclear Energy Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m. —Discussion on "The Value of Theoretical Fault Condition Analysis".

Society for Analytical Chemistry, Microchemistry Group (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting.

Thursday, December 20

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m —Mr. D. J. Batzer. "Investigation Into Jig Performance"; Dr. R. L. Stanton: "Elemental Constitution of the Black Star Orebodies, Mount Isa, Queensland, and Its Interpretation".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. A. L. S. Corner: "Topological Light on Abelian Groups".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned.

Lecturer or Assistant Lecturer in Mathematical Statistics—The Registrar, The University, Manchester 13 (December 29).

Lecturer in Zoology at Fourah Bay College (The University College of Sierra Leone)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 31).

Senior Lecturer and a Lecturer in Physiology at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 1).

Research Assistant (with an ordinary or honours degree in science, or an equivalent qualification, which must include chemistry or biochemistry, and preferably zoology at least to "A" level) in the Department of Veterinary Anatomy, for work on animal reproduction—The Registrar, The University, Liverpool 3, quoting Ref. No. CV/215/N (January 2).

Lecturers (2) (with a degree in biochemistry, organic chemistry, physiology or pharmacology) in Biochemistry—The Secretary and Registrar, The University, Southampton (January 7).

Chair of Physics at Queen Elizabeth College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 10).

Reader in Botany at Queen Elizabeth College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 10).

Chair of Medical Physics tenable at the Postgraduate Medical School of London—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 14).

Professor of Zoology and Entomology at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, January 15).

Senior Lecturer in Forensic Medicine; and a Senior Lecturer in Psychiatry at the University of Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

Lecturers (2) in Chemistry at the University of Sydney, Australia —The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 18).

Senior Lecturer in Theoretical Physics; and a Senior Lecturer in Physics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 18).

Lecturer in Histology and Embryology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 21).

Lecturer in Microbiology in the Department of Agriculture, University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 25).

Lecturer (with research experience in socio-economic geography, for preference in Asia or Melanesia, with special reference to the geography of subsistence economics) in Geography at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Australia, January 25).

Junior Lecturers (2) in Mathematics—The Secretary of the Mathematical Institute, The University, 10 Parks Road, Oxford (January 31).

Lecturer or Senior Lecturer in Inorganic Chemistry at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S W.1 (Australia and London, January 31).

Lecturer (qualified in pure or applied mathematics) in Mathematics; an Assistant in Mathematics; and a Research Fellow in Mathematics—The Secretary of University Court, The University, Glasgow (January 31).

Lecturer in the Department of Theoretical Physics—The Registrar, The University, Liverpool, quoting Ref. No. CV/208/N (February 1).

Assistant Lecturers, Lecturers or Senior Lecturers in the School of Biological Studies—The Registrar, University of East Anglia, Earlham Hall, Norwich, NOR 05G (February 11).

Chair of Archæology at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, February 15).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry, Engineering, Pharmacology or Physics —The Secretary of University Court, The University, Glasgow (February 28).

Imperial Chemical Industries Research Fellow in one of the following subjects: Chemistry, Biochemistry, Physics, Engineering, Chemo-therapy and related subjects—The Secretary, Academic Council, Queen's University of Belfast, Northern Ireland (March 1).

Assistant Lecturer (Demonstrator) in Anatomy, to undertake teaching and research—The Secretary, University College, Gower Street, London, W.C.1.

Assistant Professor (with a Ph.D. degree, some teaching and research experience, and preferably a flowering plant taxonomist) of Botany—The Dean, Faculty of Arts and Science, University of Alberta, Calgary, Alberta, Canada.

Biochemist (under 45, with a degree in chemistry or biochemistry with postgraduate experience, preferably in the problems of animal nutrition and/or physiology in the tropics) in the East African Agricultural and Forestry Research Organization, for work on biochemical problems in the Animal Husbandry Division—The Director of Recruitment, Department of Technical Co-operation, Sanctuary Buildings, Great Smith Street, London, S.W.1, quoting NF/RC. 264/348/01.

Demonstrator in Physics—The Registrar, The University, Nottingham.

Lecturers or Assistant Lecturers (2) (with experience in at least one of the following fields: microbiology, plant physiology or genetics) in the Department of Biology—Dr. G. Power, Acting Chairman, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.

Master for Physics (able to take advanced and scholarship work, with some mathematics); and a Master to take Chemistry (some advanced work available for suitably qualified man)—The Rector, Edinburgh Academy, Edinburgh 3.

Mathematician (some sixth form work available)—The Headmaster, Leeds Grammar School, Leeds, 6.

Physicist—Head, Department of Physics, Victoria College, Victoria, B.C., Canada.

Professor of Statistics at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W C.1

Research Assistant (organic chemist or biochemist) in the Department of Textile Industries, to undertake work on polysaccharides of intervertebral discs and connective tissues—The Secretary, The Royal Infirmary, Bradford 9, and The Registrar, Bradford Institute of Technology, Bradford 7.

RESEARCH ASSISTANTS (well qualified physicists, preferably with some research or industrial experience) IN THE DEPARTMENT OF PHYSICS, to join research groups working in the fields of X-ray crystallography, nuclear physics, magnetism, print recognition and ophthalmic optics—The Registrar (Dept. 61/J), Bradford Institute of Technology, Bradford 7.

SENIOR LECTURER or LECTURER (with a first- or second-class honours degree in chemistry or chemical technology or equivalent, and preferably with postgraduate research experience) IN CHEMISTRY, to lecture to university degree students and to specialized courses, and assist with laboratory classes—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 71: The Cultivation of Raspberries. Pp. 10. (Belfast: Ministry of Agriculture, 1962.) [1610

General Register Office. The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, quarter ended 30th June 1962. (No. 454, 2nd Quarter 1962). Pp. 24. (London: H.M. Stationery Office, 1962.) 2*s*. 6*d*. net. [1610

Imperial College of Science and Technology. Research Report of the Royal College of Science, 1959–62. Pp. v+96. (London Imperial College of Science and Technology, 1962.) [1610

Office of Health Economics. Progress Against Tuberculosis. Pp. 28. (London: Office of Health Economics, 1962.) [1610

The Unity of Life. By Prof. H. L. Kornberg. (An Inaugural Lecture delivered at the University of Leicester, 23 January, 1962.) Pp. 21. (Leicester: The University Press, 1962.) 3*s*. [1610

The Architect's Anguish. By Prof. Misha Black. (Harry Hardy Leach Memorial Lecture delivered 15 February 1962.) Pp. 18. (Leicester: The University Press, 1962.) 2*s*. 6*d*. [1610

Stress and Adaptation. By Prof. S. G. McK. Lee. (An Inaugural Lecture delivered at the University of Leicester, 23 November 1961.) Pp. 20. (Leicester: The University Press, 1962.) 3*s*. [1610

Science In Your Methods. Pp. 12. (London: Department of Scientific and Industrial Research, 1962.) [1610

University and College Entrance: The Basic Facts. Fourth edition. Pp. 72. (London: The National Union of Teachers, 1962.) 4*s*. [1610

Marine Biological Station—University of Liverpool. Annual Report No. 74 for 1961. Pp. 43. (Liverpool: The University Press, 1962.) 5*s*. [2210

Society for the Promotion of Nature Reserves. Handbook 1962—Forty-fifth Annual Report, year ending 31st March, 1962. Pp. 64. (London: The Society for the Promotion of Nature Reserves, British Museum (Natural History), 1962.) 10*s*. [2210

Research Defence Society. Conquest Pamphlet No. 16: The Use of Laboratory Animals in Assessing the Safety of Food Additives: Outstanding Questions Posed and Answered by the Research Defence Society. Pp. 7. (London: The Research Defence Society, 1962.) 6*s*. [2210

The British Association for the Advancement of Science. Report, 1961–1962. Pp. 100. (London: The British Association for the Advancement of Science, 1962.) 2*s*. 6*d*. [2210

Imperial College of Science and Technology, (University of London). Calendar, 1962–63. Pp. xi+439. Prospectus, 1963–64. Pp. vii+64+4 plates. (London: Imperial College of Science and Technology, 1962.) [2210

Overseas Students in Britain: a Handbook for all who are interested in the welfare of Overseas Students. Revised edition. Pp. 39. (London Standing Committee of the London Conference on Overseas Students, c/o The British Council, 1962.) 1*s*. 6*d*. net. [2210

The Natural Rubber Producers' Research Association. Technical Bulletin No. 7: Design and Use of Natural Rubber Bridge Bearings. By P. B. Lindley. Pp. 22. (London: The Natural Rubber Bureau, 1962.) [2210

Department of Scientific and Industrial Research. Forest Products Research. Bulletin No. 49: Electrical Heating for Setting Synthetic Resin Glues in Wood Assembly Joints. By J. F. S. Carruthers. Pp. iv+10+5 plates. (London: H.M. Stationery Office, 1962.) 2*s*. 6*d*. net. [2210

Other Countries

Queensland. Seventeenth Annual Report of the Council of the Queensland Institute of Medical Research for the year ended 30th June, 1962. Pp. 12. (Brisbane: Queensland Institute of Medical Research, 1962.) [1610

United States Department of the Interior: Fish and Wildlife Service. Regulatory Announcement No 64: Federal Aid in Wildlife Restoration. Pp. 21. 15 cents. Regulatory Announcement No 65: Federal Aid in Fish Restoration. Pp. 22. 15 cents. (Washington, D.C.: Government Printing Office, 1962.) [1610

United States Department of the Interior: Fish and Wildlife Service. Bureau of Commercial Fisheries. Fishery Bulletin No 204: Sexual Maturity and Spawning of the Albacore in the Central South Pacific Ocean. By Tamio Otsu and Richard J. Hansen. Pp. iv+151–161. (Washington, D.C.: Government Printing Office, 1962.) 15 cents. [1610

United States Department of Commerce: Weather Bureau. Technical Paper No. 45: Snowmelt Floods of March–April 1960: Missouri and Upper Mississippi Basins. Pp. iv+77 (9 plates). (Washington, D.C.: Government Printing Office, 1962). 1.25 dollars. [1610

United States Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Research Report No. 58: Chronic Effects of Endrin on Bluntnose Minnows and Guppies. By Donald Irvin Mount. Pp. iv+38. 35 cents. Research Report No. 59: Predicting Year-Class Abundance of Yellowstone Lake Cutthroat Trout. By Ross V. Bulkley and Norman G. Benson. Pp. iv+21. 25 cents. (Washington, D.C: Government Printing Office, 1962.) [1610

Metropolitan Life Insurance Company. Statistical Bulletin, Vol. 43, (August, 1962): Recent Trends in Diabetes Mortality. Population Growth in the Southwest. Improved Mortality Among Coloured Policyholders. Work-Time Loss from Illness and Injury. Pp. 12. (New York: Metropolitan Life Insurance Company, 1962.) [1610

Sandia Corporation. Reprints, Monographs, and Research Colloquia—Title List. Pp. 30. (Albuquerque, New Mexico: Technical Information Division, Sandia Corporation, 1962.) [1610

Canada Department of Agriculture: Research Branch. Research Report 1960 and 1961 of the Entomology Research Institute for Biological Control, Belleville, Ontario. Pp. 28. (Ottawa: Queen's Printer, 1962.) [1610

United States Department of Commerce: Coast and Geodetic Survey. Technical Bulletin No. 20: The Earth as Viewed from a Satellite. By Erwin Schmid. Pp. ii+21. (Washington, D.C.: Government Printing Office, 1962.) 20 cents. [1610

Canada: Department of Forestry. Annual Report of the Forest Entomology and Pathology Branch for year ended March 31, 1962. Pp. vii+137. (Ottawa: Queen's Printer, 1962.) [1610

Smithsonian Institution: United States National Museum. Contributions from the Museum of History and Technology. Paper 29: The Development of Electrical Technology in the 19th Century. 2: The Telegraph and the Telephone. By W. James King. Pp. 273–332. 70 cents. Paper 30: The Development of Electrical Technology in the 19th Century. 5: The Early Arc Light and Generator. By W. James King. Pp. 333–407. 70 cents. (Washington, D.C.: Government Printing Office, 1962.) [1610

Transactions of the American Philosophical Society. New Series Vol. 52, Part 4: Mediaeval Arabic Bookmaking and Its Relation to Early Chemistry and Pharmacology. By Martin Levey. Pp. 79. (Philadelphia: American Philosophical Society, 1962.) 2 dollars. [1610

Sandia Corporation Bibliography—Civilian Application Releases through 1961. By the Technical Information Division. (*SCR-500*. TID-4500. 17th Edition). Pp. 72. (Albuquerque, New Mexico: Sandia Corporation. Available from the Office of Technical Services, Department of Commerce, Washington 25, D.C.). 1.75 dollars. [1610

Field Museum of Natural History. Botanical Series, Vol. 13, Part 5–A, No. 1: Flora of Peru. By Prof. Mildred E. Mathias and Prof. Lincoln Constance. Pp. 97. (Publication 959). (Chicago: Field Museum of Natural History, 1962.) 1.75 dollars. [2210

Smithsonian Contributions to Astrophysics. Vol 5, No. 12: North–South Asymmetry in Solar Spottedness and in Great-Storm Sources. Pp. iii+187–208. 25 cents. Vol. 5, No. 13: Neutral Hydrogen between Galactic Longitudes 200° and 265°. By R. J. Davis. Pp. ii+209–230. 25 cents. (Washington, D.C.: Government Printing Office, 1962.) [2210

Central Food Technological Research Institute, Mysore. Annual Report, 1960–61. Pp. vi+112. (Mysore: Central Food Technological Research Institute, 1962.) [2210

Tanganyika: Ministry of Commerce and Industry. Annual Report of the Geological Survey Division for 1961. Pp. ii+21. Geological Maps. Quarter Degree Sheet 274: Milo (Njombe South). (Dodoma: Department of Geological Survey, 1962). [2210

Fifth Report of the West African Timber Borer Research Unit, Year 1961–1962. Pp. 96. (Kumasi: West African Timber Borer Research Unit; London: Crown Agents for Oversea Governments and Administrations, 1962.) 5*s*. [2210

Tenth Annual Report of the West African Institute for Oil Palm Research, 1961–62. Pp. 121. (Benin City: West African Institute for Oil Palm Research; London: Crown Agents for Oversea Governments and Administrations, 1962.) 2*s*. 6*d*. [2210

Proposal for an International Institute of Science and Technology: *Report of the Working Group appointed by the Secretary General of* the North Atlantic Treaty Organization. Pp. 57. (Paris: North Atlantic Treaty Organization, 1962.) [2210

Indian Forest Bulletin, (New Series). Entomology. No. 238: Sal-heartwood Borer (*Hoplocerambyx spinicornis* Newm.) and Its Control by Trap Tree Methods. By R. N. Mathur. Pp. 13. Rs. 1.35 *nP*.; 2*s*. Indian Forest Records, (New Series), Silviculture. Vol. 10, No. 10: General Standard Volume Tables and Commercial Volume Tables (Based on Palamau Division, Bihar and Rajpipla Division, Saurashtra) for *Boswellia serrata* Roxb. By S. K. Seth and S. N. Dabral. Pp. 247–256. Rs. 0.85 *nP*.; 1*s*. 3*d*. (Delhi: Manager of Publications, 1962.) [2210

Colony of Mauritius. Annual Report of the Meteorological Department, 1961. Pp. i+12. Meteorological Observations and Climatological Summaries. April, 1961. Pp. 19. May, 1961. Pp. 19. June, 1961. Pp. 19. (Port Louis: Government Printer, 1962.) [2210

Fisheries Research Board of Canada. Progress Reports of the Atlantic Coast Stations, No. 73. Pp. 73. (Ottawa: Queen's Printer, 1962.) 75 cents. [2410

Indonesia. *Department of Air Communication*: Meteorological and Geophysical Institute. Preliminary Report on Effects of Una-Una Earthquake on April 29, 1960. Pp. 9. Reporting the Flores Earthquake. Pp. 8 (16 figures). (Djakarta: Department of Air Communications; Meteorological and Geophysical Institute, 1960 and 1961.) [2410

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A NORTH ATLANTIC INSTITUTE OF SCIENCE AND TECHNOLOGY

As a result of discussions at meetings of the Science Committee of the North Atlantic Treaty Organization, a study group was appointed, with Mr. Louis Armand as chairman and including as members Sir John Cockcroft and Sir Solly Zuckerman, with the view of making an independent study of ways whereby science in the Western World could be strengthened. The study group first met in September 1959, and its report, *Increasing the Effectiveness of Western Science*, published in the autumn of 1960[*], recommended, *inter alia*, further study by an appropriately chosen group of the establishment of an international institute of science and technology. On November 2, 1960, the North Atlantic Council authorized the appointment of a small high-level working group to examine this question and to formulate recommendations on specific possibilities and their feasibility.

This working group was duly appointed, its membership including Sir John Cockcroft and Dr. J. R. Killian, jun., as chairman, and its report was presented to the Council of the North Atlantic Treaty Organization on October 13, 1961, but was only released for publication in May 1962[†]. It represents the outcome not only of the Committee's own intensive deliberations but also of numerous formal and informal discussions with many leaders in the scientific and educational community. The group was unanimous in concluding that it is both feasible and desirable to establish in Europe an international institute of science and technology devoted to graduate and postdoctoral studies and operating at the pinnacle of the university system. Furthermore, it felt that the benefits which such an institution could bring to science, engineering and education, and thus to the welfare and security of the Western countries, made its establishment urgent. The working group concluded that to-day the widely recognized advantages of international efforts in science, together with the growing facility and effectiveness with which Western countries undertake co-operative efforts, provide an unprecedentedly favourable climate for establishing such an institution. The increasing interdependence of nations and the importance to scientific progress of free and cultivated international exchange set the stage for new joint efforts. Only, in fact, by such united efforts can the European countries, in spite of their rich traditions of scientific achievement, hope to maintain their levels and enhance their traditions in face of the resources possessed by the United States and the U.S.S.R.

In presenting these conclusions, the working group stresses its conviction that such an international institute, operating at the highest international level, would supplement rather than duplicate the scientific and technological departments of existing universities and institutes, which, no matter what their strength, find it increasingly difficult to cope with the demands on teaching and research created by the growth of science and the rising numbers of students. To meet this need, Western nations must sustain and reinforce existing institutions, as well as increase their number. New academic institutions of the highest class must also be established, and the working group believes that all Western nations could gain an educational resource not otherwise achievable by jointly sponsoring one of these new institutions and giving it an international format. Such an international institution would be able to combine some of the advantages of North American, British and Continental universities, and the experiment might also assist existing universities in their efforts to modify their systems and traditions to accord with modern conditions. Through its freedom to innovate, to make a fresh start, unencumbered by traditional habits, and to exploit the opportunities unique to its international status, an international institute could be a fresh stimulus to all institutions and add to the scope and diversity of Western education at the highest academic level.

Sir John Cockcroft has already strongly urged that Britain should take the lead in establishing such an institute as a considered part of her programme for university expansion, and it is obvious that whatever contribution is made by the United Kingdom it should be considered as part of that expansion. Furthermore, the working group urges that to launch such an institution should strengthen Western morale and demonstrate the intellectual strength, vigour and unity with which Western nations can work together. Equally, it should demonstrate that only in the Free World can a truly international educational institution, attractive to first-rate scholars from all countries, be achieved.

The working group was influenced by three other broad considerations. Besides the acute need throughout the Western world for more scientists and engineers of exceptional ability, there is a growing need for leaders and scholars in these fields who combine professional excellence with that broad understanding of Western culture and languages which could be gained in an international community of scholars. This breadth of education, experience and outlook will be increasingly vital as the economic and political unity of the Western World grows.

Secondly, merely to keep peace, the Free World must double and redouble the scientific capabilities in the immediate future, and to preserve its cherished freedom, its art and the humanities will be the easier if the increasing numbers of scientists and engineers are better trained and equipped to nourish their art at the very frontiers of science. It is a matter of fulfilling the Western World's own ideals, of providing itself with a richer and better life, and of having the resources as well as the desires to behave with generosity to less-favoured nations.

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[*] Fondation Universitaire, Brussels. *Increasing the Effectiveness of Western Science*. Pp. iii+18. (Brussels: Fondation Universitaire, 1960.)
See also *Nature*, 188, 880 (1960).

[†] Proposal for an International Institute of Science and Technology: Report of the Working Group appointed by the Secretary General of the North Atlantic Treaty Organization. Pp. 57. (Paris: North Atlantic Treaty Organization, 1962.)

Thirdly, the establishment of the European Organization for Nuclear Research (CERN) and other internationally sponsored research institutions has already shown the feasibility of financing and operating such institutions, and their existence encourages the view that an international institute of science and technology is a practicable project, though the working group did not at the same time recommend the establishment of an international foundation for the development of science and technology. Such a project was regarded as outside the terms of reference of the working group, which nevertheless informally records its support for the proposal. The report, in fact, includes the outline of a scheme for organizing such a foundation and suggests that its establishment should be considered by a suitable international body.

The report sets forth in some detail the objectives and plan for the proposed institute, including its organization and academic programme, the admission of students, appointment of staff and finance. A staff of 398 is suggested, of whom 133 would be senior, with 1,055 clerical and technical assistants, and a student body of 1,025, of whom 340 would be postdoctoral. A capital investment of 55 million dollars is envisaged with recurrent annual expenditure of some 16 million dollars.

The institute is to be in the fullest sense an international institution, sponsored by a group of nations and bringing together students and staff from many countries, seeking through its organization, spirit and motivation to imbue its students with a wider understanding of science and technology, and indeed of all learning. It would recognize the growing interrelations between different fields of learning and research, stressing in its structure and all its programmes the unity of knowledge and seeking to achieve a flexibility which promotes easy interrelations among fields and to avoid rigidly organized departments. Pitching its programme at the pinnacle of university education, it would seek to build a research programme of such quality and comprehensiveness as to provide the richest possible environment for nurturing scientists and engineers of exceptional creative ability—recognizing always that research and education are inseparable and being alert to identify important new fields of research and to educate scientists in these fields.

The institute would seek a closer association between pure and applied science, and besides fostering the closer association of the education of scientists and engineers, would seek to achieve a productive relation between science and technology. It would endeavour to respond to the need for greater stress on graduate education in engineering as well as in science, and for more versatile and adaptable engineers. Through the establishment of close relations with industry it would endeavour to facilitate the rapid application of new ideas arising from basic research. It would naturally become an international centre for scientific conferences and the exchange of ideas, and formal provision of a centre for advanced study should make it a mecca for outstanding scholars. An ultimate objective would be to include a strong programme devoted to the relation between science and society and the cultivation of the humanities and social sciences as partners of science and engineering.

Generally, the institute would provide freedom of scholarly opportunity at all levels, thus encouraging individual development and initiative and achieving an environment superlatively attractive to scholars for their work and professional growth. It is in accordance with this that only exceptionally talented students would be enrolled. The academic senate would recognize successful course work and independent research of high calibre by the award of a doctor's degree, and it is hoped that formal recognition of this degree in all member countries would contribute to the reduction of existing degree barriers among various countries.

These are large objectives, many of which already shape the programmes of leading universities and institutes in the Western World, and while the proposed plan offers no particular novel methods of pursuing them, other than the international character of the institute, the working group believes that the special combination of these objectives and their pursuit in an institute sponsored internationally could provide a new pattern representing an important new resource to the West. The nature of that contribution becomes a little clearer from the outlines of the proposed plan. The institute would be a graduate institution with no undergraduate students, and all students would engage in research and some in teaching. Besides emphasizing the intimate interrelation of research and teaching, in both aspects an interdisciplinary approach is desiderated.

To this end, besides the centre for advanced study already noted, the institute would establish a small number of research centres in specified areas requiring integration of diverse disciplines. In the first instance, these are suggested for applied mathematics and theoretical physics, technological processes and systems, materials research, earth sciences, and life sciences. Stress is laid on the importance of flexible organization and procedure, and the working group hopes that the institute will have the courage to discontinue centres and programmes that lose importance if more important programmes emerge that could replace them.

It should be clear from this brief summary of the proposals that while the participation of the United Kingdom in the scheme would represent an effort of which due account must be taken in the expansion of university and technological education in Britain, the effect on undergraduate education in the first instance will be only indirect. The contribution to postgraduate education could, however, be significant, even if Britain could scarcely expect to provide even 200 of the 1,000 student places. Apart, however, from the possibilities in regard to research, particularly in interdisciplinary fields, the institute might make a valuable contribution to the provision of the teaching staff, which promises to be the real bottleneck in university expansion. Further, it is possible that, if the flexibility which the working group desiderates so firmly can be achieved in practice, a lead might well be given to the solution of the problems which arise when research teams established around some distinguished scientist in a research institute or to share some highly expensive equipment lose their leader or find the field diminishes in importance. Viewed purely from this point of view of experiment in dealing with the major problems of research to-day, the proposal deserves serious consideration and if the other members of the North Atlantic Treaty Organization are prepared to join in establishing such an institute, there should be no hesitation on the part of the British Government in making its proportionate contribution to the capital and recurrent expenditure involved.

A NEW OXFORD DICTIONARY

Oxford Illustrated Dictionary

Text edited by J. Coulson, Prof. C. T. Carr, Lucy Hutchinson and Dorothy Eagle. Pp. xvi + 974. (Oxford : Clarendon Press, 1962.) 50*s*.

THE latest dictionary from the Clarendon Press is a break with tradition; however, as the preface points out, this is a well-planned break and as such represents the culmination of more than twenty years work. The *Oxford Illustrated Dictionary* extends to nearly 1,000 pages and contains more than 30,000 separate entries with more than 600 supporting drawings. Although it is the first of the Oxford English Dictionaries to make use of illustrations, it is not this that has revolutionized the Oxford Dictionary system, for from its own definitions this latest publication cannot truly be classified as either a dictionary or an encyclopædia. On one hand, it goes much farther than the traditional idea of a dictionary, and on the other it is not sufficiently extensive in its definitions to be used just as an encyclopædia. All in all, it represents an intermediate stage between the two.

The fact that a printing house steeped in tradition, as is the Clarendon Press so far as its dictionaries are concerned, should feel that there is a need for such a work is a consequence of the ever-increasing strides of science and technology. No longer is it sufficient to have a lexicon that deals solely with words, their use, spelling, significance, etc.; instead such a work must be supplemented by definitions of 'things'. The preface states that "the vocabulary has been chosen with an eye to the needs of one who may require either type of information. Information about words, however, is more often sought by the average user of a reference book than information about things and the vocabulary has therefore been based on that of the *Concise Oxford Dictionary* . . . ".

Special consideration has indeed been given to many terms in everyday use which would normally be excluded from an ordinary dictionary because of their technical and scientific character. For example, the entry for 'bacterium' carefully defines the organism, outlines the occurrence and activities of this kind of organism and, by illustration, indicates the differences between the coccus and the bacillus forms, giving as examples *Staphylococcus*, *Streptococcus*, *B. anthracis*, *B. typhosus* and *Spirilla*. Generally the entries for zoological and botanical types are under the common name; but details of the classification and common species are also supplied, and in some cases information concerning physiology and origin is also included. Treatment of the chemical elements is extensive, and details are given of the atomic number, atomic weight, common isotopes, occurrence and the more generally known compounds of that element. Included in the appendixes are lists of the chemical elements and also British, American and Continental weights and measures.

Even though the present pace of development in science and technology is likely to make it impossible for any general reference book to be completely up to date, such terms as 'fall-out', 'radiocarbon dating', 'computer', 'rocketry', 'sputnik' appear, but not 'laser', 'maser', 'chromatography' or 'Van Allen belt'. However, it is good to be reminded that the Leyden jar was invented at the University of Leyden in 1745 "for storing electric charge, consisting of a glass jar coated inside and outside with tinfoil, the electric energy being stored in the glass dielectric (non-conducting substance) between the tinfoil electrodes"; that Birkbeck College was " . . . formerly the Birkbeck Mechanics' Institution, founded 1824 by Dr. George Birkbeck (1776–1841), a physician and professor of natural philosophy"; that Uranus is "The 7th of the major planets, farthest from the sun except Neptune and Pluto; discovered in 1781 by Sir W. Herschel . . .". From these examples it can be seen that the *Oxford Illustrated Dictionary* goes much farther than dealing with words and things. In fact it also deals with famous people, the names of important places and events—the Clarendon Press is to be congratulated for extending the services of the *Dictionary* to such ends. It is inevitable that there should be some omissions, but it is to be regretted that, among the scientists, while Dalton, Fleming, Banting, Ross, Curie, Bohr, Raman, Rayleigh and Röntgen (to mention but a few) have been included, Leeuwenhoek, Rutherford and Thomson (J. J.) have been omitted.

The *Oxford Illustrated Dictionary* goes far to meet a need that has developed during the past half-century and still remains within the financial reach of the average individual. The layman has now only himself to blame if he remains ill-informed of the trends in world science, for whereas before he could cry out that he could not understand the language of the scientist, he now at least has a means to help him interpret it. At 50*s*. the *Oxford Illustrated Dictionary* should find a place on all shelves of reference books.

CONCRETE

Concrete

Properties and Manufacture. By T. N. W. Akroyd. Pp. vii + 336. (London and New York: Pergamon Press, 1962.) 50*s*. net.

THERE is no doubt whatever that concrete holds a very high rank among building materials. In the past concrete was regarded as a useful but cheap substitute for other materials, and before the introduction of the now well-known and fundamental water–cement ratio law first introduced by Abrams, the requirements for good concrete were not fully understood or appreciated. Consequently concreting was often carried out in a more or less haphazard way and generally by unskilled labour.

To-day, the design and control of concrete have become specialized activities founded on principles which are becoming so well established that the production of inferior concrete is now almost inexcusable. This does not mean that concrete is a complex science involving the use of intricate formulæ and complicated procedure; on the contrary, in spite of the confusion of literature on the subject, it is a fairly straightforward and practical art.

With a thorough understanding of some basic principles, the observation of a few fundamental and often common-sense rules, and the exercise of certain reasonable precautions, the production of high-quality concrete becomes a relatively simple task.

There are many excellent books available on the subject of concrete, but the first impression given by Mr. Akroyd's book is that he has achieved a good balance between theory and practice. The book is well set out in a logical sequence, and although in his preface the author indicates that it is intended for students and graduate engineers, I am quite certain that this book will find a very much wider range of reader.

The opening chapter provides some concise and useful information on the properties of concrete and this is followed by a rather lengthy but important chapter dealing with the materials used for concrete manufacture. The chapter on mix design sets out some useful examples based on recognized methods. The chapter on concrete manufacture: handling and batching of materials, mixing and placing, compacting and curing, is most informative and always keeps the practical aspect well in mind. The short chapter on quality control is adequate for the purpose of introducing a statistical treatment of strength test results, and in again reminding the reader of the importance of good workmen and efficient plant. The remaining chapters dealing with resistance to deterioration, surface treatment, and special concrete are based on reliable sources of information.

The provision of a general index and a list of references after each chapter is extremely useful.

To summarize, I think this book will be very helpful not only to students and graduate engineers but to other concrete workers. I compliment the author on producing such a splendid balance between theory and practice. A. J. NEWMAN

ENTOMOLOGY AT HIGH ALTITUDES

Introduction to High Altitude Entomology

Insect Life Above the Timber-line in the North-West Himalaya. By Dr. M. S. Mani. Pp. xix+302+10 plates. (London: Methuen and Co., Ltd., 1962.) 42*s*. net.

PROF. M. S. MANI of Agra University has led a number of expeditions to the Himalaya and has found, in what at first appears to be an inhospitable expanse of snow or wind-swept rock at heights of 3,500–5,000 m, a surprisingly rich insect fauna.

The most conspicuous insects are the big alpine butterflies like *Papilio machaon* and the many species of *Parnassius* which feed on the bright flowers of the short alpine summer. Their larvæ take shelter in rock crevices and feed on the low herbage. A few plant-feeding bugs and beetles are also dependent on these plants for food.

Readers unfamiliar with these places will be surprised to hear that the most numerous insects are scavenging Carabid and Staphylinid beetles, springtails and earwigs which are especially numerous in rock crevices near the melt line of the snow.

These are not so much dependent on the local plants for food as on the vegetable debris, pollen and insects originating in the subtropical plains to the south carried on the high air currents and deposited in cold storage on the snow-fields and glaciers. Careful watch gave a figure of two insects deposited per sq. m per min. At times the snow was littered with the corpses of flies, moths and aphids, and occasionally migrating swarms of butterflies were overtaken by bad weather and brought down.

As the snow-fields melt in summer, their surfaces and edges provide a rich harvest of preserved food which is taken by the active scavengers.

Another significant source of food is provided by the migrating ladybirds and other insects which form great aggregations at heights of over 4,000 m and return to lower levels after diapause. These are eaten by birds and even bears. The glacier snouts also disgorge in the short summer their accretions of food which at least partly explains the richness of the fauna of the torrents the water of which abounds with early stages of mayflies, stone flies, and countless fly larvæ, many of which are filter feeders.

The fauna of the mountain sides is greatly influenced by aspect. North and south faces are characterized by different groups of species. The slopes remain very barren except where melt water or snow-fields are adjacent. The animals swarm under the rocks which are warmed by the Sun. Only a few centimetres down in crevices temperatures of 10°–25° C and high humidities may be available for long periods, while outside it is very cold and dry. Insects of the north slopes cannot find these hot-house conditions, but many of them can maintain activity close to freezing point or even at −10° C. Collembola may become so numerous and active on snow as to impart a sooty appearance to the snow-fields.

This book is in the nature of an interim report. Perhaps three-quarters of the insects caught have as yet not been named beyond the genus (the butterflies and a few groups of beetles being the only groups easily named to species). Doubtless there are many new species in the collections still to be named, and as more and more groups are worked out in the various museums many curious new facts of distribution and ecology will emerge.

Zoologists who have tended to equate the mountain flora and fauna in the tropical zones with the tundra of the far north will find the broad generalizations of this book very stimulating. It serves to emphasize that the mountains differ not only in the physical attributes of the environment such as the relative constancy of day-length, but in the amount of nutriment derived from the richer environments close by.

The book is attractively illustrated by photographs, some of which are in colour, and by many clear line drawings and diagrams. There is a detailed bibliography and a good index. G. C. VARLEY

SPECULATIVE IMMUNOLOGY: NEW MAPS FOR OLD

The Integrity of the Body

A Discussion of Modern Immunological Ideas. By Sir MacFarlane Burnet. (Harvard Books in Biology No. 3.) (Cambridge, Mass.: Harvard University Press and London: Oxford University Press, 1962.) Pp. vi+189. 30*s*.

SIR MACFARLANE BURNET is at once the elder statesman and the *enfant terrible* of immunology. No one knows better than he how to assume an imposing air of magisterial authority while cutting the most outrageous capers.

His latest book, *The Integrity of the Body*, is a new triumph for his special blend of magniloquence and mischief. The general scientific reader, if unprotected against the exhilaration of Burnet's style by any special knowledge of the topics discussed, is assured of having the time of his life. The professional immunologist, if he is worth his salt, may derive a benefit of a different kind: in all likelihood he will be goaded into fresh experimental attempts to refute, once and for all, this irrepressible sage.

The first four chapters cover in a clear and breezy style the elementary background of disease-resistance, antibodies, blood groups and hypersensitivity reactions. Chapter 5, which is about cells, suffers from a misfortune of timing. Important recent advances made by J. L. Gowans and others in charting the life-histories and metamorphoses of the cells responsible for immune reactions were made public after Burnet's book went to press, and the same is true of much of our new knowledge of the immunological role of the thymus gland. A similar mishap, which overtakes the following chapter on "Self-recognition", reveals the unwisdom of dogmatism in scientific exposition. The chapter contains among other things a gallant attempt to expound the principles by which protein primary structure can be coded in the base-sequence of the DNA molecule. The description appears, however, to be based on the now disproved 'commaless code' of Crick, Griffith and Orgel. The account of immunological tolerance given in this same chapter constitutes a more serious case of the same nature—more serious because it is a topic on which Burnet may claim a special title to have his views heard. The statements that "tolerance must be induced in the first day of life or before birth" and "no animal can produce antibody till it is at least 14 days old" are both incorrect. First, states of immunological unresponsiveness which are indistinguishable from tolerance can be induced in adult mice. Secondly, in sheep and opossums antibody-formation has been successfully elicited in embryonic life. These facts are exceedingly damaging to Burnet's own particular theories, according to which tolerance results from confrontation with antigen during a special 'self-recognition' process supposed to occur at a critical phase of ontogeny. Indeed, they suggest a quite different interpretation of tolerance in terms of the well-known phenomenon of 'immunological paralysis' first demonstrated in adult mice by Felton. Yet Burnet omits from this book all mention of Felton's work.

In the next chapter, "The Clonal Selection Theory of Immunity", Burnet vaults astride his own special hobby-horse, which soon, Pegasus-like, carries him far away from earthbound mortals. In the remainder of the book a few interesting descents to Earth are made, notably in the discussion of auto-immune diseases. He remarks, for example, on the occurrence of these diseases in patients suffering from congenital agammaglobulinæmia, and makes the important point: "Since in agammaglobulinæma antibody in the blood is never produced, we can feel sure that rheumatoid arthritis and the other so-called auto-immune diseases are not primarily due to anomalous antibody production, even though it may be easy to show in most cases that abnormal antibodies are present." But in the final chapter, entitled "The Deeper Problems", contact with established fact ceases, for we have come to the peroration. This takes the form of a plea for Burnet's present ideas concerning the evolution of immunological systems. He sees their function not primarily as that of a defence force against microbial invasion; rather they constitute a sort of secret police for the liquidation of deviationist (for example, cancerous) cellular elements which may arise from within by somatic mutation. This is, Burnet admits, "no more than a hypothesis. But it is in accord with the whole trend of modern immunology that it should be true". This statement has surprise value, if nothing else, and the book concludes on a lively note.

DONALD MICHIE

ADVANCES IN STEREOTAXIS

A Stereotaxic Atlas of the Cat Brain

By Ray S. Snider and William T. Niemer. Pp. 132. (Chicago and London: University of Chicago Press, 1961.) 95*s*.

A Stereotaxic Atlas of the Monkey Brain (Macaca Mulatta)

By Ray S. Snider and John C. Lee. Pp. 132. (Chicago and London: University of Chicago Press, 1961.) 95*s*.

THE brain is one of the most inaccessible of the body's tissues and in both the clinical and experimental fields means have been sought of gaining access, without excessive trauma, to its deeper parts. An early attempt to take advantage of the reasonably close relationship between the bony landmarks of the skull and intra-cranial structures was made by R. A. Clarke in 1908, who built the first stereotaxic instrument based on rectangular co-ordinates for use by Sir Victor Horsley in the study of cerebellar function in the monkey. The potential of this technique for the localization and study of discrete cerebral structures was overlooked until 1936, when the late S. W. Ranson stimulated a renewal of interest in a tool which has since proved invaluable to the neuro-anatomist, neurophysiologist, neuro-pharmacologist and neurosurgeon. Though originally designed for the positioning of circumscribed electrolytic lesions the stereotaxic method has more recently been used for the administration of focused ultrasound and ionizing radiations and for the study of changes in neuronal activity following the electrophoretic application of drugs.

Several atlases have been designed for studying, by stereotaxic methods, such species as the cat, rat, rabbit, dog and monkey, and among these the present volumes are quite the most complete and sophisticated to date. Presentation is in the form of paired photomicrographs of beautifully prepared coronal sections of the brain extending, at intervals of 0·5 mm, from the anterior corpus striatum to the anterior limit of the nucleus gracilis. The plates are large in size and of exceptional clarity. One of each pair is stained and labelled to show nuclear and the other to show fascicular structures, and co-ordinates measured from the sagittal and inter-aural planes are clearly marked.

These atlases represent a considerable advance over their predecessors, which have concentrated mainly on the diencephalon, and the inclusion of the co-ordinates of medullary structures will be of considerable value to students of the reticular formation of the brain stem. One may perhaps regret that, in work of this quality, no attempt has been made to provide a guide to the identification of nuclei in terms of their finer anatomy other than to refer the reader to "original research articles in the various anatomical journals" which incidentally are not contained in the bibliography; and since the authors direct attention to the dangers of fouling the tentorium cerebelli with the advancing electrode tip it would have been helpful had, at the same time, the co-ordinates of this structure been illustrated on the appropriate sections. A further, and surprising, omission is a failure to define the horizontal plane of orientation in terms of extra-cranial structures—a feature which may prove disadvantageous to users of non-standard stereotaxic instruments. But such criticisms are small by comparison with the value of these two companion volumes whose appearance will be welcomed by all working with the cat and the monkey whether or not they are concerned with stereotaxic methods.

THE GIFTED CHILD

The Year Book of Education 1962

The Gifted Child. Joint Editors, Prof. George Z. F. Bereday and Prof. J. A. Lauwerys. Pp. xiv+541. (Published in Association with the University of London Institute of Education and Teachers College, Columbia University, New York, by London: Evans Brothers, Ltd., Twenty-fourth edition, 1962.) 63*s*.

THIS book contains sufficient educational dynamite to turn the education committee of every local education authority into a fratricidal bear garden. For there is war—and war to the death—between the views of Sir Cyril Burt and those of Prof. J. P. Guilford of the University of Southern California.

Sir Cyril Burt, the convinced Platonist, in a fine historical account of the development of the intelligence quotient (I.Q.) idolatry which some of us consider is slowly destroying the educative value of all conventional 'education', tells us that the gifted child, as defined by him and those who share his views, is "as a rule, taller, stronger and healthier than the average. He is on the whole free from abnormalities of whatever kind, physical, intellectual, emotional and nervous. Until they begin to specialize, most gifted children are gifted all round". They are, indeed, the very people whom Plato would have set aside to provide the upholders and preservers of the changeless State, and also, of course, of the small core of philosopher-kings. They are those whom Samuel Smiles would also have approved of most.

In contrast take this paragraph from Prof. Guilford's very important article. "And which children should be regarded as gifted? The current answer, at least in many places, is the student with a high I.Q. and with high grades (the two indicators usually strongly correlated). Such children may be those who please their teachers most because they learn more rapidly under conditions that call for uniformity of thinking and acting within a group. The more creative child, who may be higher in divergent thinking abilities and not so high in cognitive abilities emphasized in present tests and examinations, may be a source of annoyance and not recognized as gifted. And how many children who are potential composers or artists, who are very high in concrete intelligence but not so high in academic intelligence, are missed when the 'gifted child' is selected."

It was worth publishing this fascinating volume even if only to make the epoch-making researches of Prof. Guilford known to a wide non-specialist world public, and his article on parametry and categories of talent is doubtless the most exciting in this book. The wide researches carried out under his guidance on young adults, and supported by funds from the U.S. Office of Naval Research and the U.S. Office of Education, have established that there are already fifty-five different primary abilities which have been recognized; there are probably many more. For the past thirty years thousands of educationists and millions of ordinary people, believing that there is more faith in honest doubt, have found it hard to accept the view that the I.Q. test could really be adequate as a measure of human intellectual endowment. But we have been unable, through lack of the necessary research funds and research workers, to prove this scientifically. But thanks to Prof. Guilford and the U.S. Office of Naval Research we can now say with Prof. E. Paul Torrance, who writes on "Measurement and Development of the Creative Thinking Abilities", that educators "are beginning to recognize that the human mind is far more complex than can be inferred from present-day methods of identifying 'the gifted', and, in particular, that traditional measures of I.Q. tap only a few of man's thinking abilities". Indeed, Torrance is able to affirm "if present methods of identifying talent had been applied they would have eliminated many of the great men of the past".

The tragedy for England and Wales at this time is that the development of "Beloe" examinations in all secondary modern schools and streams is going to confine education for an ever-greater percentage of our pupils within the cast-iron boundaries of the traditional academic syllabus and examination. The opportunity for the teacher to develop in every one of his pupils the peculiar and valuable endowment that nature has given him is slowly being eroded away. As Prof. Guilford states, "If each child is to be given the opportunity to make the most of his outstanding assets, those outstanding assets will have to be recognized. They cannot be recognized by the use of an I.Q. test whose coverage of that great complex of intellect is severely limited to a few qualities that have predicted grades that are also severely limited in intellectual scope".

There are forty articles in this volume and they survey the developments in the chosen field from many points of view and discuss selection procedures in many countries. Every educational thinker and educational politician should dip into it.

C. H. DOBINSON

Logic and Existence

By Prof. Martin Foss. Pp. x + 240. (New York: Philosophical Library, 1962.) 4.75 dollars.

THE author of this book writes with a deep conviction of the part which philosophy has played not only in history but in present-day affairs in bringing discipline into the realm of thought. Much of what is said amounts to a strong plea for responsibility in mankind's work of ratiocination. There are two main parts, entitled "Abstraction" and "Reality". Running through the argument is a marked axiological thread, courageously facing the problem of value and what it entails. The outcome of this, as might be expected, is that a class of experiences will emerge, the members of which are individual, and, to that extent, unique. They are art, ethics and religion. Naturally, East and West have gone their separate ways, but there is no doubt that Prof. Foss perceives in a picture or a piece of music an emotional event, but subject to some measure of mathematical law. This is only to be loyal to his thesis that philosophy lives doing battle against subjective thinking. Abstraction is almost taken as a crutch to help us along uphill towards metaphysics.

All this is admirable, if slightly unfashionable. What would weld it together a little more convincingly might have been to introduce the '*Gestalt*-concept': then, with the help of the law of *prägnanz*, a minimal energy is shown to be implicit (akin to potential theory) in works of art. This method—if teleological considerations are permitted—could allow for (almost demand) the stark element of sacrifice which the writer's 'sacramental' principle requires.

F. I. G. Rawlins

Quantitative Chemical Analysis and Inorganic Preparations

By Dr. R. M. Caven. Second edition. Revised by Dr. A. B. Crawford and W. A. Alexander. Pp. vi + 428. (London and Glasgow: Blackie and Son, Ltd., 1962.) 35*s.* net.

THIS book, first published in 1923, and afterwards reprinted twenty times, now appears in a second revised edition. Intended as a teaching manual, it is divided into two sections—Part 1 at the elementary level, and Part 2 at a more advanced level for college and university students, the general effect, enhanced by some duplication of chapter headings, being one of two text-books in one binding. Part 1 is described as covering the preparation of inorganic salts and simple exercises in gravimetric and volumetric analysis, and Part 2, volumetric analysis, gravimetric separations, analysis of minerals and alloys, and preparation of inorganic compounds.

The treatment is on conventional lines and, on the analytical side, is intended to give the student a thorough grounding in classical principles, while the preparations ensure practice in other than purely analytical manipulations. Recent developments in analytical chemistry are represented in Part 2 by a chapter on chelatometric titrations, with six exercises in the use of ethylenediaminetetra-acetic acid, and one of three pages on some applications of ion exchange resin, with two practical examples. The chapter on colorimetric analysis is limited to three determinations by the Nessler technique. In view of the widespread use of the simpler colorimetric instruments and the growing importance of colorimetric methods of analysis, some amplification here might seem desirable, but the present authors state their intention not to include instrumental methods, which, in their opinion, are adequately dealt with elsewhere and which in any event provide very little opportunity for the student to acquire the basic analytical skills.

The style and manner of the original text have to a large extent been retained in the new edition, with the emphasis on readability as opposed to the almost peremptory conciseness of some present-day text-book writing.

G. A. Sergeant

Advances in Clinical Chemistry

Vol. 4. Edited by Harry Sobotka and C. P. Stewart. Pp. xiv + 378. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1961.) 86*s.*

AS in earlier volumes of this series, the contents of Volume 4 can be sharply divided into two classes—those dealing with methodology and those dealing with chemical substances or clinical conditions.

The sections on methodology cover flame photometry by I. MacIntyre, immunoelectrophoresis by C. Wunderly and ultramicro methods by van Haga and de Wael, and are given with a wealth of detail and an abundance of useful diagrams.

The other sections include a miscellaneous collection of topics ripe for review, such as the non-glucose melliturias by J. B. Sidbury, jun., organic acids in blood and urine by J. and R. Nordmann, ascorbic acid in man and animals by W. E. Knox and M. N. D. Goswami and parathyroid function by B. E. C. Nordin. The high standard of presentation which one is now accustomed to in this series has been maintained throughout.

J. N. Davidson

Advances in Spectroscopy

Vol. 2. Edited by Dr. H. W. Thompson. Pp. xi + 483. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1961.) 13 dollars.

THIS is the second volume of a collection of short surveys of developments in all the important branches of spectroscopy over the past few years. These are intended to be easily read and comprehended by spectroscopists working in other fields. The present volume contains nine such surveys on atomic absorption, flame emission, X-ray analysis, nuclear magnetic resonance, infra-red absorption of crystals and of micro-organisms, refraction of gases in the infra-red, ultra-violet absorption of proteins, and the theory of molecular energy levels. Each of these articles is an up-to-date and fairly comprehensive review by an authority in that particular field, although in some cases much of the subject-matter has been recently reviewed in other publications.

In order to cover the vast range of practical and theoretical topics embraced by the term 'spectroscopy', many volumes will be needed to complete this work, and with the present spacing of two years between the volumes, it may never be completed. While there exists a great need for surveys of this sort, it is surely necessary for each volume to be composed of a greater number of shorter articles confined to advances in the preceding two or three years and with a minimum of introductory material.

With these general reservations, this volume is authoritative and well produced, and should certainly be read by every spectroscopist who wishes to keep a grasp on the subject as a whole.

A. Knowles

CONSERVATION AND OUTDOOR RECREATION IN THE UNITED STATES

By J. J. MACGREGOR

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White House Conference on Conservation

EVEN the most casual observer of the American scene is aware of the present concern over the use of the nation's resources; nor are the broad reasons for the disquiet difficult to account for. In many ways the United States is an under-developed region of the world: its population has been, and is, low in relation to its extensive and very valuable natural resources, and this at times has given the appearance of wastefulness and of callousness over their usage. There was lavish use of land, of forests and of water under industrialization as the frontiers were pushed farther west. To-day there are no frontiers in this sense and the nation is taking stock of the way in which the "free gifts of Nature" have been, and are being, used. In an era when there was little local or regional planning, private enterprise had free-planning for exploitation of the local environment to suit its own particular, financial gain—this appears to have been a feature of early industrialization in most countries. Even if others paused to count the social cost there was little that could be done to stop the malpractices which opportunities for quick profits often engendered. Now it is different, and there is a growing recognition that unthinking exploitation must be countered as the social cost of soil erosion, water pollution, air pollution, silting up of reservoir and river basins, destruction of amenities and so on, is being added up. The rules of the game are being clarified. A distinction is being made between something which can be mined and eventually exhausted and one which is a renewable resource and can exist or be improved more or less in perpetuity if given good management. The antithesis of conservation is the depletion of natural resources.

Symptomatic of the present concern is the weight given by the Administration to conservation. In March of this year President Kennedy sent to Congress a message which underlined the urgency of conservation policy. The growths of population and of industrial activity and productivity implied greater demand and opportunities for leisure. While much of the affluence of the country was attributable to the abundance of many physical resources, these were not inexhaustible and did not automatically replenish themselves. Conservation policies implied wise use of the natural environment—an approach which could be described as the highest form of thrift. Special effort would be necessary to deal with the problems of air, water and land; fuels, energy and minerals; soils, forestry and forage; fish and wild-life and human resources. In his message, the President reminded Congress of his suggestions at the start of his administration and referred to the "heartening progress" made to counter water pollution and flooding; to the development in water resources partly through the saline-water programme; to the establishment of recreation areas; to long-term plans for wild-life and forestry and so on.

This message gives a very useful clue to the range and scope of a conservation policy within the American setting since it reviewed progress and made recommendations under each of the main heads in the following order: outdoor recreation (see next heading); water; public lands; soil; watershed and range resources; timber; power; and research and technology. Proposals for the formation of an Outdoor Recreation Advisory Council, a Bureau of Outdoor Recreation and programmes of 'matching' grants to help individual States to provide facilities are measures of their present importance. In January the Outdoor Recreation Resources Review Commission made its report after intensive investigation and its recommendations included the advisory council and the bureau which are now urged as part of national policy.

Other proposals emanating from the President's message were the creation of a land conservation fund; the setting up of new national parks; new legislation to plan water resources and to deal with land sales; amendment of the Watershed Protection and Flood Protection Act; development of national multiple-purpose forest roads; and the listing of a great variety of problems for research.

To maintain the momentum of the present activities outlined in his message to Congress, the President mentioned that he would convene a White House Conference on Conservation in order "to provide an opportunity for the exchange of further ideas—and to permit those who have dedicated their efforts to the principles of conservation to participate in evaluating the progress that has been made—and to seek the best possible advice in presenting what must be done in the future".

When the Conference was duly convened, towards the end of May, I was able to attend and learnt that it was the first White House Conference since Theodore Roosevelt had called a meeting of Governors for the same purpose in 1908. On this occasion it appeared that most of the speakers in the two-day programme were representatives of the executive or legislatives of Federal and State Departments. Appropriately enough the Conference began with an address from Mr. W. W. Heller, the chairman of the Council of Economic Advisers. He made it clear that economic considerations had come within the purview of the Conference theme of "Conservation for the 'Sixties" and exposed the futility of having large national incomes if, in the process of achieving them, the foundations were undermined. Subsequent speakers made different approaches to the same threat: the Secretary of the Interior, Mr. S. L. Udall, emphasized the limitations of the former piecemeal approach and that in the new efforts it was necessary to see things in perspective and to look at the resources as a whole rather than as watertight, individual compartments. Conflicts between diehard advocates should be avoided if conservationists were to be effective politically; simple solutions in complex matters were inappropriate and would lead to lost opportunities in a period of 'last chances'.

The Secretary of Agriculture, Mr. O. L. Freeman, dealt with the relationship of Government aid and land-use adjustments. He felt that it would be possible to balance the productivity of farming with the use of land and water resources to meet the growing demand from an expanding urban population—to reconcile food plenty with a recreation shortage. Clearly, agricultural and conservation policies had to be compatible and this object could be found in the conservation principles and multiple use of private lands.

Other speakers directed attention to significant tendencies or proposals. The encroachment by urban expansion was about one million acres per annum—the urban area had doubled in the past decade. The Army's Corps of Engineers takes a very active part in the civil development of the country and was carrying out a billion-dollar-a-year conservation mission in river basins, coastal harbours and the Great Lakes. With water resources it was not only essential to have adequate supplies for the future but also that they should be effectively sited and where wanted. The Senate Select Committee on National Water Resources had estimated that by 1980 more than 400 million acre-ft. of extra reservoir space would be required. In addition to the actual supply of water, reservoirs have multiple-purpose functions: from river regulation, storage of flood-water for release in dry seasons; recreation; fishing and wildlife. The increased opportunities for power production and for use by shipping were other features of water storage policy. This multi-purpose function is, indeed, one of the main lessons which the development of the Tennessee Valley Authority has effectively demonstrated.

The conservation movement undoubtedly has adherents who accept rather uncritically some of its limitations as a political panacea. Unlike those who take the economic view they tend to overlook the problems associated with the institutional constraints, evaluation and uncertainty. Economists realize that a nation has to make a choice over allocations of resources and over time. Conservation is only one choice which may merely mean that posterity's inheritance will be altered from capital goods to natural products.

To many who regard conservation as a rather vague or complex concept it is probable that few original ideas emerged from the Conference although those who are aware of some of its implications would welcome the publicity given to a subject which they regard as topical and urgent. At least the supporters of the movement can take comfort from the fact that some of the ideas of the conservationist are finding their way into legislation.

At the end of the Conference President Kennedy spoke of the need to apply scientific discovery to conservation. As an example he believed that the first country which could produce cheap and abundant fresh water from the sea would have even greater advantages than the one that would be first to conquer space and, as he put it: "Harnessing science to conservation is going to be the big accomplishment of our day".

Outdoor Recreation for America

At present very great interest is being shown in outdoor recreation in the United States. This is apparent, for example, from President Kennedy's message to Congress at the beginning of March 1962, where, indeed, recreation appears first in the order of proposals and recommendations made to Congress. It was in this message that the President intimated that he was going to have the White House Conference on Conservation because he wanted "to provide an opportunity for the exchange of further ideas—and to permit those who have dedicated their efforts to the principles of conservation to participate in evaluating the progress that has been made—and to seek the best possible advice in prescribing what must be done in the future".

Some of President Kennedy's proposals were based on recommendations of the Report by the Outdoor Recreation Resources Review Commission which was submitted at the end of January*. The Report is a well-documented and comprehensive survey of 246 pages, including the Commission's terms of reference, which were set out as follows:

"That in order to preserve, develop and assure accessibility to all American people of present and future generations such quality and quantity of outdoor recreation resources as will be necessary and desirable for individual enjoyment, and to assure the spiritual, cultural and physical benefits that such outdoor recreation provides; in order to inventory and evaluate the outdoor recreation resources and opportunities of the Nation, to determine the types and location of such resources and opportunities which will be required by present and future generations; and in order to make comprehensive information and recommendations leading to these goals available to the President, the Congress and the individual States and Territories, there is hereby authorized and created a bipartisan Outdoor Recreation Resources Review Commission".

After a review of the outdoor recreation resources, the Report measures present and likely demands on them over the next forty years, and recommends actions to ensure their availability to all Americans of present and future generations. In the past Americans had "responded to the need for protecting their unparalleled outdoor heritage" and it was now necessary to follow their lead at a time when the country was growing faster and becoming even more urban and requiring a new scale of effort and ingenuity. The Commission believed that a great deal could be accomplished by well-directed and vigorous action and by co-ordinated public and private activity and investment. The Commission's investigation was greatly assisted by the individual States some twenty Federal agencies and their own advisory council.

The main parts of the Report are concerned with an introduction with summary of recommendations. Part I, "The Facts", has five chapters headed: the outdoors in American life; the demand; the supply; the economics; and the needs. Part 2, "Recommendations", has nine chapters: guidelines for management; organizing for the task; Federal policies and programs; the key role of State Governments; recreation for metropolitan America; the private role; financing outdoor recreation; water—a key element; and research—an essential foundation. The appendixes cover the Act, State contact officers, the Commission's study reports—including 27 useful abstracts, contractors, consultants and statistical tables.

To measure present resources for outdoor recreation an inventory of all the non-urban public recreation areas which number more than 24,000 for the country as a whole and more than 100 items were analysed for 5,000 of the larger areas in order to evaluate existing uses, capacity and potential for development. Special studies were made to cover wilderness, water recreation, hunting and fishing, the densely populated north-east and sparsely populated Alaska. In order to estimate the existing and future pressure on the resources, a series of studies was made on the demand for recreation and a National Recreation Survey involving about 16,000 people was carried out for the Commission by the Bureau of the Census. This survey was also supplemented by studies by the Commission on the effects on outdoor recreation of present and prospective changes—sectionally and nationally—in personal income, population, leisure time and in travel facilities. A State contact officer, at the request of the Commission, was appointed by each Governor so that all requests could be channelled to him. They were usually the head of the State conservation, recreation, fish and game, or planning agency and played a major part in the inventory; in other studies they provided financial, legal and administrative data. The Federal Agencies in Washington and their field officers put their valuable experience before the Commission and also provided specific data on their programmes. In most of the studies the Commission started off by consulting these agencies to see what information was already available. Needless to say, the Commission was helped by a considerable staff which was recruited at the end of 1958. Apart from the editorial, administrative, secretarial and clerical staffs there were specialists on policy and programme, inventory and evaluation, forecasts and economics.

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* *Outdoor Recreation for America.* A Report to the President and to the Congress by the Outdoor Recreation Resources Review Commission. January 1962. (Washington, D.C.).

Under a heading on "Some Findings of the Study" the following conclusions were listed about outdoor recreation: the simple activities are the most popular; outdoor opportunities are most urgently needed near metropolitan areas; across the country, considerable land is now available for outdoor recreation, but it does not effectively meet the need; money is needed; outdoor recreation is often compatible with other resource uses; water is a focal point; it brings about economic benefits; it is a major leisure-time activity and it is growing in importance, and more needs to be known about its values.

Recommendations. In developing its recommendations the Commission was greatly assisted by its own Advisory Council, composed of 25 individuals representing other rural interests as well as recreation and conservation and also top-level representatives of 15 Federal agencies. The State contact officers also had an important influence in shaping the decisions. Many of the recommendations are general but some are specific.

A national outdoor recreational policy. "It shall be the national policy, through the conservation and wise use of resources, to preserve, develop and make accessible to all American people such quantity and quality of outdoor recreation as will be necessary and desirable for individual enjoyment and to assure the physical, cultural, and spiritual benefits of outdoor recreation.

"Implementation of this policy will require the co-operative participation of all levels of government and private enterprise. In some aspects, the government responsibility is greater; in others, private initiative is better equipped to do the job."

Details are given of what the Federal Governments and the State's functions should be, and what the various interests should do.

Guidelines for Management. All the agencies administering outdoor recreation, both public and private, are urged to adopt a system of classifying recreation lands designed to make the best possible use of available resources in the light of needs. The following system of classification is proposed: Class I, high-density recreation areas; Class II, general outdoor recreation areas; Class III, natural environment areas; Class IV, unique natural areas; Class V, primitive areas; Class VI, historical and cultural sites.

Expansion, modification and Intensification of present programmes. Under the head of "Planning, Acquisition, Protection and Access" more than a dozen detailed suggestions are made, a central State agency should be set up to develop long-range plans; local Governments should be more active in supplying the need; inter-State arrangements should be systematic; continuing research, acquisition of more access to water areas, including reservoirs, establishment of certain primitive areas as 'wilderness', certain rivers of unusual scientific, æsthetic and recreation value should be allowed to remain in their free natural state, educational programmes should be intensified; etc.

Several suggestions are also made under the heading of "Promoting Recreation Values in Related Fields" and these include suggestions for obtaining recreational benefits from other major activities such as water developments, pollution control, flood-plain zoning, highway reconstruction, watershed and other agricultural conservation programmes, State encouragement of public use of private lands, for example, by leases for hunting, fishing, scenic easements and landowners' protection.

The final heading in this section is "Meeting the Costs" and the suggestions here are that all levels of Government must provide continuing and adequate funds, mostly at increased levels, use of bonds, season user fees, dedicated funds, and use of uncollected refunds of petrol taxes, easements, zoning, cluster developments, and open land tax policies to supplement the supply of recreation opportunities. Public agencies should stimulate desirable gifts of land and money from private sources, and private enterprise should be encouraged, and concessions to provide facilities.

A Bureau of Outdoor Recreation. This Bureau should be established in the Department of the Interior and would have over-all responsibility for leadership of a national effort by co-ordinating the various Federal programmes and assisting other levels of Government to meet the demands for outdoor recreation, but it would not manage any land, which would remain with the existing managerial agencies. The main functions foreseen for the Bureau were classified as follows: (1) Co-ordinate the recreation activities of the more than 20 Federal agencies the activities of which affect outdoor recreation; (2) Assist State and local Governments with technical aid in planning and administration, including the development of standards for personnel, procedures and operations; (3) Administer a grants-in-aid programme to States for planning and for development and acquisition of needed areas; (4) Act as a clearing-house for information and guide, stimulate, and sponsor research as needed; (5) Encourage inter-State and regional co-operation, including Federal participation where necessary.

To assure that recreation policy and planning receive attention at a high level and to promote interdepartmental co-ordination, there should be established a Recreation Advisory Council, consisting of the Secretaries of Interior, Agriculture and Defense, with the Secretary of the Interior as chairman. Other agencies would be invited to participate on an *ad hoc* basis when matters affecting their interests are under consideration by the Council.

The Recreation Advisory Council would provide broad policy guidance on all matters affecting outdoor recreation activities and programmes carried out by the Bureau of Outdoor Recreation. The Secretary of the Interior should be required to seek such guidance in the administration of the Bureau.

Initially, the new Bureau should be staffed where possible by transfer of experienced personnel from existing Federal agencies, and it would have regional offices.

A Research Advisory Committee consisting of professional people from Government, academic life and private business should be established to advise the Bureau on its research activities.

It is urged that each State designate a focal point within its governmental structure to work with the Bureau. This focal point, perhaps one of the existing State agencies, could also serve to co-ordinate State recreation planning and activities and be responsible for a comprehensive State outdoor recreation plan.

A grants-in-aid programme. This would be a Federal Programme and would be set out to stimulate and assist the individual States in meeting the demands for outdoor recreation and would be administered and reviewed by the proposed Bureau of Outdoor Recreation. The programme itself would be supplementary to the provisions of recent legislation. The level of grants envisaged were up to 75 per cent of total cost for planning in the first year and would be reduced later; for acquisition and development the grants could be up to 40 per cent or to 50 per cent where the acquisition was part of an inter-State plan. The criteria for giving priorities in grants would be based on State population, area, needs and amount of Federal land and Federal recreation programmes in the State and region. This programme of grants-in-aid, it was recommended, should be supplemented by loans to the States in order to help where matching funds were not available and where the need was urgent, and where the grants-in-aid funds were not adequate.

The main body of the Report provides considerable elaboration under each of the main chapter headings although some of them are less fully explored than others. For example, no very deep assessment has been made of the economic aspects, and it is not thus very surprising that in the research recommendations the economic assessment should have strong support. Some of the statistical data are presented in a more palatable form for those who prefer visual aids, and these help to stress what is most significant in the individual chapters: one of the regional maps directs attention to the position that whereas most of the recreation is in the west most of the people are not, and this is particularly true of Federal lands. Forest agencies at all levels of Government manage the greatest number of acres but a small percentage of the total number of areas. Most of the acreage is in large tracts and conversely most of the units are small—less than 40 acres. One of the map diagrams illustrates the variety of ways that recreation can be built into an environment; another shows examples of each of the six classified recreation areas and there are associated photographs indicating the great variety of activity or type of interest. There is also a useful diagram which shows how the Federal agencies, with programmes involving some aspect of outdoor recreation, are linked to each other and to the individual Government departments; the same outline shows which agencies have major land management responsibilities and also how the proposal to have an Advisory Council and the Bureau of Outdoor Recreation are built into the Department of the Interior.

On research proposals the report distinguishes between three related but distinct categories: data collection, inventory and fact-finding; applied management research; and fundamental research. One of the most urgent needs was for more knowledge on the direct benefits derived from recreation. Public recreation facilities are not usually sold for a price, and Federal agencies and others have sought for some time to devise methods of evaluation. Some methods were described, and their limitations emphasized: these included flat rate daily charge basis, cost of travelling at various distances from the recreation area and in terms of alternate use. One of the most promising methods is based on market area surveys. One of the Commission's studies developed an analytical framework to assist in orderly planning and development of recreation facilities in order to provide a guide to the kinds of data needed to solve problems of benefit-cost analysis in outdoor recreation. A general conclusion, however, was that regardless of the technique used no single value can be assigned to the wide variety of recreation activities. The net benefits of recreation could only be judged on the merits of the case and in the present state of knowledge the best procedure for making judgments appeared to be based on a detailed analysis. One of the main functions of the proposed Bureau of Outdoor Recreation would be to act as a central clearing house of information, and where there is a lack the Bureau would stimulate or sponsor appropriate investigation.

ELECTRONIC DELOCALIZATION AND BIOCHEMICAL EVOLUTION

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IN one of his recent books Szent-Györgyi[1] states that "research is to see what everybody has seen and think what nobody has thought". When a quantum chemist, used to thinking in terms of σ- and π-electrons, localized and delocalized molecular orbitals, saturated and conjugated compounds, comes for the first time in contact with biochemistry he is bound to be attracted by aspects of this science which escaped the attention of other types of investigators. What struck us as one essential, although apparently hitherto unnoticed, aspect of biochemistry is the fact that all the essential biomolecules which are related to, or perform, the fundamental functions of the living matter are constituted of completely, or at least partially, conjugated (resonating) systems, rich in delocalized π-electrons[2,3].

Thus, the three fundamental structural units of the cell are undoubtedly the nucleic acids, the proteins, and the energy-rich phosphates. Now, the most significant constituents of the nucleic acids which impose on them their biological personality and determine their specific functions are the purine and pyrimidine bases, which are conjugated heterocycles. In the biologically important energy-rich phosphates, the mobile electrons of the phosphoryl group always interact either with those of another similar phosphoryl group (the case of the pyrophosphates, adenosinetri- and adenosinedi-phosphate) or with those of a π-electron-possessing organic radical (the case of the guanidino-, acyl- and enol-phosphates). No such interaction is present in the energy-poor phosphates, in which the phosphoryl group is attached to a saturated carbon. As to the proteins, although they appear, at first sight, to be essentially non-resonating entities containing only isolated conjugated fragments (each peptide link is such a fragment[4]), there is a number of indications that their over-all supramolecular structure involves (as foreseen by Szent-Györgyi[5]) some degree of general electronic delocalization[6]. This last situation arises from the fact that the peptide bonds, although separated from each other in the backbone by saturated carbons, are, however, united to each other by secondary cross-linkages, namely, hydrogen bonds. Inasmuch as these bonds may participate in electronic delocalization and transmit conjugation effects[7-9], the existence of such a network represents the possibility of an extended electronic conjugation involving the whole molecular framework of the protein.

Nucleic acids, proteins and energy-rich phosphates, although the most important representatives, are not the only conjugated constituents of the cells.

Pteridines, porphyrins, quinones, carotenoids, retinenes, melanins, etc., are other important structural components of biomolecules belonging to the same type of conjugated compounds.

Another most striking observation in the same field concerns enzymes. There are hundreds of enzymes and they are essentially proteins. However, if one excepts the hydrolytic ones, most of the enzymes exert their catalytic activity in conjunction with a coenzyme. There is only a very limited number of essential coenzymes, about twenty, and practically all of these coenzymes are conjugated organic compounds. Such is the case, in particular, with the oxidation-reduction coenzymes, DPN, TPN, FAD, FMN, the hæm prosthetic groups of the cytochromes, and the quinones. Moreover, it is also the case with the coenzymes involved in group transfer reactions: tetrahydrofolic acid, pyridoxal phosphate, thiamine pyrophosphate, vitamin B_{12}, etc.

As a matter of fact, among the fundamental organic constituents of the living cell, carbohydrates, fats, and steroids seem to be the only non-conjugated type of molecules. Among these, carbohydrates and fats represent merely the fuel for the driving of the machinery without being functional constituents of it. The steroids generally possess a π-electron section, and, anyway, seem to be in some way involved in electron transfer phenomena[10,11].

It is thus obvious that the basic manifestations of life are intimately connected with the existence of highly conjugated compounds, which for some decisive reason have been 'chosen by Nature' for being the vehicle of life.

When looking for such a reason one can scarcely think of anything else than electronic delocalization, which represents the most specific and at the same time the most important characteristic of such compounds. In what way can this outstanding feature of the resonating molecules account for their occurrence as the principal building stones of the living matter? We may distinguish at least two major factors responsible for this occurrence, related, respectively, to stability requirements and to functional advantages.

Stability

One of the major and most apparent results of electronic delocalization is the increment of stability which this delocalization confers on compounds in which it occurs. Quantitatively this increment is defined as resonance energy, and is nowadays one of the most popular concepts of quantum chemistry. Particularly strong in pentagonal and hexagonal rings it may amount in the common previously quoted biochemicals from a few tens to a few hundreds of kcal/mole (for example, it is of the order of 30–45 kcal/mole in the pyrimidines, of the order of 50–80 kcal/mole in the purines and of the order of 200 kcal/mole in the porphyrins). It represents an unexpected economy for the energy expenses in anabolic processes. Thus, for example, the *de novo* synthesis of purines from simple constituents may be considered as a series of ATP dependent synthesis of carbon–nitrogen bonds[12]. Eight such bonds are established during the formation of hypoxanthine, the first purine ring to be formed in this synthesis, and in every case but one the formation of such a bond is associated with the hydrolysis of an energy-rich bond of ATP. This represents a cost in energy for the synthesis of the purine skeleton of about 50–60 kcal/mole. The gain of resonance energy associated with this synthesis, calculated by the molecular orbital method[3], is 3,4β which with $\beta = 16$ kcal/mole represents about 55 kcal/mole and compensates thus this expenditure.

It seems natural to assume that this thermodynamic stabilization must have played a non-negligible part in the evolutionary selection of this type of molecules for biochemical processes. It is widely acknowledged to-day[13-16] that the biological evolution through the natural selection of species was preceded by a biochemical (or prebiological) evolution, a long period during which a great number of molecules and aggregates were formed, altered and destroyed and during which the molecules competed between themselves for reaction and function networks. This was the period of struggle for the survival and selection of biomolecules. Although the adaptability to the developing function was, of course, highly important it seems nevertheless that this condition must have been subordinated to the requirements of stability and that the molecules which finally gained must have been those which were the most stable and the biochemical possibilities of which must have, in fact, oriented or at least influenced the function itself.

A confirmation of this point of view may be found both in the extraordinary unity of biochemistry, the same limited number of compounds being used all over the plant and animal kingdoms and in the multiplicity of functions linked with some of the basic skeletons. This last statement may be illustrated by two striking examples.

The first one concerns the case of porphyrins. These very strongly resonance-stabilized molecules (in which four cyclic pyrroles are united by supplementary double bonds into a conjugated super-cycle) are utilized in photosynthesis, in the respiratory (electron transfer) enzymes and in the oxygen-carrying enzymes of higher animals. It seems as if Nature having discovered this particularly stable skeleton has found means of utilizing it in a number of different functions. This point of view is substantiated by the observation that the chlorophyll molecule is to-day manufactured by a sequence of reactions almost identical with the sequence of reactions used to manufacture the hæm. The branching between these two syntheses only occurs at the stage when the metal cation (ion or magnesium) is introduced into the porphyrin skeleton[16]. Following the opinion of Gaffron[17], a number of other dyes could have been utilized with just as much efficiency than porphyrins in the photosynthesis apparatus. Gaffron infers, therefore, that the porphyrins owe their predominant position essentially to the fact that they came first and were much more stable than other pigments. On the other hand, Calvin[15] has indicated strong reasons why, once the porphyrins formed, more of them will have a tendency to be formed by an autocatalytic self-selection mechanism due to the evolutionary pressure of peroxides.

The second example of polyfunction concerns the problem of adenine. This molecule seems to play an apparently unique part in Nature. It is one of its most important single compounds and it enters into the constitution of a great number of complex biomolecules. Thus, adenine, besides contributing to the structure of the nucleic acid, is also a part of several fundamental coenzymes (DPN, TPN, FAD, CoA) and other important transfer agents (*S*-adenosylmethionine), and it is also the carrier of the pyrophosphate chain in the energy-rich ATP and ADP. The clue to this omnipresence may perhaps reside in the fact that, as shown by calculations[18,19], adenine has the greatest resonance energy among all the biochemical purines. It may not be unreasonable to imagine that, inasmuch as Nature was induced to utilize a purine or pyrimidine base as a constituent of these fundamental biochemicals, a phenomenon of natural selection played a part, leading to the choice of the energetically most stable base. (This is, however, certainly not the only reason for the use of adenine, for example, in coenzymes. In these compounds adenine probably serves for the binding between the coenzyme and the apoenzyme. An essential role in this binding seems to be played by the NH_2 group of adenine[20]. This is a very reactive amino-group (more reactive than that of guanine or of cytosine)[18,19]. A highly-reactive group, fixed on a very resistant skeleton, obviously represents a most successful combination for reactions involving this group. The same reason may be operating in ATP.)

Until now we have considered the resonance stabilization of conjugated biomolecules as a thermodynamic advantage of their ground-state. In fact, this stabilization seems to be equally effective in a second field in which its role must have been particularly important for the biochemical evolution. This second field concerns the effect of ionizing and ultra-violet radiations. As demonstrated in details in the particular case of biochemical purines and pyrimidines, there is a very close parallelism between the resonance stabilization of biochemicals and their resistance to damage produced both by ionizing and ultra-violet radiations[21,22]. The intimate nature of this correlation is not entirely elucidated, but it seems highly probable that one of its predominant factors is the possibility for resonating molecules to produce, under the influence of the radiation, relatively long-lived stable excited states. This possibility must have been particularly advantageous for the evolution of life under the early conditions of the Earth in which the energy derived from the Sun in terms of radiation or electronic discharges was the primary source of energy for the synthesis of molecules and even for the polymerization processes leading to macromolecules. The conjugated systems, the loosely bound π and lone-pair electrons of which may be relatively easily raised to the excited states were among those which could make the most efficient use of the available light energy[23]. It is possible that the $n \rightarrow \pi^*$ transitions of resonating heterocycles have been particularly advantageous in this respect, as they also seem to be nowadays in bioluminescence[23]. (We shall see later other reasons which point to the importance of the lone-pair electrons and of their participation in electronic delocalization for the processes of life.) The involvement of resonating molecules, retinenes, as primary receivers of light in vision, is, of course, self-explanatory. Less self-evident, but no less significant, is the involvement of retinenes also in the sense of smell[24].

This same factor of stabilization through electronic delocalization might probably have played also a part in the evolutionary production of the huge macromolecular polymers, in particular nucleic acids and proteins. Such a formation is undoubtedly associated with complementary stabilization. As already mentioned, the hydrogen bonds of proteins connect the otherwise isolated π-electron possessing peptide links into a one quasi-conjugated π-electron system. A more quantitative observation concerns the complementary resonance stabilization through hydrogen bonding of the purine–pyrimidine pairs of the nucleic acids. Calculations have predicted that this stabilization should be, by about 1 kcal/mole, greater for the guanine–cytosine pair than for the adenine–thymine pair[25]. This prediction is remarkably confirmed by experiment[26] which indicates that the denaturation temperature of the nucleic acids rich in guanine–cytosine is appreciably higher than that of the nucleic acids rich in adenine–thymine. In fact, so far as our present knowledge goes, the purine–pyrimidine pairs which actually exist in DNA seem to be more stable than the artificial combinations recently created between other related polynucleotides[27]. In the nucleic acids there is probably moreover, besides the hydrogen binding between the complementary purine–pyrimidine bases, the possibility of electronic interactions among the π-electrons of the parallel stacked base-pairs, either by direct σ-type overlap of their electronic orbitals[28,29] or by a charge transfer complex formation between them[30,31] or by polarization binding. Moreover, such surprising selection phenomena as the exclusive use of D-sugars in the nucleic acids and L-amino-acids in the proteins correspond also to stability requirements, as it is only such a selection that permits the construction of regular helices with minimum strain[16].

Nevertheless, it seems certain that in fact this elaboration of biological macromolecules, which may be considered as the second important stage in the pre-biological evolution (the first one being the selection of the appropriate conjugated molecules) offered also appreciable functional advantages over the use of smaller compounds[32], and this situation brings us to the discussion of this second factor responsible for the evolutionary selection of conjugated molecules in biochemistry.

Functional Advantages

Life is a delicate, dynamic equilibrium between a series of synthesis and decomposition phenomena. Among its main characteristics are the constant mobility of its mechanisms, the interdependence of its constituent biochemicals, the long-range transmission of its manifestations and its numerous transducing activities. It appears obvious that the mobility, the fluidity, the great polarizability of the electronic cloud of conjugated molecules, the possibility that the existence of such a unique, large and deformable cloud offers for the rapid and distant transmission of perturbations (which in biological language may mean an order or a warning) represent important functional advantages which make the conjugated systems particularly suited to perform the functions required by the existence of the living matter. The essential fluidity of life agrees with the fluidity of the electronic structure of these compounds.

As mentioned before, a number of functional advantages may be found for the utilization of macromolecular polymers as the fundamental substances of life. Among those some are very explicitly related to the enhanced and extended electronic delocalization which occurs in such compounds.

Thus, although the exact nature of the semiconductivity observed in such polymers and in other organized, solid state like biological structures[6,33] is still uncertain (intrinsic electronic conductivity or extrinsic electronic conductivity or ionic conductivity) it nevertheless obviously represents a most interesting procedure for the long-range transmission of electronic perturbations. Inasmuch as catalysis by semiconductors involves the formation of charge transfer complexes between the catalyst and the substrate, the theoretically predicted decrease in ionization potential and increase in electron affinity of a protein chain with respect to these properties in an isolated peptide link may have an important significance for the catalytic activity of enzymatic proteins[3,9].

In the very important field of bioenergetics, the utilization of the 'energy-rich' phosphates as the principal coupling agents for the driving of anabolic reactions springs out from some advantageous characteristics of these compounds which, again, are due to the conjugation of their mobile electrons[34]. Thus, the principal sources of the 'energy-wealth' of these phosphates are: (1) the resonance stabilization of the products of the hydrolysis; (2) the electrostatic repulsions due to the particular electronic distribution in the phosphates (consisting generally of a main chain of at least three and frequently more atoms carrying net positive charges surrounded by a cloud of negatively charged atoms), distribution which is a direct result of the delocalization of the mobile electrons; (3) the keto-enol tautomerism of the products of the hydrolysis; (4) the free energies of ionization. The 'energy-wealth' is therefore directly dependent on the manifestations of electronic delocalization.

However, the most striking example of the functional advantages associated with the utilization of conjugated molecules in the living systems is offered by the case of coenzymes. Biochemical reactions need enzymes to proceed and enzymes are essentially proteins. However, the great majority of enzymes exert their activity in conjunction with a coenzyme in which case the essential reaction occurs at the coenzyme, the function of the protein (or apoenzyme) consisting mainly in ensuring the high efficiency and the specificity of the reaction, by suitably orienting and polarizing the reactants. Now, there are hundreds of enzymes but, as already mentioned, there is only a very limited number of coenzymes and practically all these coenzymes are conjugated organic molecules. This is in no way an accident but a deeply significant situation arising from the simple fact that these conjugated molecules are particularly well adapted for being the sites of biochemical transformations.

This may be illustrated by examples which we shall take in the field of two of the most important groups of coenzymes, the oxidation-reduction coenzymes and the group-transfer coenzymes.

Thus it has been shown[35] that the mechanism of functioning of the oxidation-reduction coenzymes (DPN, TPN, FMN, FAD, the hæm prosthetic groups of cytochromes) in electron transfer may be related to the values of the energies of the lowest empty molecular orbital (*lemo*) of their oxidized form and of the highest occupied molecular orbital (*homo*) of their reduced form and to the variation of the energies of these two essential orbitals in the course of the oxidation-reduction. It is observed that, in each case, the oxidized form of the coenzyme has a very low-lying *lemo* and the reduced form of the coenzyme a particularly high-lying *homo*. The oxidation-reduction of these compounds is thus associated with an instantaneous redistribution of these two essential orbitals in such a way that in each case the oxidized form of the coenzyme has a relatively great electron affinity or electron-accepting tendency and the reduced form of the coenzyme a particularly low ionization potential or a particularly strong electron-donating tendency. This oscillation makes these coenzymes particularly suitable for being the vectors of electron transfer. Now, obviously, only conjugated molecules can involve such a specific displacement of molecular orbitals and satisfy the conditions imposed on the efficient electron-transfer agents. In fact, even among the conjugated molecules themselves, only very specific ones can fulfil these requirements. Thus, for example, if we replace the nicotinamide ring of the pyridine nucleotides by benzene or the isoalloxazine ring of the flavin coenzymes by anthracene, nothing useful will happen. This remark brings us to an essential complement to our general thesis about the importance of conjugated molecules in the processes of life. Namely, that what is needed and essential are not simply conjugated molecules but, mostly, conjugated heterocycles disposing of atoms with lone-pairs, such as nitrogen or oxygen, which by undergoing suitable changes in their valence states are able to bring about the most economical and spectacular transformations. Thus the previously discussed redistribution of the molecular orbitals in the respiratory coenzymes, which apparently is so well adapted to the function of these coenzymes as electron transfer agents, is only possible because the oxidation-reduction is associated with changes in the valence states of the nitrogen atoms of these compounds, which alternatively involve their lone-pair in the overall π-delocalization or exclude it from such a participation. It may even be observed that owing to this modification in the valence state of the nitrogen atom the reduction of DPN+ to DPNH does not involve any loss in the total number of the π-electrons of the system (in DPN+, N_1 contributes one π-electron, while in DPNH it contributes its lone pair) and that the reduction of FMN to $FMNH_2$ involves even an increase in the total number of π-electrons (in FMN, N_1 and N_{10} contribute one π-electron each, in $FMNH_2$, they contribute two electrons each, namely, their lone pairs). Although the reduction is in each case accompanied by a certain loss of resonance energy[36], this loss is probably the minimum possible one.

Our second example comes from the field of group transfer coenzymes. We may specifically quote, as an illustration, the case of pyridoxal phosphate, although exactly the same or very similar considerations apply to other coenzymes of this category, such as thiamine pyrophosphate or tetrahydrofolic acid.

Pyridoxal phosphate, which is a heterocyclic aldehyde, catalyses a great number of group transfer reactions involving the α-amino-acids of the proteins[37]. The mechanism of functioning involves, in the first place, the formation of a Schiff's base (an imine) between pyridoxal phosphate and the α-amino-acid. This formation labilizes the departure of the COOH+, H+, and *R*+ groups attached to the α-carbon, permitting thus the involvement of the α-amino-acid in a series of reactions. It may be shown that the essential electronic factors connected with the catalytic properties of pyridoxal phosphate reside in[38,39]: (1) the strong resonance stabilization of the transitional form deriving from the initial Schiff's base through the labilization of any of the COOH+ or H+ or *R*+ groups, stabilization due to the increase in dimensions or the reorganization or both of the total conjugated system; (2) the creation in this transitional forms of prominent centres of extremely high reactivity (for example, great local concentrations of electronic charges or of free valences), which ensures the rapid consecutive development of the reaction towards its final products.

Thus the catalytic activity of this and similar coenzymes is essentially related to the transformation of the initial coenzyme–substrate complex into a resonance stabilized and, at the same time, electronically activated intermediate which is the essential active form of the reaction. Only conjugated molecules, with their cloud of mobile and deformable electrons and containing heteroatoms capable of oscillating between different valence states, can bring about such results.

These different phenomena definitely seem to point out that there were deep reasons for the evolutionary choice by Nature of conjugated molecules for biological purposes. We know, nowadays, owing to the work of a series of authors, that all the essential biomolecules, in particular the amino-acids[15,40,41] and even the fundamental purines[42,43] and pyrimidines[44], could have been produced and probably were produced in the early stages of the history of the Earth in a random, irregular, abiotic way. (An interesting point to investigate is to determine the probability factors or entropy contributions related to this formation. This unfortunately seems, at present, difficult for quantum chemistry.) It seems highly probable that the evolutionary selection tended then towards the biological utilization of the most stable compounds which, not by chance but by the particular virtue of the π-electrons, must have also been, at the same time, the most efficient and best adapted compounds for the biological purposes of Nature. We cannot say and do not want to say that life has originated with the appearance of the conjugated compounds but what may probably be safely said is that the possibility of life, in the form in which it is present to-day, has been rendered more probable and its development hastened through the utilization of such compounds. This fact is to some extent inherent in the very elementary chemical composition of the living matter[45]: 99 per cent of it is composed of hydrogen, carbon, nitrogen and oxygen, the last three first-row elements being among all the elements those which most easily form multiple bonds. The next two most abundant and important elements to enter into the structure of the cell are sulphur and phosphorus and these are practically the only ones other than first-row elements to form multiple bonds. One can also imagine[45] that carbon rather than silicon has been 'chosen' by Nature as an essential component of organic matter (although silicon is much more abundant on the surface of the Earth than carbon) possibly because carbon may form chains of conjugated double bonds while silicon cannot and possibly because carbon bonds are much stronger than silicon bonds. The choice and utilization of conjugated heterocycles as structural components of life appears thus as one of the most important quantum effects in biochemical evolution.

Resonance and Biological Evolution

We have considered so far the role of electronic delocalization or resonance in pre-biological, chemical evolution. It may be pointed out that the same factor might have had also a decisive influence on the biological evolution of species[46]. Thus, as is well known, the purine and pyrimidine bases of the nucleic acids are considered as existing essentially in the lactam and amino tautomeric forms. This situation is in agreement with quantum-mechanical calculations about the relative stabilities of the different possible tautomeric forms of the bases[3]. However, the involvement of rare tautomeric forms is considered as possibly playing an essential part in mutations[47]. The presence of a rare tautomeric form may produce an erroneous coupling, through hydrogen bonds, of unusual bases and may lead to an induced, self-perpetuary, perturbed sequence of purine–pyrimidine bases. As the genetic information is probably enclosed in this sequence, the result is a mutation. The situation concerns in the first place spontaneous mutations the occurrence of which appears as a physical necessity due to the mere existence of tautomeric equilibria and, secondly, to induced mutations, produced, for example, by base analogues or antimetabolites capable of increasing the frequency of the abnormal pairings.

Now, it can easily be shown[46] that one of the principal factors responsible for these tautomeric shifts towards rare forms and thus possibly responsible for the occurrence of mutations is a resonance effect. This is true in particular for spontaneous mutations Thus, the essential varying factor responsible for the relative tendency of the bases to exist in a rare tautomeric form is the variation of the resonance energy which accompanies the tautomeric transformations. There are two such transformations to be considered: the lactam–lactim and the amino–imine transformations. In the lactam–lactim tautomerism, the transformation of the lactam form (the most stable) to the lactim form (less stable) is accompanied by an increase of resonance energy so that the proportion of the lactim form will be the greater, the greater this increase. In the amino–imine tautomerism, the transformation of the amino form (the more stable) to the imino form (less stable) is accompanied by a decrease in resonance energy and will therefore be the greater, the smaller this decrease.

These considerations indicate clearly the predominant role of resonance energy variations in the production of spontaneous mutations and thus in the biological evolution. Explicit calculations carried out for the resonance energies of the different tautomeric forms of the purine and pyrimidine bases of the nucleic acids lead, moreover, to the prediction that the base which, from that point of view, should have the greatest tendency to exist in a rare form, either lactam or imino, is cytosine (a prediction which seems to be confirmed from the purely chemical point of view). So far as the starting hypothesis about the cause of spontaneous mutations is correct, it is the tautomerism of cytosine which may be expected to have the greatest probability of being involved in such mutations. Of course, from the practical point of view, it is evident that the electronic factor considered here cannot be the only one to play a part in this phenomenon. For example, the steric adaptability of the rare tautomer into the general architecture of the nucleic acid may play an important part in determining its aptitude to subsist and to be involved in coupling, through hydrogen bonds, with complementary bases. Löwdin[48] has pointed out recently the possibly important contribution of the mutual stabilization of two rare tautomeric forms engaged in such complementary binding.

If we add that the same phenomenon of tautomeric shift and erroneous coupling may possibly be one of the causes of 'ageing' (considered as a continuous loss of genetic information and an accumulation of sequence errors) and also of 'spontaneous' cancer, that induced mutations and perhaps also chemical carcinogenesis may be approached from a point of view very similar to that just described, that important drugs, whether harmful or therapeutic, are frequently conjugated or at least partially conjugated compounds, in fact, in the majority of cases, heterocyclics[49], we clearly realize to what a decisive degree is life depending on the properties of the mobile electronic systems of resonating molecules. In a recent paper on biochemical evolution, Rich[50] states that unfortunately, "at the present time, we do not have any guide lines for our thinking, that is general concept about molecular evolution which may prove of utility in understanding and interpreting the results". We hope that the reasons indicated here for the orientation of life towards the utilization of conjugated systems, in particular oxygen- and nitrogen-containing heterocyclics, may form such a concept.

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WHY AFTER-IMAGES ARE NOT SEEN IN NORMAL CIRCUMSTANCES

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AFTER-IMAGES are not seen in normal circumstances. If you look at the clock on the mantelpiece and then at the painting above it, you will not see any aspect of the clock superimposed on the painting. This fact is fundamental to the visual process.

Goethe considered this fact, and suggested that it was due to lack of attention on the part of the observer[1]. Helmholtz thought that it was due to the slow roaming of the eye, which occurs involuntarily, and which ensures that any such after-images will be out of focus[2]. A recent authority, in summarizing previous work, takes these two factors as the two main reasons for it[3]. However, the following experiments indicate that there is one important aspect which was touched on by Nechkovitch[4], but has otherwise received scant attention[5].

The experiments were designed to be related to vision in normal circumstances. Consequently they involved looking at a scene full of objects with highlights and shadows, and afterwards looking at a different scene (or a different part of the same scene) of approximately the same brightness. The first scene will be called here the primary scene, and the second scene the secondary scene. The experiments were confined in this way because it is quite rare in normal life that one looks from a scene of one brightness to a scene of another substantially different brightness. Generally one moves in an environment in which the average luminance in one direction is the same as that in another. When one does look at a part of a scene of one luminance followed by another part of the scene of a much lower luminance, one does see after-images. Looking at a light bulb or a photographic flash are examples. This type of situation will not be considered here.

The stimulus for these experiments came from the work of E. H. Land, who has demonstrated how a pair of transparencies, one projected in red light and the other in white light, can give the sensations of red, white, pink, grey, yellow, blue, green, brown, orange and black[6]. Some authors have suggested that these colours are partly due to successive contrast, which is equivalent to saying that they are due to after-images[7,8]. However, the colours are found to be independent of the part of the scene which the observer has been looking at previously, and, further, the colours seen in a flash of light are found to be much the same as those seen in continuous illumination[9]. The same question arises here as arises in the ordinary world, namely: is it really true that our perception is confused with after-images and successive contrast?

The primary scene in the first experiment was a coloured scene. It was made up from a red colour separation positive (Fig. 1) projected in Wratten No. 24 red light, superimposed on a green colour separation positive (Fig. 2) projected in tungsten light. The observer was asked to fixate on the end of the teapot spout for 12 sec. At the end of this time the red light was turned off, so that the secondary scene consisted of an ordinary black and white photograph.

The observer saw a coloured after-image if he continued to look at the end of the teapot spout. The colours of this after-image have been described by Land[10]. The pillow behind the teapot is a deep cyan (it was red in the primary scene); the decanter and the other pillow (which were cyan in the primary scene) appear a pale brown, or pink; the stapler appears grey, as it was in the primary scene. The colours are the same if other points of fixation are used, provided the same point of fixation is used for both the primary and secondary scenes. Some observers found it a little difficult at first to describe the colour of the decanter while fixating on the end of the teapot spout, but were able to do it after practice.

If the observer did not continue to look at the end of the teapot spout, the coloured after-image disappeared. The whole scene became black and white,

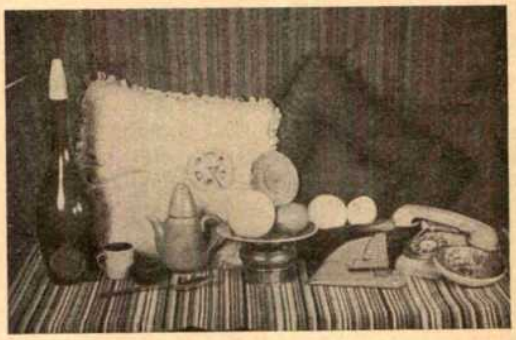

Fig. 1. Red colour separation positive for the first experiment

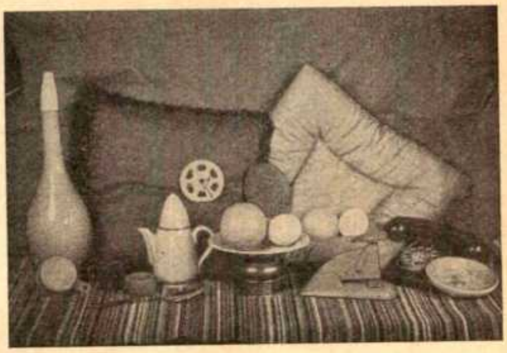

Fig. 2. Green colour separation positive for the first experiment

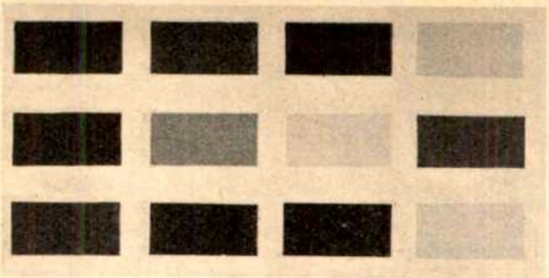

Fig. 3. Red colour separation positive for the second experiment

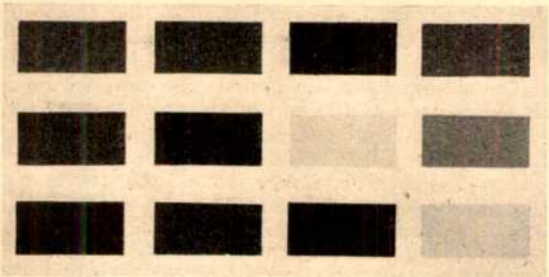

Fig. 4. Green colour separation positive for the second experiment

not cyan, as one might expect from the fact that the observer had previously been staring at a scene illuminated with wave-lengths predominantly from the long end of the visible spectrum.

If the observer looked away from the end of the teapot spout and back again, the after-image disappeared when he looked away and re-appeared when he looked back again, provided the time elapsed since the change from the primary scene to the secondary scene was not too great. He had to look back to exactly the same point for the after-image to re-appear. The end of the teapot spout was chosen as a point of fixation because it was clear, small, and well defined. An observer who fixated carefully on it during the primary scene found, while he was looking at the secondary scene, that a slight movement to either side of the point was enough to destroy the whole after-image.

The after-image took about 12 sec to disappear totally. During this time the observer could look away from the end of the teapot spout and have the after-image disappear, look back again and have it re-appear, and repeat this several times. Each time the after-image re-appeared it would be fainter, according to the time which had elapsed since its formation, but quite readily apparent.

The period of 12 sec fixation on the primary scene was carefully chosen. A longer period gave a stronger and more lasting after-image, but was inconvenient because it took the observer several minutes afterwards to recover fully, and necessitated a long delay before the next observation could be made. In addition, most observers found it hard to fixate for more than 12 sec. A shorter period gave a weaker and less-lasting after-image (as a rough guide we found that the after-image lasted about as long as the fixation time taken to form it). This was also inconvenient, because it did not give the observer time to look away from the end of the teapot spout and back again several times before the after-image had faded. Twelve sec fixation was found to be a good compromise.

Occasionally an observer would see an after-image when looking at some point of the secondary scene away from the end of the teapot spout. The after-image would appear in this case only after 1–2 sec of concentration on the new point of fixation, and may have been what Goethe was referring to when he said that an attentive observer will see after-images everywhere. The sensation was ephemeral compared with that obtained on looking back at the original point of fixation, when the after-image re-appeared immediately and unhesitatingly.

The primary scene for the second experiment consisted of a coloured geometrical figure. As in the first experiment, it was made up from a red colour separation positive (Fig. 3) projected in Wratten No. 24 red light, superimposed on a green colour separation positive (Fig. 4) projected in tungsten light. The observer was asked to fixate on a point *A* for 12 sec. Then Fig. 3 was occluded. Thus the primary scene was a coloured set of rectangles, and the secondary scene a black and white set of rectangles.

If the observer continued to fixate on point *A*, he saw a coloured after-image, as in the first experiment. He also saw a coloured after-image when he looked at the top right-hand corner of any of the other rectangles, where the geometry of the after-image fitted the geometry of the secondary scene. The colours were different, because the after-image was registered with a different set of rectangles of different lightnesses in the secondary scene, but they were

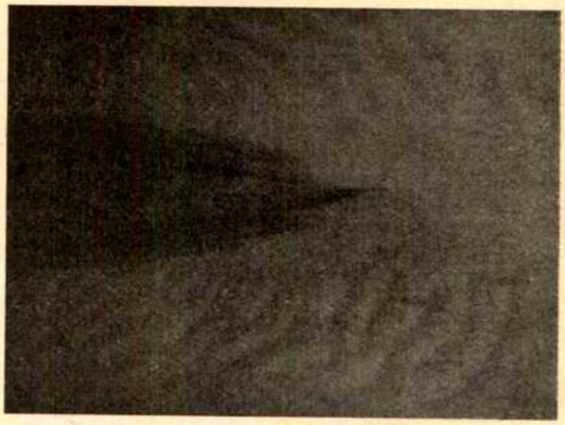

Fig. 5. Scene with soft contours

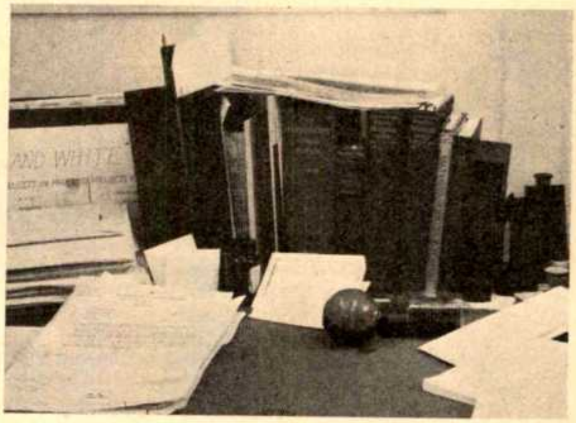

Fig. 6. Scene with hard contours

readily apparent. The observer saw them immediately, whenever his eye paused on the top right-hand corner of a rectangle.

In the third experiment the shapes of the objects in the primary scene differed from those in the secondary scene. The primary scene was Fig. 1 illuminated by light of 615 mμ (slightly different from the primary scene in the first experiment, which contained both Fig. 1 and Fig. 2). The secondary scene was one of a variety of transparencies (Figs. 2, 5 and 6 are examples) illuminated by white light. These were chosen to give a variety of types of scene, some with hard contours, some with soft contours, and one with no contours at all. The purpose was to find out if they would all inhibit the after-image to the same extent.

We found that the after-image was far more apparent with Fig. 2 as the secondary image than with any other. Using Fig. 6 very little after-image was apparent; using Fig. 5 the portion of the after-image due to the black/white contours in Fig. 1 was apparent; using a plain grey background some out-of-focus contours were apparent. In none of these cases, however, was the after-image as stable and colourful as it was with Fig. 2; the after-image would appear to a certain extent immediately the scene was changed, but a movement of the eyes would destroy it, and concentration for a second or two was required to bring it back again.

These three experiments were all observed by a number of people on various occasions at an average luminance of about 1 foot-lambert, which corresponds to a not very highly lighted room. Two observers looked at them, in addition, at average luminances of about 15, 100 and 500 foot-lamberts, which correspond to a moderately well-lighted room, a dull day outdoors, and a moderately bright day respectively. In the last two cases different pictures were used, but essentially the same series of experiments was performed. The results were the same, except that the after-images tended to fade more rapidly at the higher brightnesses.

The first conclusion from this is that the after-image is inhibited rather than destroyed. The first experiment showed that it will re-appear whenever the eye goes back to the original point of fixation. The second experiment showed that it will appear whenever the eye moves to a new point of the scene where the scene fits the after-image. As the eye moves around the scene the after-image stays with it, invisible and intact, in spite of all the other impressions made on the retina. It re-appears when the eye is rotated to a position in which the after-image is in geometric registry with the scene which originally produced it, provided the time elapsed since its formation is not too great.

The second conclusion is that when the contours of the after-image are inhibited, its colour is also inhibited. In the first experiment, when the observer looked away from the end of the teapot spout, and the after-image disappeared, there was no significant overall cast of cyan. The secondary scene appeared black and white.

The third conclusion is that one needs contours and detail in the secondary scene to do the inhibition with complete effectiveness. Some parts of the after-image may re-appear to a small extent when the secondary scene consists of weak and ill-defined contours. When it has no contours at all, that is, when it is a uniform white or grey, the after-image may appear fairly complete. One way of expressing this is to say that the after-image is always in registration with a uniform surface, whatever the point of fixation. However, even in this case, it is out of focus and ill-defined. Only when the contours of the secondary scene fit the after-image closely, does the after-image appear with its maximum stability and colour.

What is the significance of these conclusions for vision in normal circumstances ? One might argue that they are irrelevant, because one does not ordinarily fixate on anything for as long as 12 sec, so that after-images are never formed. However, it is possible to form an after-image in much less than 12 sec. We found, in the first experiment, looking at the end of the teapot spout, that less than 1 sec of fixation was sufficient to form a weak after-image. In ordinary life, one quite frequently looks at an object for 1 or 2 sec. We only chose 12 sec fixation in this experimental work, as explained here, to bring out the points clearly and concisely. Consequently, I believe that the conclusions from the experiments are applicable to normal vision.

Thus we have three explanations of why we do not see after-images in normal circumstances: Helmholtz's, Goethe's, and that described in this article. In my opinion, Helmholtz's explanation, and the explanation given in this article, are the true ones. Helmholtz says that one does not normally form an after-image because one does not normally look at any point of the scene long enough to do so: this paper shows that one will form an after-image after more than 1–2 sec fixation, which is quite common; but that it will be inhibited by any subsequent pattern of stimulation the contours of which conflict with it. Goethe's explanation is not so much an explanation as a prescription for the kind of procedure one should follow in order to see after-images. He says that the attentive observer will see after-images everywhere. So he will, if he does an unusual amount of fixation; but this would be abnormal behaviour.

In summary we may say that we are continually forming after-images, even at quite moderate levels of illumination. Some are quite strong, and some are extremely weak, depending on how long the eye rests on a point in the scene it is looking at, before moving on around the scene. The eye carries these after-images with it as it moves, and they will be seen whenever the eye alights on a point where the scene is in geometric registry with the after-image. However, this rarely happens in the ordinary world, where we are faced with trees and houses, with faces and dresses, and not with simple geometrical figures. The first experiment corresponds to the kind of scene we see in the ordinary world—full of objects of various shapes, with highlights and shadows on them. In these circumstances the after-image is completely inhibited.

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MODELS FOR SUPERPOSED LAMINAR FLOW FOLDING

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IN recent papers[1-3] I described the experimental development of laminar models to demonstrate geological concepts of surface and internal shape transformations resulting from the mutual interference of superposed systems of defined similar folds. These models have been extended and adapted to show other aspects of laminar flow transformations in a general study of rheid[4] morphology related to specified strains.

The accompanying Figs. 1–4 depict an improved laminar model in which the relief of a bi-component (sinusoidal) interference surface is automatically delineated by true contour lines resulting from the combined effect of a regular succession of horizontal lines pre-printed on the card laminæ. With sufficiently thin laminæ the successive traces of these lines produce the effect of continuous contour planes emerging in correct position at the slopes of the interference surface.

Fig. 1 shows a specimen contour card lamina imprinted with horizontal contour lines, and with a specified profile curve, *BX*, representing the first fold system with its axial planes parallel to *BQ*.

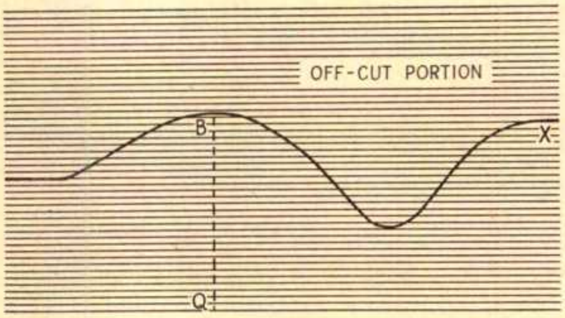

Fig. 1. Contour card lamina printed with horizontal contour lines. The profile *BX*, drawn on the outside card only, is cut through the pack by band-saw

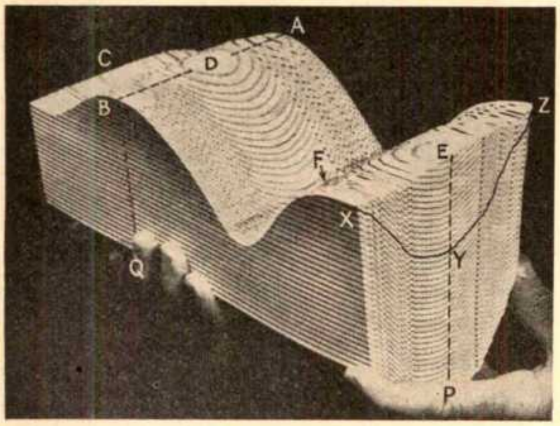

Fig. 2. Contour model showing two fold systems on upper and lower surfaces prior to their mutual interference

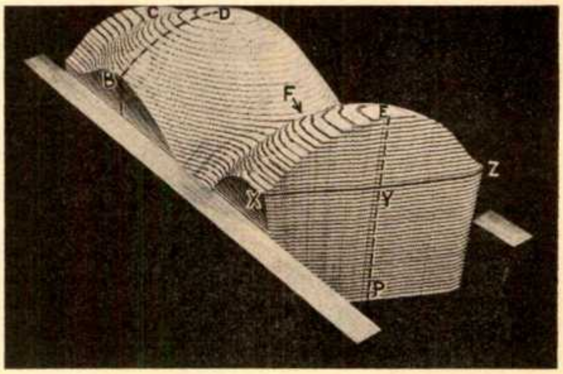

Fig. 3. Interference surface from superposed deformations shown in contoured relief

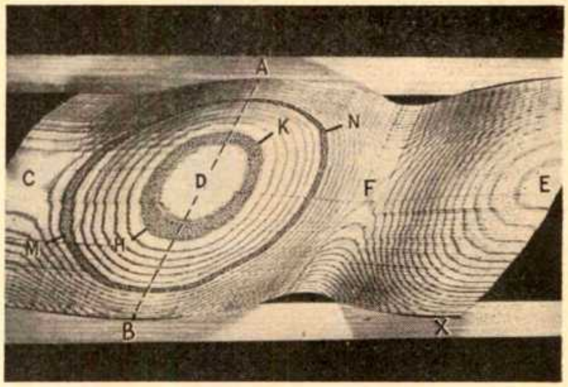

Fig. 4. Vertical view of interference surface showing elliptical contours in sigmoidal echelon. Component fold axes are *AB* and *CF*

In contrast to the operational sequence previously described[1], the profile curve *BX* is not cut until after the second specified fold system has been moulded in reverse into the bottom of the card pack.

Fig. 2 shows the model at the stage where a simple second-fold anticline has been moulded in reverse (that is, as a syncline) into the bottom surface of the pack causing the contour lines to form a succession of internal surfaces parallel to the bottom surface according to the profile *XYZ*, which represents the second-fold system having its axial planes parallel to the plane containing *P*, *E* and *D*. The profile *BX* has been cut to represent a first-fold system having its axial planes parallel to the plane containing *A*, *B* and *Q*. At this stage, both systems of folds exist in the model as two separate and independent deformations, one at the top and one at the bottom of the pack. Their mutual interference is effected by flattening the bottom of the card pack as in Fig. 3, where the model is shown supported in a wooden jig. This movement not only produces the interference relief on the top surface, but also restores the contour

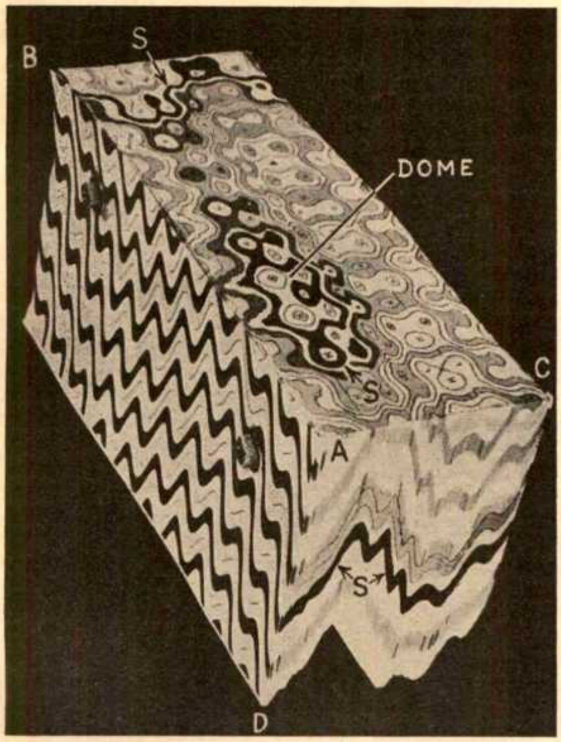

Fig. 5. Horizontally truncated model showing 'outcrop pattern of multi-wave interference complex

lines to positions where they produce the effect of correctly spaced horizontal contour planes within the pack parallel to XYZ (now a horizontal straight line). The specified curved profile formerly represented in reverse by XYX in Fig. 2 is now represented correctly in Fig. 3 as an anticlinal profile passing through X, E and Z. The points D and E represent the crests of interference domes circumscribed by true contour lines which automatically fall into position on the slopes.

Fig. 4 shows a vertical plan view of the resulting contoured interference surface. The non-orthogonal axial directions of the two interfering fold systems are respectively AB and CF, and the elongation of the resulting interference domes, D and E, are oriented into the acute angle between them. The connecting cols, or saddles, are seen at C and F. Two contour intervals at different levels of dome D have been stippled to emphasize the change of domal elongation direction from MN at the lower contour to HK at the higher. This is a right-hand sigmoidal elongation as will be seen by tracing the locus of corresponding points of maximum curvature on all successive contours.

Fig. 5 shows an elaborated version of a fold profile card model which demonstrates the plane 'outcrop' pattern of two orthogonal 'multi-wave' systems in mutual interference. The two component profiles can be seen respectively as the curve printed on the lamina BAD, and the moulded shape of the bottom of the card pack as seen on the end section CAD. One particular stratum, S, has been accentuated and its 'outcrop' pattern on the horizontal plane is shown on the top surface BAC. The combination of axial and quadrantal directions is evident, and the gross trend of stratum S in the vicinity of B is at right angles to its gross trend in the elongated dome near A.

I have also prepared simplified model presentations of laminar flow concepts to show the two-dimensional transformations of specified initial shapes by simple shear strain. This is achieved by inscribing such shapes on the surface formed by the edges of the laminæ. Subsequent simple shear movement of the laminæ causes this surface to deform as if by laminar flow, and the inscribed shapes are transformed accordingly.

Figs. 6*a*, *b* and *c* show a succession of such shape transformations under the influence of uniform homogeneous simple shear acting in a clockwise direction. The observed surface, which has been inscribed with shapes common to sedimentary rocks, is shown in Fig. 6*a* in its initial unsheared state. AB represents an irregular rippled stratum in which the top and bottom ripples are unequal and out of phase. CD represents a stratum containing elliptical elements, such as concretions or pebbles, with their long axes along the bedding. The thickness of the stratum CD is arbitrarily defined as 10 units. EF represents a stratum with regular approximately symmetrical ripples. MN represents the approximate bedding direction and PM an approximately orthogonal element in a bed, such as a vertical worm burrow or a perpendicular fracture. S represents a reference circle in the unsheared position which becomes the conventional strain ellipse after shearing.

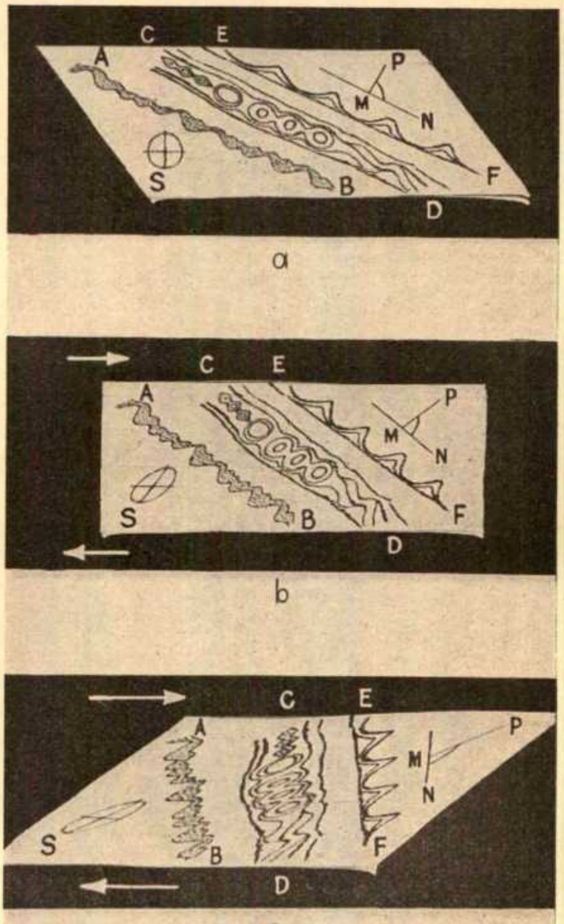

Fig. 6. *a*, *b* and *c*, Formal elongations and symmetry reversals produced by uniform continuous shear

Fig. 6*b* shows the same laminar model after the application of clockwise shear (indicated by arrows). The resulting strain, seen in the strain ellipse *S*, has produced some remarkable transformations in the inscribed elements. The ripples in strata *AB* and *EF* have become accentuated in amplitude and asymmetrical in a dextral sense, such as might lead to the wrong supposition of original depositional or slump flow from *A* to *B* and from *E* to *F*. Stratum *CD* has increased its thickness from 10 to 15 units, and the enclosed elliptical elements have been re-oriented at large angles to the bedding plane where they lie imbricated dextrally or in right-hand echelon with respect to the stratum. Their elongations are not coincident with the strain ellipse elongation. Cross-bedding near *D* has become decidedly condensed. The angle *PMN* is now conspicuously less than 90°.

Fig. 6*c* shows remarkable accentuations and formal reversals following the continued application of the same initial clockwise shear. The increased strain elongation is shown in *S*. The ripples in strata *AB* and *EF* are now greatly accentuated in amplitude and resemble intraformational slumping and flame casts respectively. Their asymmetry has reversed to a sinistral sense such as might suggest flow from *B* to *A* and from *F* to *E*, contradicting the earlier supposition based on the previous figure. The stratum *CD* has increased its thickness to 25 units, and the original slight irregularities in its top and bottom boundaries have become much exaggerated. The intraformational elliptical elements, while remaining oriented at large angles to the bedding plane, are now imbricated in a sinistral sense, or in left-hand echelon with respect to the stratum, an attitude conventionally associated with anticlockwise rotation, but shown here to be produced, paradoxically, by clockwise rotation. The original cross-bedding at *D*, now scarcely recognizable as such, might lead to the supposition of aberrant tectonic discordance. The angle *PMN* has become greater than 90°, showing marked attenuation in the line *PM* and corresponding condensation in the line *MN*. Between the stages of deformation in Fig. 6*b* and Fig. 6*c*, there was a point of transition where the angle *PMN* became 90° for the second time, restoring, at that point, the initial orthogonal relationship. Coincidently, the ripples in *EF* became symmetrical for the second time, but with exaggerated amplitude. Observations based on shape criteria at the transition point would have wrongly suggested tangential compression without shear rotation. It follows that if the intensity of the clockwise shear movement varies, the analogues of Figs. 6*b* and 6*c* may co-exist and falsely indicate independent opposing tectonic influences.

Additional models similar to Fig. 6 have shown that very slight initial sedimentary disharmonies can become so exaggerated and rotated by continuous simple shear as to produce entirely misleading evidence of origin based on assumptions of shape. A laminar model representing a simple curvilinear granite surface of unconformity overlapped by sedimentary 'pinchouts' can, when sheared, simulate an intrusive contact in which the beds of the overlapping sediments appear to be orthogonally truncated by the granite surface, or even engulfed in re-entrant angles of the granite. This produces the effect of a large-scale gross concordance prevailing over a succession of small-scale discordances. In this context also, any contrasting linear element, such as a quartzitic seam, provided it has the slightest initial undulate form, can be transformed to classical ptygmatic folds by the one uniform homogeneous shear. If inclined shearing of periodic intensity be applied to independently undulate surfaces, opposing overturns can be produced which simulate conventional compressional criteria in spite of the fact that the deformed surfaces have been attenuated.

Analogues of Fig. 4 may be seen in most cross-folded sedimentary sequences. Surficial expressions of Fig. 5 may be seen abundantly in the Proterozoic rocks of South Australia. Shear transformations of the type shown in Fig. 6 are observable both in the Precambrian rocks of Broken Hill, New South Wales, and particularly in the lower Paleozoic (Kanmantoo) rocks of South Australia.

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PARALYSIS CAUSED BY THE DIGGER WASP *PHILANTHUS*

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WITH one or more stings, the females of almost all Sphecidae can paralyse other insects or spiders, which they then carry into brood cells as food for the larvæ (Fig. 1). The problem of stinging had been considered by earlier investigators, but the controversy whether the sting reaches the central nervous system of the prey or whether injection of the poison into the hæmolymph is sufficient for the paralysis has not been settled. Two histological investigations of the nervous system of paralysed insects produced quite different results[1,2].

In the case of the digger wasp, *Philanthus triangulum*, F., this issue for the most part has now been resolved. Moreover, indication of the specific action of the poison involved has also been obtained[3].

To discover whether or not the central nervous system is reached it is necessary to locate the actual place of puncture. When *Philanthus* was allowed to sting honey-bees in small containers, the sting was always observed on the unsclerotized membrane on the ventral side of the bee behind the first pair of legs (Fig. 2). Following the stinging, which lasts about 30 sec, the wasp turns its victim venter up tightly beneath the body. With the rectangular shaped tip of its abdomen the wasp squeezes the abdomen of the bee telescopically. As a result of the pressure the honey-stomach bursts, the contents of which are immediately licked up by the wasp (Fig. 3). Nearly always the wasp drums the spot where it has stung with the proboscis and frequently kneads the bee's legs with its mandibles. This action, performed by a few other digger wasps, is known as 'malaxation'.

Also under natural conditions, the sting is given behind the right or left coxa of the fore legs. Histological sections through this area showed in ten out of

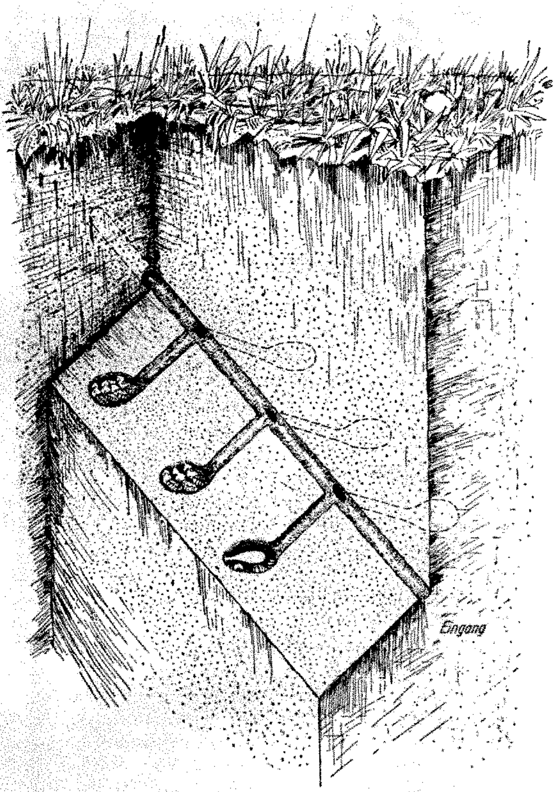

Fig. 1. Nesting place of the digger wasp *Philanthus triangulum* F. Paralysed bees are stored in the brood cells. In the first chamber a larva has already spun its cocoon

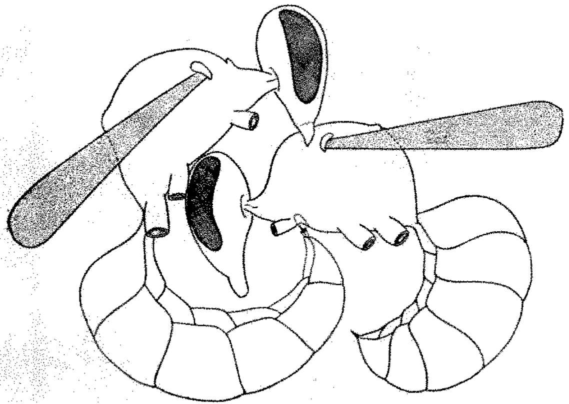

Fig. 2. The sting of *Philanthus* is always placed behind the first pair of legs on the ventral side of the bee

twelve cases studied that the sting had not reached the first thoracic ganglion. In the other two cases, the perilemma of the ganglion in the region of the endosternum I was damaged. Presumably the wasp's sting had broken off and in this way reached the ganglion.

For *Philanthus* to locate this very small area for stinging without optical means requires specialized receptors on the sting apparatus. The sheath lobes of the sting are not attached to the oblong plates as two unsclerotized processes—as in the Apidae—rather they are chitinous and serve to guide the shaft of the sting itself. The distal ends of the lobes are enlarged and club-shaped and bear a pad of some seventy *sensilla chaetica*. These sensitive hairs range from 3 to 4μ in diameter and from about 45 to 90μ in length, and articulate proximally in the chitin. Fig. 4 shows the sting apparatus with the gland system as a whole. Furthermore, two additional types of *sensilla campaniformia* are found in the dorsal wall of the bulb of the sheath (Fig. 5, *S.c.A.* and *S.c.B.*). They are probably sensitive to tension stimuli in the chitin. This sense organ at the tip of the sting represents a highly specialized tango-receptor for soft unsclerotized spots and has been found with certain modifications in all species of digger wasps examined.

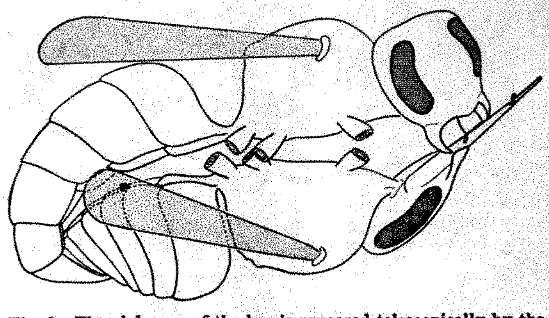

Fig. 3. The abdomen of the bee is squeezed telescopically by the wasp; in this way the contents of the honey-stomach are burst off

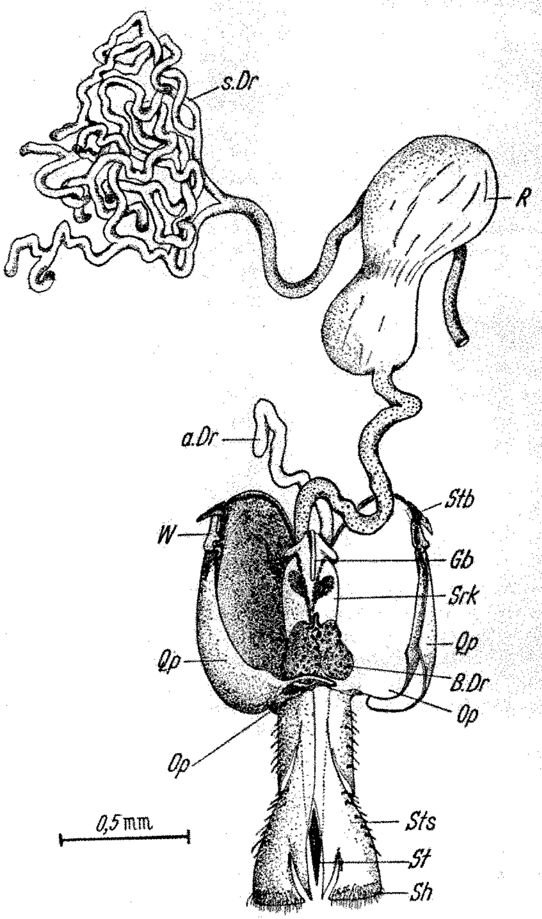

Fig. 4. Sting-apparatus of *Philanthus triangulum* with the various glands. Parts of the oblong and quadrate plates of sting are cut on the right side to show their anatomy. *aDr*, alkaline gland; *B.Dr*, Bordas gland; *Gb*, furcula; *Op*, oblong plate of sting; *Qp*, quadrate plate of sting; *R*, poison sac; *s.Dr*, acid gland; *Sh*, sense organ at the tip of the sheath lobes of sting (*Sts*); *Srk*, bulb of stylet; *St*, sting; *Stb*, lancet of sting; *W*, triangular plate of sting

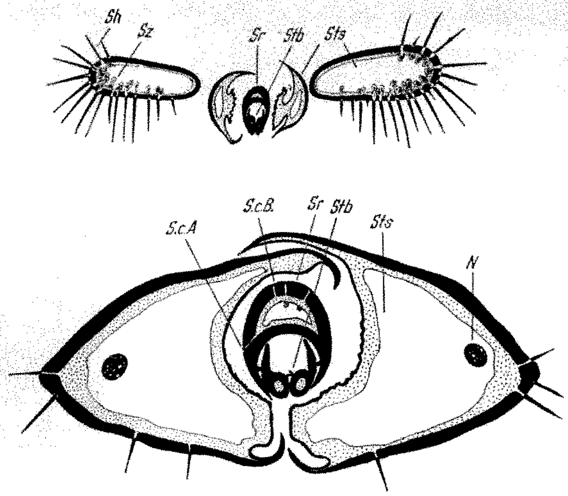

Fig. 5. Sections through the distal and middle part of the sheath lobes of sting. *S.c.A.* and *S.c.B*, two types of sensilla campaniformia; *N*, nerve from the sense organ *Sh* of Fig. 4 to the last abdominal ganglion. For other abbreviations see Fig. 4

The next important point to be considered is whether *Philanthus* chooses this particular spot behind the fore legs because it is one of the few places which can be pierced by the sting, or, whether it is necessary for paralysis that the stinging be near the thoracic ganglia. For a suitable explanation bees were stung experimentally by wasps at various distances from the ganglia. The spots were predetermined by cutting windows in the chitin with a very fine scalpel. After a normal stinging, the front and middle legs are immediately paralysed, the hind legs, after 1 min and the prætarsus after 30–40 min. Following an experimental stinging in the mesonotum, the bees are able to move forward for some 30 sec and only after this interval become paralysed. After 50–90 sec, the bee can no longer move about. When examined, it is seen that coxæ and femora of all legs are paralysed, but the tibiæ and basitarsi still kick vigorously. After some 15 min the basitarsi show evidence of paralysis.

When stinging occurs in front of the right tegula, the legs of the right side become paralysed faster and more thoroughly than those of the left side, and vice versa. Thus, there is a correlation between the side stung and the paralytic effect. Even stinging in the abdomen of bees will, after a certain time, produce complete paralysis. But in this case the onset of paralysis has been delayed by a few minutes. In the case of abdominal stinging, some bees recover after initial paralysis, that is, paralysis can be reversible. Relationship between place of stinging and the speed of paralysis can be shown. Following a stinging in the mesonotum, paralysis will appear after 65 sec; in the episternum, after 32 sec. On the other hand, if the bees are stung in the abdomen, paralysing effects occur after 220 sec.

Thus, for positive results the poison need not be injected near a thoracic ganglion, but may produce paralysis when it reaches the hæmolymph. Moreover, it does not produce a sudden paralysis but takes effect gradually. The theory that the poison is a neurotoxin which affects the central nervous system[1] cannot be experimentally sustained. The nerve cells for the different muscles of one leg are located very close to one another in the same thoracic ganglion and must be reached by the poison at practically the same time. Yet observations prove that the coxæ and femur are paralysed while the tibia and basitarsus of the same leg are still movable. If the poison causes accelerated degeneration of the central nervous system to the extent that Nielsen indicates, it is even inexplicable how individual bees can recover from paralysis. Obviously, another mechanism of poisoning is involved.

There are two methods for investigating where the poison of *Philanthus* primarily takes effect. Besides a histological examination of the central nervous system of paralysed bees, experiments involving smaller dosages of poison are most informative. Theoretically, if the poison does not affect the ganglia, assumed from previous experiments, then application of small amounts of the poison may cause a local paralysis to the actual effectors.

Newly hatched *Philanthus* possess very little poison. Experiments with such wasps showed that a sting near the tegula of a bee immediately paralyses the wings. Otherwise, the bee can move about quite normally. Only after a few minutes do the coxæ and femora become paralysed; the tibiæ, not at all. After 1 h at the most, the bees are again able to fly.

Consequently, the poison cannot be a neurotoxin which blocks the ganglion centres. If it were, the fore-wing and the middle legs, both of which are innervated by the second thoracic ganglion, would be paralysed at practically the same time and not after a maximal interval of 7 min. The poison has a peripheral effect, spreading first around the puncture and only by means of the hæmolymph reaching the leg musculature. The amount now present is insufficient for paralysis of the tibiæ. The progressive paralysis of the leg segments can be explained in the following manner. Those muscles which control movements of the coxæ and femora lie within the body proper and are bathed by the hæmolymph, which is responsible for a quick transport of the poison. The muscles of the tibiæ are located within the leg, namely, in the femora, and those of the basitarsi, in the tibiæ. It takes a considerable amount of time for sufficient poison to reach these muscles through the action of the accessory pulsating organs on the basis of the leg. The fact that the tarsus retains its mobility the longest of all supports this assumption.

Injection of small amounts of poison into the head of a bee will paralyse the antennæ and the proboscis, while the bee otherwise is unaffected. If the poison primarily affects the central nervous system, then the co-ordination necessary for movement would be disturbed. This is explained by the fact that the overlying centres for movement are located in the brain and subœsophageal ganglion. Whether the onset of peripheral paralysis is located in the muscle or in the axon or in the synapsis (most probably the latter case) is not as yet determined. Further investigations are planned.

Observations of the life-span of a paralysed bee show that 37 h after stinging heart activity can be demonstrated. But the frequency of heart contractions is disturbed, often bursts of a constant beat occur. Maybe this phenomenon is a result of a disturbance or blocking in the neurogenic pacemaker while the myogenic automatism, controlling the bursts, is unaffected[4].

To support the conclusion that the poison of *Philanthus* does not primarily affect the central nervous system, but first attacks the periphery, ganglia of paralysed bees were examined histologically. When sections were prepared 2–5 h after stinging, the brain and subœsophageal ganglion, likewise the second and third thoracic ganglion, showed no change. Only in the first thoracic ganglion, which lies in the immediate vicinity of the puncture, did glia cells and the neuropilem manifest alterations. Although the nuclei of the glia cells were present and the cell boundaries discernible. The plasma region of these cells appeared empty and the neuropilem did not evince its usual fibrous ground structure. The ganglion cells, however, remained unchanged. Only 24 h after stinging do the changes of the neuropilem and the glia cells spread to the other thoracic ganglions, but nerve cells and the cells of the perineurium are almost never affected. Not until three days after the stinging is shrinkage in the brain and the subœsophageal ganglion noticeable.

It is possible that after peripheral paralysis has set in, the poison disturbs the metabolic functions of the glia cells which are responsible for the nutrition of the neurones. The lack of essential nutrients could be the cause for the viscous degeneration of the neuropilem. The histological examinations have shown that the poison does not attack primarily ganglion centres, for those ganglions at some distance from the puncture, for example, the second and third thoracic ganglion, exhibit degeneration only after a day. The respective extremities, however, are immovable within a few seconds.

The potent effect of *Philanthus* poison is not confined only to the honey-bee. On the contrary, positive results were obtained with all insect orders, spiders and even closely related Sphecidae. Just as remarkable is the fact that *Philanthus* is immune to its own poison, toxicity being destroyed by a particular compound in its hæmolymph. Only in very strong concentrations can the poison cause a momentary paralysis in the musculature. Within a very short time the local paralysis is checked by the hæmolymph. If the poison is injected directly into a thoracic ganglion of a *Philanthus*, the legs will become paralysed. Whether in this instance the structure of the ganglion is damaged or a nerve–nerve synapsis is blocked has not as yet been determined. Since insect ganglia are not bathed by hæmolymph, the poison in this case cannot be rendered ineffectual.

Philanthus can be killed by its victim's poison if the bee successfully introduces its sting into the heavily sclerotized body of the wasp. This is, of course, an exceptional case. There is a Mediterranean species of digger wasp, *Palarus variegatus*, F, which preys among other aculeate hymenoptera on *Philanthus*. The apparent immunity of this species to the poison of the latter has obvious selective advantage. Whereas the victim's poison may be fatal to *Philanthus*, in this case, the hunter is immune to the poison of its prey.

The illustrations used in this article are from *Z. vergl. Physiol.*, **45**, 413; 1962 (Springer-Verlag: Berlin, Göttingen, Heidelberg).

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OBITUARIES

Sir Irvine Masson, M.B.E., F.R.S.

Sir Irvine Masson, M.B.E., formerly vice-chancellor of the University of Sheffield, died in Edinburgh on October 22, 1962, at the age of seventy-five. He was a grandson of David Masson, professor of English literature at University College, London, from 1853 until 1865, and at the University of Edinburgh from 1865 until 1895, and the only son of Sir David Orme Masson, professor of chemistry at the University of Melbourne during 1886–1923.

James Irvine Orme Masson was born on September 3, 1887, in Melbourne, where he was educated at the Grammar School and the University before coming to Great Britain in 1910 as an 1851 Exhibition Scholar. After a year with Sir James Walker at Edinburgh, Masson moved to University College, London, where he worked with Sir William Ramsay, with whom his father had been associated some thirty years earlier at University College, Bristol. In 1912 Masson became assistant in chemistry at University College, London, and during the First World War he served first with the R.N.V.R. and later at the Research Department of the Royal Arsenal, Woolwich. He was appointed reader in inorganic chemistry at University College in 1920, and served as secretary of the Chemical Society during 1921–24. He became the first professor of chemistry and head of the Department of Pure Science of the University of Durham (Durham Division) in 1924, and in 1938 he went as vice-chancellor of Sheffield, where he remained until retirement in 1952. At the outbreak of the Second World War he added to his duties as vice-chancellor the active direction of a Ministry of Supply team which was housed in the Chemistry Department and engaged in research on explosives. He was also a member and chairman of several committees of the Ministry.

Irvine Masson was a man of varied talents. His chemical work, which led to election to the Royal Society in 1939, was not voluminous, since he never had a large team of helpers and much was done with his own hands. But his work is characterized by elegance and breadth; problems were reduced to the simplest possible terms and then approached by neat, ingenious and well-considered methods. He belonged to the group of chemists—now non-existent—who could tackle equally well problems in the inorganic, physical and organic sections of chemistry. His early publications were concerned with the theory of solution and the gas laws, and his use of the inert gases was clearly an outcome of association with Ramsay, for whom he had unbounded admiration. But his most significant contributions were his studies of nitration and of the organic compounds of iodine. The nitration work is a classic, paving the way for later researches which have had an important bearing on the development of theoretical organic chemistry. The study of organic iodine compounds had been neglected since the work of Victor Meyer and Willgerodt some forty years earlier; Masson developed this work in an important series of researches, in which he demonstrated once more his remarkable ability to apply physical chemistry to problems in both organic and inorganic fields.

Masson was interested in the teaching of science, frequently serving as external examiner at other universities, and his book, *Three Centuries of Chemistry*, published in 1925, shows a profound knowledge of the historical and philosophical aspects of his subject. While science was his profession, classics and the arts were hobbies in which he reached professional standing. He was a great lover of books and an expert in the early history of printing. His major contribution in this field—the fruit of research during the latter years of his vice-chancellorship—is an outstanding monograph on the *Mainz Psalters* and *Canon Missae*.

Masson was tireless in his efforts on behalf of the University of Sheffield, and his flair for the lucid presentation of complex matters of finance and statistics proved invaluable. No one knew better than he what education was or what a university should and could do. As vice-chancellor he steered the University through its war-time problems and through the equally difficult period of development which followed. He made plans to guide the expansion from 850 students to a well-balanced and effective University of some 2,500 students (a target now increased to 4,750); temporary structural alterations were made and building programmes initiated; Stephenson Hall and the new Chemistry and Engineering Departments were almost completed before his retirement in 1952.

Though invariably courteous and dignified, Masson was nevertheless a determined fighter, often outspoken in formal debate. In contrast, his natural shyness in personal relationships was a handicap frequently mistaken for aloofness. Rarely at his best in large gatherings, he was an excellent host of a small party, with a turn of wit which would have surprised his detractors. Those who knew him well found him a lively companion and a true friend.

Masson became M.B.E. in 1918, was knighted in 1950, and received the honorary degree of LL.D. of the Universities of Edinburgh and Sheffield. His retirement, spent in bibliographical work in Edinburgh and in advisory posts to the Carnegie Trust and the University of St. Andrews, was saddened by the long illness of his wife Flora Lovell, daughter of Prof. G. Lovell Gulland, whom he married in 1913 and who died in 1960. He leaves one son, who is sub-librarian in charge of the Brotherton Collection at the University of Leeds.

R. D. HAWORTH
A. H. LAMBERTON

Prof. Kh. M. Abdullaev

THE death of Khabib Mukhamedovich Abdullaev, president of the Academy of Sciences of Uzbekistan and former vice-premier of the Uzbek Council of Ministers, has been reported from Tashkent. Prof. Abdullaev visited Britain in 1960 and lectured on the geology and mineral resources of the Central Asian republics at several British universities. Although only fifty years old, he was the author of many geological monographs on the ore deposits of Central Asia, among them his doctoral dissertation on the scheelite deposits in the mountains of Uzbekistan and a number of lengthy works on the genetic connexion between mineralization and igneous intrusions. His book on *Dykes and Mineralization* (1957) brought him a Lenin prize; an English translation has just been published by the American Geological Institute.

Apart from his activities as an economic geologist, Abdullaev took a prominent part in the post-war organization of scientific affairs in Central Asia, in recognition of which he was awarded many Russian decorations. He spoke only Uzbek and Russian; but as a representative (and corresponding member) of the U.S.S.R. Academy of Sciences he latterly travelled widely on scientific missions to Western Europe, Mexico, China and India. He was a member of the Mineralogical Society of Great Britain.

NEWS and VIEWS

Biochemistry at Adelaide

SIR MARK MITCHELL, who has held the appointment of deputy vice-chancellor in the University of Adelaide since 1948, has resigned his chair of biochemistry in order to devote the whole of his time to the office of deputy vice-chancellor. Prof. R. K. Morton, Waite professor of agricultural chemistry in the University of Adelaide, has been appointed professor of biochemistry. Prof. Morton is a Fellow of the Australian Academy of Science. He recently returned to Adelaide after spending several months in England as a visiting Fellow under the auspices of the Commonwealth Fellowship and Scholarship Plan. Morton is distinguished for his work on purified enzymes and cytochromes including the first example of a protein containing two coloured prosthetic groups both necessary for enzyme activity. His method of purifying enzymes was a valuable contribution. He has also done important work on nucleic acids and plant proteins. During the Second World War he served in the Royal Navy and from 1949 until 1952 worked at Cambridge in the laboratories of Dr. Malcolm Dixon.

Demand for Graduates in Agriculture in Britain

IN a written answer in the House of Commons on November 27, the Minister of Agriculture, Fisheries and Food, Mr. C. Soames, announced that, with the Home Secretary and the Secretary of State for Scotland, he had decided to set up a committee of inquiry to assess the present and future demand, at home and abroad, by agriculture (including horticulture), its associated industries and public services, for graduates from agriculture and other faculties or departments of universities in the United Kingdom. The chairman of this committee would be Dr. C. I. C. Bosanquet and its members would be: Sir Arthur Benson, Mr. A. S. Cray, Mr. J. L. Davies, Mr. R. A. Hamilton, Prof. D. S. Hendrie, Dr. W. G. Humphrey, Prof. H. G. Lamont and Mr. Maitland Mackie.

The International Association for the Exchange of Students for Technical Experience: Annual Report

THE fifteenth annual report of the International Association for the Exchange of Students for Technical Experience covering the year 1962 has recently been issued (Pp. 88. Bad Godesberg: Dr. Klaus Wyneken, General Secretary, IAESTE, Frankengraben 50, 1962). The report records an increase of 185 over 1961 in students exchanged, 60 of whom were sent abroad by the new member country, Ireland, the remaining increase over 1961 being mainly from five countries. The main part of the exchange is still concentrated in the European area, where Germany, Sweden and the United Kingdom account for more than 50 per cent of the total exchange. Exchanges in the fields of architecture, chemical, civil and electrical engineering increased considerably and some 50 per cent of the 31 members (21 in Europe, 3 in Africa, 3 in America and 4 in Asia) have already arranged long-term training exchanges, part of which is with non-European countries. The present non-European countries increased their number of students sent from 585 to 681, and of foreign students received from 337 to 426. There was some exchange with non-member countries, and during its fifteen years' activity the Association has been able to arrange training periods abroad for 67,432 students. Of the 7,663 students sent in 1962, Germany (1,222), the United Kingdom (839), Austria (776), Netherlands (577) and Turkey (535) sent the most, and Germany (1,092), United Kingdom (749), Austria (746), Netherlands (538) and Sweden (471) received most. The United Arab Republic sent 345 and received 342 students. Of countries with an excess of students accepted over students sent, Germany (830) and Sweden (496) had much the largest, and of those with an excess of students sent over students accepted, Austria (416), Turkey (307), the United Arab Republic (230) and Yugoslavia (114) had the largest. Reports from the participating countries are included.

United Kingdom

A SEPARATE report for the United Kingdom (Pp. 13. London: International Association for the Exchange of Students for Technical Experience, United Kingdom, 178 Queen's Gate, S.W.7, 1962) includes notes on the first annual general meeting of the United Kingdom Association and on the fifteenth annual conference of the main Association at the Imperial College of Science and Technology during January 7–11, 1962. In 1962, 52 universities and colleges were affiliated in the United Kingdom Association, compared with 48 in 1961, and sent 839 students to 24 countries, receiving 848, compared with 894 and 965, respectively, in 1961. The Imperial College of Science and Technology (169), the University of Nottingham (55), the University of Cambridge (38), the University of Durham (37), the University of Manchester (35) and the Universities of Birmingham and Bristol (33 each) supplied the most students.

Overseas Students in the United Kingdom

MORE than 60,000 students from 140 countries were studying in Britain in 1961–62 according to *Overseas Students in Britain: a Handbook for all who are interested in the welfare of Overseas Students.* (Revised edition. Pp. 39. London: Standing Committee of the London Conference on Overseas Students, c/o the British Council, 1962. 1*s*. 6*d*. net.) More than 70 organizations concerned with overseas students are represented on the London Conference on Overseas Students, a permanent body with a secretariat provided by the British Council. The chairman, Sir John Macpherson, points out that the Conference is concerned with overseas students in Greater London, where some 34,400 are living and studying, and that there are similar co-ordinating conferences or committees in Birmingham, Bristol, Edinburgh, Glasgow, Liverpool and Manchester. The booklet contains tables of estimates of the numbers of full-time overseas students from each country, their subjects and places of study. Reference is made to the accommodation and personal problems of overseas students, and the booklet describes what is being done to help overseas students by the British Council and other British organizations, their own Governments, universities, technical colleges and other places of study. Attention is directed to the acute shortage of good accommodation available to overseas students and to the scheme which provides for Government grants for hostel projects.

The British Association for the Advancement of Science

THE annual report for 1961–62 of the British Association includes a report of the Council to the General Committee, the General Treasurer's statement and account, references to the publication of papers presented at the Norwich meeting and the report of the Seismological Committee on seismological investigations in 1961 (Pp. 100. London: British Association for the Advancement of Science, 1962. 2*s*. 6*d*.). The latter includes a note by J. H. Wavish on determination by electronic computer and one by Dr. A. E. M. Geddes on microseismic storms recorded at Aberdeen during the winter 1960–61. The Council's report notes the establishment of a new Area Committee at Southampton and the recognition of the Education Sub-Committee of the Manchester Federation of Scientific Societies as an Area Committee for the Manchester district and north Lancashire. More than 1,100 lectures were arranged by the Area Committees during the year to audiences totalling about 55,000 and the Junior British Association meetings have continued to show their value. Further science fairs were held at Middlesbrough in January 1962 and at Leeds in March 1962, and it is believed that the time is ripe for a major effort in Youth Clubs when the Government assistance for which the Association has already applied is forthcoming. The Mathematical Research Unit for the study of the generating of mathematical insights continued its work and the demand for visual aids again increased, but the major stress in the report is on the growth of the work connected with schools and young people.

Scientific Methods and Industrial Operations

THE work of the Industrial Operations Unit of the Department of Scientific and Industrial Research is concisely but effectively described with examples from the Unit's case-book studies and cartoons by Peter Kneebone in a booklet, *Science in Your Methods*, recently issued by the Department (Pp. 12. London: Department of Scientific and Industrial Research, 1962. Gratis). The booklet shows how scientific methods have already improved techniques and organizations in a varied range of industry and indicates some of the opportunities which industrial surveys afford. The Unit, which was established in 1952, does not normally undertake investigations for individual firms. Its three major functions are to demonstrate techniques that simplify industrial operations; to conduct instructional courses; and to examine the cost aspects of novel techniques which improve productivity. To this end it assists research associations and other trade and industrial organizations in applying scientific methods to problems arising in managerial and industrial practice, and its services are given free for the specific purposes of acquiring case material, demonstrating techniques and holding appreciation courses. More than 1,000 men from middle and top management in a wide range of industries have already attended the special courses for management, more than 150 the work simplification courses, and 50 the seven courses in decision-making techniques.

Location of Industry

NEARLY two-fifths of the people in Great Britain live in one or other of seven large conurbations, and during the past thirty years population has grown rapidly on the edges of these conurbations or in districts round about them. Rural areas, on the other hand, have continued to lose population, and even good farming regions, notably parts of East Anglia, have suffered. These trends have given rise to important contemporary problems of congestion, soaring property values and pressure on open spaces, not forgetting the lengthening of the commuter's journey. The greatest expansion in population has been in South-East England. Birmingham and London, in particular, have been the major foci for growth and development. Greater emphasis on foreign trade has increased the attractiveness of locations which provide good opportunities for access abroad, and the advantages of the South-East would be increased if a Channel tunnel or bridge were constructed. In a broadsheet entitled *Location of Industry*, issued by Political and Economic Planning, Mr. Michael Chisholm argues that the existing machinery for planning industrial location needs overhaul and that the economic climate of the regions should be so adjusted that economic forces can be harnessed to social ends (*Planning*. Vol. 28, No. 466; 29 October, 1962. Pp. 323–366. London: Political and Economic Planning, 1962. 5*s*.). The increasing scale of manufacture and improved communications are giving industrialists a greater choice of location within those general areas that provide good market access. He suggests that many "foot-loose" industries could be attracted to the older, less fortunate areas if they were thoroughly renovated. Much of the social capital (houses, schools, public utilities) in the older areas has outlived its time and is, in fact, often a liability. What is wanted are improved amenities and development in these older areas.

The National Institute of Sciences of India

THE Year Book of the National Institute of Sciences of India for 1961 includes, besides the report of the Council for the year ended March 31, 1961, in which the Institute celebrated its Silver Jubilee and the number of Ordinary Fellows was raised from 350 to 400, brief reports on the work carried out by the Research Fellows, of whom seven senior and nine junior were appointed during the year. The Year Book also lists members of Council, senior staff, Fellows, Committees, and includes an account of the foundation of the Institute as well as its rules and regulations and the accounts for the year (Pp. viii+247. New Delhi: National Institute of Sciences of India, 1962).

The Office of Health Economics

LACK of information about the economic aspects of medical care makes it difficult to run medical and other social services in the best interests of the patient and the State. To help fill this need, the Office of Health Economics has recently been founded by the Association of the British Pharmaceutical Industry. The director is George Telling-Smith. The Office will investigate the economic aspects of health problems, commission original research investigations and reports, collect and collate information on experience in other countries and publish a series of papers based on these inquiries. It will have an advisory panel of independent experts and hopes to receive the support of individuals and professional bodies who are interested in the problems of health economics. The first paper issued by the Office, *Progress Against Tuberculosis*, forecasts the disappearance of tuberculosis as a cause of death in Britain by 1975 if present trends continue and if there is no relaxation of the two-pronged curative and preventive attack on the disease (Pp. 23. London: Office of Health Economics, 1962. 2*s*.). Until 1939, available treatment did no more than delay the fatal outcome, but since then the introduction of the new anti-tubercular drugs has revolutionized treatment. In a preface, Prof. D. Dunlop, Department of Therapeutics and Clinical Medicine, University of Edinburgh, points out that the economic benefit to England and Wales resulting from the use of the anti-tubercular drugs alone is approximately £55 million a year, or more than half the cost of the expenditure of the National Health Service on all drugs to treat all disease.

Mental Health and Disease in General Practice

Two recent publications of the General Register Office deal with different aspects of morbidity. The first, *Supplement on Mental Health*, has become a permanent feature of the Registrar-General's publishing programme, and the present volume is the sixth of the series (Pp. vii+88. London: H.M.S.O., 1962. 7*s*. 6*d*. net). It contains new tables showing sex and age distribution of patients admitted to mental hospitals, and deaths and discharges by age at admission and duration of stay in hospital. The number of patients in mental hospitals, which stood at 148,000 in December 1954, had fallen to 138,000 in 1958 (or 142,000 if informal patients were included) and to just less than 140,000 in 1959. At the same time, direct admissions have risen steadily and in 1959 stood at 106,000 compared with 72,000 in 1954 and 59,000 in 1951. Information is provided about rates of admission and duration of stay for various groups of mental disease. *Disease in General Practice*, the third volume of the *Morbidity Statistics from General Practice* (*Studies on Medical and Population Subjects*, No. 14. By the Research Committee of the Council of the College of General Practitioners. Pp. v+144. London: H.M.S.O., 1962. 16*s*. 6*d*. net), brings together some of the results of the earlier two volumes in essays on different aspects of illness written in narrative form by some of the practitioners who took part in the survey. There is no new material which has not previously appeared, but it is hoped that the form of presentation will appeal to those interested in the subject, who are not statistically minded, and particularly to readers who are concerned with the training of students of medicine.

The Teaching Problem in Britain

IN a pamphlet, *Revalue the Teacher!*, the National Union of Teachers outlines the case for a substantial increase in the salaries of teachers, emphasizing the overall shortage of teachers, as well as the shortage of honours graduates in teaching (Pp. 16. London: National Union of Teachers, 1962). While the pamphlet answers a few of the arguments and quotes freely from the Crowther report, it does not attempt to deal with the difficulty of differential salaries, the serious loss of women teachers, and the problem of part-time employment and other measures require to ensure the most efficient use of highly trained professional men and women—a problem that becomes the more important as their salaries are raised. A good case is well presented, which would be strengthened by evidence of more flexibility and more vision and of recognition that teaching is, above all, a vocation.

The Teaching of Biology

In his inaugural lecture at the University of Leicester, Prof. H. C. Kornberg, professor of biochemistry, first discussed the unity of life and then went on to examine the implications in the present provisions for biological education (*The Unity of Life*. Pp. 21. Leicester: The University Press, 1962. 3*s*.). The subdivision of living organisms into bacteria, plants and animals rests on the differences which are emphasized in their academic study. In consequence, universities in general tend to train specialists in one or two of these branches of the life sciences and, in order to gain admittance to the universities for their pupils, schools adopt the same procedure. There is from the outset a fragmentation of biology, which will become more severe as new biological specialties arise. The multiplication of specialties will face the would-be student of biology with an increasingly bewildering multiplicity of degree courses, all of which will inevitably tend, more and more, to overlap one with another. If this trend continues, it is not inconceivable that students might ultimately graduate with a detailed knowledge of biophysics, biochemistry and molecular biology but with only a hazy knowledge of what plants and animals look like. This multiplication of specialties, with the concomitant tendency to create a corresponding multiplicity of specialist departments, will also impose an increasingly severe strain on the universities themselves, with the result that the smaller universities, at least, may soon find themselves unable to provide the staff, space and equipment necessary to accommodate this mushroom growth. These dire tendencies can be reversed if, in the future development of biology, the present divisions based on the differences between living organisms are complemented by academic units based on the fundamental similarity of life processes. This might find its physical expression in the creation of schools of biological science which might house, under one roof, the specialties which operate at the cellular and functional level at which a unitary approach has already been achieved.

Standardization of Reporting Dental Diseases and Conditions

It has been realized for a long time that in any medical examinations carried out for comparative purposes the most careful standardization is necessary, and this involves control of such factors as the method of examination and the exact definition of diagnostic criteria. This is a particular problem in the field of dental epidemiology, because of the difficulty in defining the threshold levels at which it is considered that undoubted dental or gingival pathology is present. A recent report issued by the World Health Organization is concerned with establishing definitions and criteria for measuring the prevalence of dental caries, gingivitis, periodontal disease, dento-facial anomalies, tooth eruption, and prosthetic requirements (Technical Report Series No. 242. *Standardization of Reporting Dental Diseases and Conditions*. Report of an Expert Committee on Dental Health. World Health Organization, Geneva, 1962). The criteria are simple and well defined; and it is emphasized that the methods recommended are mainly for large-scale studies of differences between populations. If finer comparisons have to be drawn the Committee considers that refinements can be added to the basic data according to the requirements of the particular investigation. There is also a brief section on the planning, reporting and writing of results and examples of dental survey forms. The report fulfils its stated purpose very well and represents a useful contribution to the advancement of dental epidemiology.

Natural History Museum in Alma-Ata

I. G. Galuso, V. S. Bazhanov and T. N. Nurumov (*Priroda*, 3, 62; 1962) provide a brief description of a new Natural History Museum in Alma-Ata, the capital of Kazakhstan. It contains more than 2,500 exhibits in the fields of geology, botany and zoology, and its aim is to represent all the varied nature of Kazakhstan. The Museum was built and organized by the Kazakh section of the U.S.S.R. Academy of Sciences.

The British Museum (Natural History): Recent Acquisitions

Among the more important and interesting items recently acquired by the British Museum (Natural History) is a collection of young stages of British grasshoppers (Acrididae), presented by Prof. O. W. Richards and Miss N. Waloff. It is unique, comprising about 1,000 specimens in spirit, including every nymphal instar of all ten British species. The Department of Entomology has also been presented with 242 slides of Streblidae, the major part of the unique collection of Mr. Boris Jobling, of the Wellcome Foundation.

The Bernice P. Bishop Museum

Under the attractive title of *Science and Education* and with an arresting format, the activities for 1961 of the Bernice P. Bishop Museum in Honolulu are described in a far from formal manner (Pp. 29. Honolulu, Hawaii: Bernice P. Bishop Museum, 1962). The chief event was the completion of Kilolani—the Hawaii Planetarium and observatory. This was the climax of eight years of planned improvement of educational facilities of the Museum. Kilolani consists of three separate but connected units. The first is an appropriate entrance exhibit gallery, then the Planetarium proper, seating about 100 visitors and equipped with a Spitz instrument. The third unit is an observatory containing a 12·5-in. reflecting telescope, but capable of accommodating a larger instrument if this proves desirable in the future.

U.S. Federal Aid in Wildlife Restoration

The Federal Aid in Wildlife Restoration Act was approved in September, 1937, and popularly known as the Pittman–Robertson Act. It was designed to help check the depletion of the wildlife in the United States and stimulate its restoration. Since wildlife is a product of the land, its restoration depends on providing suitable living conditions for the desirable species. The developmental work that favours the increase of wild birds and mammals contributes also to the conservation of soil and moisture, which are essential to the maintenance of a prosperous agriculture. The income from the sale of hunting and fishing licences is practically the only revenue available to finance the activities of State fish and game departments. Lack of adequate funds in the past prevented the conducting of much-needed restoration work. Under the Federal Aid in Wildlife Restoration Act, funds are made available to the States for co-operative programmes to increase wildlife populations, to procure information through field studies for improving the management of this natural resource, and to provide an annual surplus of game birds and mammals to be collected by hunting and trapping. The Act provides that the United States may pay 75 per cent of the cost of work performed on approved projects. Projects are confined to the purchase and development of lands, the restoration of natural environment, the maintenance of completed projects and the management of wildlife areas and resources (United States Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Regulatory Announcement No. 64: *Federal Aid in Wildlife Restoration*. Pp. 21. Washington, D.C.: Government Printing Office, 1962. 15 cents) (see also p. 1134 of this issue of *Nature*).

Wildfowl Research and Conservation

THE typically attractive report of the Wildfowl Trust for 1960–61, by Hugh Boyd and Peter Scott, is important for a number of accounts designed to show what has been happening in various parts of Europe with regard to wildfowl research and conservation (Pp. 204 + 65 photographs. Slimbridge: The Wildfowl Trust, 1962. 17*s*. 6*d*. net). The general picture emerging is that, in the field of conservation, legislation restricting shooting and other forms of destruction is now fairly severe. Continued reduction of 'shooting pressure', where this may be found necessary, is likely to call for improvements in the enforcement of existing legislation and for self-education and restraint by wildfowlers, rather than for further extensive restrictions. The pressure on wildfowl exerted by the continued loss of wetlands, offset to some extent by the construction of new reservoirs, cannot be relieved in any simple way. It calls for constant vigilance, for the early detection of major threats, and for dogged but not unreasonable resistance to changes which are unnecessarily harmful. Though this is certainly an international problem, shortly to be discussed at a full-scale European conference, it is also one against which people whose concern is primarily local can protest more effectively. It is to be regretted that the international problem of oil pollution of navigable waters has not been solved. The Trust and related organizations have no shortage of important and fascinating problems for investigation, but there are few people to carry them out—there are fewer than forty biologists in the whole of Europe able to give a substantial part of their time to research on wildfowl. Yet, in relation to the work which has to be done, the need for more workers is even more important and urgent in the practical application of research, in education and the other tasks of conservation. It is gratifying to read of the increased number of visitors to the Trust's collections at Slimbridge and Peakirk.

The Society for the Promotion of Nature Reserves

IN several ways 1961–62 was remarkable in the history of the Society for the Promotion of Nature Reserves. Early in the year the Nature Conservancy was enabled, through a grant of £1,500 from the Society, supplemented by £500 from a generous donor, to acquire 123 acres of downland at Wye and Crundale in Kent as a National Nature Reserve. The reserve lies on the southern escarpment of the North Downs and is an excellent example of the product of long-continued grazing by sheep. It is cut into deeply by the conspicuous Devil's Kneading Trough. Entomologically and botanically, the area is of considerable interest. The year saw a further impressive expansion of the County Naturalists' Trusts' movement. New Trusts were established in Gloucestershire, Northumberland and Co. Durham, the Lake District, Cornwall and Shropshire, and the process of formation was started in Wiltshire, Cheshire, Lancashire, Hertfordshire and Middlesex. In Northamptonshire, Nottinghamshire, Herefordshire and Somerset, discussions about forming Trusts are going on, and within eighteen months almost every county in England, and possibly Wales, should be covered by a Trust. To the Society's co-ordinating and advisory functions has now been added the important responsibility of acting as a channel for the Trusts' applications for financial aid to the Society, the World Wildlife Fund and the Nature Conservancy. Full details of its activities are available in the forty-fifth annual report of the Society for the year ended March 31, 1962 (Pp. 64. London: The Society for the Promotion of Nature Reserves, British Museum (Natural History), 1962. 10*s*.).

The Lake District

THE case for resisting encroaches on the amenities of the Lake District is eloquently pleaded in the July issue of the *Report and Newsletter* of the Friends of the Lake District (Pp. 21. Ulverston: Friends of the Lake District, 1962). Of recent attempts to remove more water from the Lakes, the society contends that no more water should be taken away until it has been established beyond all doubt that the consumers who need water cannot be supplied from sources outside the National Park. The society offers total and determined opposition to the damming of lakes and the flooding of valleys, and to the heightening of existing embankments on such lakes as Crummock Water or Ennerdale. If further water has to be taken from Lake District sources, then the method of intakes in the lower courses of rivers (that is, below the respective lakes), with or without pumping, is that least injurious, always providing that the interests of agriculture and of communities lying downstream are safeguarded by the reservation of adequate compensation water. That the Lake District sources could be developed more cheaply by more ruthless methods is not sufficient argument for their adoption. For cheapness must not be the chief criterion. The values that National Parks exist to serve must be given their full weight in the equation; neither considerations of mere convenience nor money values should be allowed to become the deciding factors. The *Report and Newsletter* also records the public-spiritedness of the North-Western Electricity Board which, despite approval from the Minister of Power, changed its policy with regard to a long stretch of overhead mains and decided, at its own expense, to put them underground.

Handa Island Nature Reserve

THE Royal Society for the Protection of Birds recently announced an important new reserve on the uninhabited island of Handa, off the west coast of Sutherland (*Council for Nature. Intelligence Unit*, 30, August 1962). The proprietors have made an agreement with the Society to manage this magnificent sea-bird colony as a nature reserve and bird sanctuary. Puffins, razorbills, common and black guillemots, fulmars, shags, kittiwakes and herring and great black-backed gulls all breed there. A grant from the Helena Howden Trust has enabled the Society to repair and furnish the bothy on the Island to provide accommodation for up to six members wishing to stay there to watch and study the birds. Bookings should be made through the Society's Scottish Office, 21 Regent Terrace, Edinburgh 7. Access is by fishing boat from Tarbet or Scourie, and there are no restrictions on landing.

The Lundy Field Society

THE fourteenth annual report of the Lundy Field Society includes a list of the birds recorded during the year, the species ringed during 1959, 1960 and 1961, and some interesting ornithological notes on the more unusual observations (Pp. 40. Barnstable: Hon. Secretary, Lundy Field Society, 8 Rock Avenue, 1962). It is also of importance to note that fourteen recoveries have been made of ringed birds, two having reached the U.S.S.R. There is a preliminary account of the archæological investigations in the "Bulls Paradise" by Mr. K. S. Gardner and a report on the discovery of the 'Tulocus' Stone in Beacon Hill Cemetery.

The International Congress of Mathematicians (Edinburgh) Fund

THE British National Committee for Mathematics has been asked by the Trustees of the International Congress of Mathematicians (Edinburgh) Fund to allocate grants from the Fund for the following purposes: (*a*) award of travel grants to mathematicians in the United Kingdom to attend international conferences; (*b*) donations to mathematical symposia, meetings or conferences to be held in the United Kingdom. Applications in respect of (*a*) should be made on forms of application obtainable from the Executive Secretary of the Royal Society (Burlington House, Piccadilly, London, W.1). Applications in respect of (*b*) should be submitted by letter to the Executive Secretary of the Royal Society, supplying the following information: (i) name and address of sponsoring organization; (ii) details of proposed scientific meetings: (*a*) place, (*b*) dates, (*c*) total amount of financial assistance required; (iii) brief statement of scientific programme of the meeting including principal speakers; (iv) exact purpose for which grant is required (giving estimates with details where possible); (v) whether the applicant has applied for assistance from any other source and, if so, with what results. Applications in respect of (*a*) or (*b*) should be sent to the Executive Secretary by December 31.

The Cement and Concrete Association's Training Courses

THE Cement and Concrete Association has issued its prospectus and programme, *Training Courses in Concrete*, for 1963. The Association's courses, held at their Training Centre and Research Station at Wexham Springs in Buckinghamshire, are well known as an integral part of the training of those concerned with the design, making or placing of concrete. The programme of courses offered in 1963 follows a similar pattern to previous years, but a new feature is the appearance of two combined courses and overseas visits, one for architects, the other for engineers concerned with motorway and major road construction. Participants on these courses will spend two days at the Training Centre and the remainder of the week visiting outstanding jobs on the Continent. Copies are obtainable from the Training Centre, Cement and Concrete Association, Wexham Springs, Framewood Road, Stoke Poges, Slough, Bucks.

Announcements

DR. E. M. PATTERSON, senior lecturer in mathematics in the University of Aberdeen, has been awarded the Makdougall Brisbane Prize for the period 1960–62 by the Royal Society of Edinburgh, for his papers on linear algebras published in the *Proceedings* of the Society during the period of the award and earlier papers on cognate subjects published by the Society.

ENTRIES are invited for the 1963 Maccabaean Prize of 25 guineas and a bronze medal, which is awarded annually by the Faculty of the History of Medicine and Pharmacy of the Worshipful Society of Apothecaries of London. Competitors, who must be under thirty years of age on March 15, 1963, will be required to submit before that date an essay of not more than 6,000 words on any subject connected with the history of medicine or pharmacy. Entry forms and further information can be obtained from the honorary secretary of the Faculty, Dr. F. N. L. Poynter, the Wellcome Historical Medical Library, the Wellcome Building, Euston Road, N.W.1.

THE eleventh inter-university geological congress on "Geological Aspects of Commonwealth Economy" will be held at University College, Cardiff, during January 3–5. Further information can be obtained from K. Howells, Department of Geology, University College, Cathays Park, Cardiff.

A SYMPOSIUM on "The Evaluation of the Degree of Hydrogen Bonding in Biological Molecules", supported by the British Biophysical Society, will be held at the South-West Essex Technical College and School of Art, Walthamstow, on February 16. The opening lecture will be delivered by Prof. J. A. V. Butler. Further information can be obtained from Dr. S. Lewin, South-West Essex Technical College and School of Art, Forest Road, Walthamstow, London, E.17.

POSTGRADUATE courses in "Inorganic Stereochemistry" and "Nuclear Metallurgy", suitable for graduates and research workers in the fields of chemistry and metallurgy, will be held at Brunel College. The first course will consist of seven lectures to be given on consecutive Mondays commencing on January 14, and the second of nine lectures, to be given on consecutive Thursdays commencing on January 10. The fee for each course will be £1. Further information and forms of application can be obtained from the Registrar, Brunel College, Woodlands Avenue, Acton, London, W.3.

THE fourteenth Pittsburgh conference on "Analytical Chemistry and Applied Spectroscopy" will be held in Pittsburgh, Pennsylvania, during March 4–8. The programme will include symposia on: solution techniques in X-ray and emission spectroscopy; techniques related to infra-red spectroscopy; nuclear magnetic resonance—nuclei other than hydrogen; gas chromatographic analysis of metallo-organics and related compounds; uses of reaction-rates in analytical chemistry; the analysis of refractory metals. Further information can be obtained from the programme chairman, Dr. W. A. Straub, Applied Research Laboratory, United States Steel Corporation, Monroeville, Pennsylvania.

THE VEGETABLE AND GLASSHOUSE RESEARCH STATION, IRELAND

By Dr. DIARMUID MURPHY

ON September 20, Mr. Sean Lemass, Taoiseach (Prime Minister) of Ireland, opened the Vegetable and Glasshouse Research Station of An Foras Taluntais (Agricultural Institute), Kinsealy, Malahide, Co. Dublin. Mr. John G. Litton, chairman of the Council of An Foras Taluntais and Dr. T. Walsh, director, welcomed An Taoiseach to the Station. The opening was attended by members of the Council of An Foras Taluntais, senior staff members and 150 guests.

Kinsealy is in the centre of the vegetable growing area of North County Dublin, and An Foras Taluntais acquired 150 acres in the area in 1959. The farm is 70 ft. above sea-level and is about 8 miles from Dublin. The solid geology consists of pale grey, coral reef limestone, overlain by gravelly boulder drift of predominantly limestone composition deposited by ice during the Weichsel glaciation. 90 per cent of the soil on the farm is a well-drained loam to clay loam texture of high base status. Available phosphorus-levels are moderate, but available potassium-levels are low (due to fixation by large amounts of illite).

The station serves as the headquarters for the Horticulture and Forestry Division. In addition, the following Departments of other divisions of An Foras Taluntais have research facilities at the station: Soil Physics (Soils Division); Cereal Chemistry and Nematology (Plant Sciences and Crop Husbandry Division). Staff of the National Soil Survey and of the Plant Nutrition Department (Soils Division) use Kinsealy as a centre of operations, and the Grassland Management and Animal Physiology Departments (Animal Production Division) also conduct experimental work at the Station. The following buildings are now complete: the main laboratory and administrative block, the glasshouse and propagating unit, the boiler unit, and the nematology unit. It is proposed to build a mushroom research house.

The main laboratory and administrative block contains a library and assembly hall (570 sq. ft.), canteen to seat 50, administrative offices (2,200 sq. ft.), and laboratories for cereal chemistry (2,040 sq. ft.), soil physics (2,380 sq. ft.), horticultural chemistry and biochemistry (2,720 sq. ft.), plant pathology (1,100 sq. ft.) and mushroom research (850 sq. ft.). In addition, there is a workshop, storage space, dark room and provision for growth rooms.

The main laboratory building runs north–south with the main entrance in the middle. The library and conference hall are over the main entrance, making for ease of access for lectures or conferences. A corridor runs the length of the building with laboratories and offices opening off. The building was originally planned as a single story structure but due to the topography of the site it was found more desirable to build a semi-basement under the North Block, thereby increasing the available floor space by almost 50 per cent. The entire building, which is 310 ft. long by 42 ft. wide, is planned on the modular principle, 10 ft. being the standard module width. The depth of the laboratories is 17 ft. 6 in. Adequate office space separate from the laboratories has been provided for graduate staff.

The glasshouse unit consists of 13 houses, each 100 × 30 ft., and two propagating houses, 75 × 20 ft. One of the glasshouses is designed as a lysimeter house, allowing exact nutritional and moisture studies on a wide variety of plants. It is proposed to build more glasshouses in order to extend the research programme.

A boiler-house serves the entire heating needs of the glasshouses and laboratory buildings. The main boiler uses compressed peat briquettes and is the first completely automatic peat-burning boiler in Ireland.

Because of the nature of the work involved, the nematology laboratory (2,560 sq. ft.) has been placed at a distance from other buildings. It is planned for a smooth flow of work and contains the following rooms: soil reception, drying, extraction and microscope rooms, plus offices, research laboratory and dark room.

The greater part of the research effort at Kinsealy is on horticultural crops, under two headings: vegetable crops and glasshouse crops. A great deal of information is still lacking on the needs of vegetable crops as regards climate, soil suitability, varieties, husbandry methods and nutritional requirements

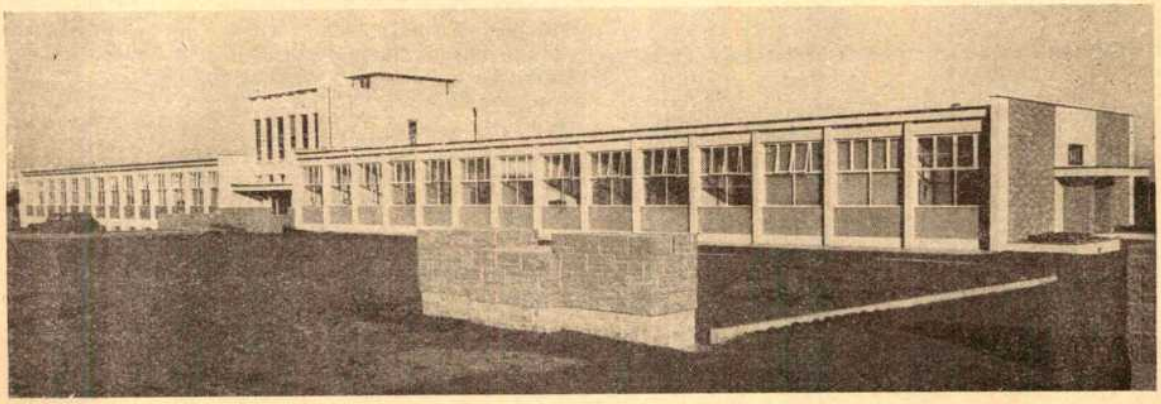

Fig. 1. New research laboratory and administrative building at Kinsealy, Co. Dublin

under Irish conditions. All the common vegetable crops are being grown in a series of field trials. Aspects also being investigated are variety breeding (with emphasis on new varieties suitable for packaging and processing), and weed control.

The glasshouse vegetable work is at present concentrated on tomatoes, cucumbers and lettuces. Already valuable information has been obtained on methods of extending the tomato season. Flower crops under glass are also being examined so that information may be gained which will enable growers to cater for the home and export market.

The Chemistry and Biochemistry Department serves the needs of the vegetable and glasshouse crops sections and a comprehensive research programme is being developed, including work on manurial and trace element problems. It is proposed to build up a large laboratory programme dealing with the chemical and biochemical factors of 'quality' in fruit and vegetables. In addition, work is being done on the chemical aspects of Irish honey—a product capable of great development.

The mushroom research unit is concentrating on the cytological and genetical aspects. A close liaison is maintained with growers and many problems of a practical nature have received attention. To supplement the research facilities at Kinsealy a production unit is available at another centre of An Foras Taluntais. It is proposed to erect a commercial-scale mushroom house at Kinsealy.

A feature of the research work which is of special practical value is that, due to the nearness of the Dublin market, test marketing projects can be carried out on vegetables and flowers in conjunction with the Rural Economy Division of An Foras Taluntais.

The work of the Soil Physics Department deals with research in soil texture and structure, and drainage. The Department also uses Kinsealy as a centre from which soil studies are conducted at a number of other stations. Facilities provided include equipment for soil mechanics and physics, and soil moisture studies. Particular attention is being paid to the water requirements of a number of vegetable crops. In addition, the lysimeter house provides facilities for accurate water balance studies.

The Cereal Chemistry Department has a comprehensive research programme on both the applied and fundamental aspects of wheat and other cereals. Equipment includes drying facilities for 50 samples simultaneously, Brabender grain cleaner, Buhler semi-automatic mill, dough-testers (Brabender farinograph and extensograph) and complete experimental bakery. Factors such as yield, early maturity, resistance to sprouting (of fundamental importance in Ireland because of high rainfall), milling and baking quality, and protein content of wheat are under investigation. Studies on malting and feeding barley are proceeding, and studies on oats are projected.

The Nematology Department undertakes such work as the study of bionomics, host-range and economic importance of eelworms and population studies of eelworms. An important aspect of the work is certification and quarantine of seeds and plants. Other work undertaken includes search for resistant plant varieties, and testing the mode of action and efficiency of nematicides under Irish conditions.

While the research station at Kinsealy is comparatively young, it presents a unique opportunity for co-operation between research workers of many disciplines. As a result of the research programme in hand, much information has already been gained and has been made available to the horticultural and agricultural industry in Ireland.

NUCLEAR CHEMISTRY

An informal discussion on nuclear chemistry, organized by the Atomic Energy Research Establishment, Harwell, was held in the Inorganic Chemistry Department at the University of Oxford during September 18–21. The meeting followed similar discussions previously held, at the University of Durham in 1960, and at CERN, Geneva, in 1961. On this occasion the subject was "Fission and other Low Energy Nuclear Processes". Sixty-one papers were presented covering fission, spallation, radioactive decay; the energy released in these processes; and the general techniques for observing them. Abstracts of the papers have been published as a Harwell memorandum number *AERE–M* 1078, but in view of the informal nature of the meeting no other record has been made. About 180 delegates attended, representing fifty different laboratories in Europe and the United States.

In the opening paper W. J. Swiatecki (University of California) described recent developments in the liquid drop model of the nucleus, and these were applied by T. Sikkeland (Berkeley) to his work on fission induced by heavy ions. Here the compound nuclei formed by the interaction of heavy ions carbon-12 and oxygen-16 on targets from praseodymium-141 to plutonium-240 cover a wide range of charge to mass ratios. The kinetic energy released was shown to agree closely with detailed predictions of the liquid drop model. In the course of the discussion it was pointed out that the fission energy, which arises from the coulomb repulsion of initially contiguous nuclei, is extremely sensitive to the shape of the nuclei, and provides a valuable means for studying nuclear deformation.

S. G. Thompson (Berkeley), R. H. Tomlinson (McMaster University, Canada), and R. Vandenbosch (Copenhagen) all reported experimental support to the theoretical picture that at fission one fragment tends to assume a closed shell configuration, whereas the other fragment is more deformable, takes up excitation, and emits neutrons.

Discussion on fission yields concentrated mainly on the relative amounts of isomers of different spin. Radiochemical measurements of the yields of the isomers reported by I. F. Croall (Atomic Energy Research Establishment) implied that the initial fragments were of high angular momentum, and this conformed with the measurements of the directional correlation between prompt γ-rays and primary fission fragments reported by M. M. Hoffmann (Los Alamos).

Perhaps the most important contributions to the meeting were those on semi-empirical mass formulæ based on the new tables for nuclear masses recently published by Everling, Koenig, Mattauch, and Wapstra. H. Kummel (Max-Planck Institute, Mainz) accounted for the systematic trends in the masses of all known nuclei to an accuracy of 1 MeV by the use of fifty parameters. W. J. Swiatecki showed that the major trends in the deviations of the masses and shapes of nuclei, from those predicted by a liquid drop formula, could be accounted for in a rough way on the basis of a semi-empirical expression with three parameters. These new formulæ should make possible a better assessment of the mass and energy balances in fission which, as discussed at the meeting, are seriously wrong for uranium-235 and plutonium-239 fission on the basis of the old mass formulæ.

In the session on spallation, K. F. Chackett (Birmingham) interpreted his results from (p,n) reactions on the basis of the optical model of the nucleus, and N. Lefort (Paris) described proton reactions in terms of α-particle structures in the nucleus. G. R. Martin (Durham) reported cross-sections for $(n,{}^3\text{He})$ reactions, and for (n,pn) processes; the latter seemed to agree well with a 'direct interaction model', rather than with an evaporation mechanism. Again, the excitation functions of $(d,2n)$ reactions reported by L. J. Gilly (Louvain) failed to agree with predictions of neutron evaporation from compound nuclei. In discussion the general view was expressed that for spallation phenomena the theoretical background is at present very inadequate.

Among the papers on radioactive decay some new isotopes were reported, notably radon-223 (half-life 39·8 min.) and radon-224 (114·8 min) by V. J. Robinson (Liverpool). A paper was given by J. Maly (Dubna) on a new method for the preparation of mendelevium by the bombardment of uranium with neon ions, and some details of the chemistry of this element were reported for the first time. Interesting accounts were given of activation processes (M. Lindner, Livermore), and of the production of transuranic elements (J. D. Knight, Los Alamos), in underground nuclear explosions.

The session on chemical effects was restricted to studies in which different nuclear events could be compared. A. G. Maddock (Cambridge) compared the condition of chromium-51 formed by the (n,γ) process on chromium-50, with its condition when formed by the $(n,2n)$ process on chromium-52 in solid potassium chromate. While no significance could be seen in the initial retention, the subsequent annealing of the material formed in the more highly energetic $(n,2n)$ process was shown to be slower than the annealing of that from the weaker (n,γ) process. In subsequent papers by I. G. Campbell (Manchester), L. Lindner (Amsterdam), and G. E. Miller (Oxford), the importance of the annealing or recombination processes was again emphasized. In the discussion attention was directed to the pressure effects which may influence the 'rigidity' or 'softness' of crystal structures in solids, and 'cage' effects in liquids. Four papers were given on the effects of fission recoil, and in discussion particular attention was given to the sputtering processes that occur when fission fragments pass through surfaces.

In sessions on techniques consideration was given mainly to the problems of flux monitoring. In particular the accurate measurement of reactor neutrons by cobalt activation was described by T. A. Eastwood (Chalk River, Canada) and of neutron spectra from cyclotron targets by A. H. W. Aten, jun. (Amsterdam). New methods for conducting fast chemical separations for measuring short-lived radionuclides were also described. For example, G. Herrmann (Mainz) showed that thin layers of fresh crystalline precipitates on filters retain a large fraction of their own and related ions from solutions filtered rapidly through them. The most notable advance in recent years in radiochemical techniques for the analysis of mixtures of radioactive isotopes is the use of a mass separator in which the relative amounts of the activity on the different target areas where the different nuclides are deposited is measured. Papers by G. Andersson (CERN), and T. A. Eastwood, described this technique, and a third paper was given by H. Ewald (Munich) on the application of a similar technique to the mass separation of highly charged fission fragments during their formation. A refined method for the measurement, by mass spectrometry, of stable fragments (lithium-6 and lithium-7) formed in high-energy proton irradiations was described by E. Gradsztajn (Orsay).

The meeting concluded with descriptions and comparisons from Livermore and Aldermaston of the computer programmes used in the large-scale analyses of radioactive decay data. G. N. WALTON

FUTURE OF TEACHING MACHINES

PROF. B. F. SKINNER, of the Department of Psychology, Harvard University, delivered an address on "The Future of Teaching Machines" on October 19 in the University of Oxford, at the invitation of Prof. R. C. Oldfield. Prof. Skinner began by commenting on the present state of confusion in this field, which is now barely eight years old. This confusion was partly due to commercial interest in producing programmed texts, partly to the enormous growth in the needs of education, with ever more students to be taught and ever more to teach them, and partly to the activities of 'gadgeteers' who delighted in inventing a variety of devices which could be referred to as 'teaching machines' but which were not based on a knowledge of the learning process.

Though education is often considered a preparation for the real world, it is difficult to bring the real world into the classroom; consequently, education has in practice mainly concerned itself with setting up verbal repertoires of one sort or another, teaching the student to speak a language, to perform mathematics, essentially a verbal procedure, or to command the facts of a science. The first learning machines which had been developed in his department had similarly been directed to the development of verbal skills. They required the student to fill in a missing letter in a word, a word in a sentence, or a term in an equation; if right, the student moves on to a new frame; if wrong, he must try again. In this way he works through the programme. Present-day programmes advance by very small steps, so that students make few errors and very little repetition is required. Though the material seems very simple the students move on to advanced technical material very rapidly. The students in his course at Harvard spent their first month using the machines in the 'self-instruction room'. At the end of that period he could lecture to them secure in the knowledge that they had mastered the technical material necessary for the course.

Two effects of the use of these machines were worthy of note. The first was the improvement in the pupils' motivation which they produced. In most schools children studied because they did not dare do otherwise; but children using the machines became absorbed in the work. He had visited an eighth-grade class in Roanoke engaged in studying a programme on algebra in an experiment by Dr. A. Calvin. Not a head was raised from a machine while he wandered through their classroom talking to their teacher. In Toronto an experiment came awry because the children would not restrict their studying to the prescribed hours but smuggled the programmed text (on English grammar) out of school for further study. The children became confident and interested; on returning to ordinary teaching methods they were alert and ready to offer comments. The second effect depended on the fact that the machine allowed each student to go at his own pace. It has been said that what would astonish Plato most to-day is that we attempt to teach two students at the same time. One result of teaching large numbers at the same rate is that the slow student cannot keep up and rationalizes his failure. Yet there is no evidence that the slow student cannot do first-class work if he is given the time to do it. In one experiment, by Dr. D. Cook, an 800-frame programme on chemistry was presented to a class whose intelligence quotients ranged between 100 and 140. It was found that the least intelligent children took approximately twice as long to finish the programme as the brightest, but when tested at the end there was little difference in their achievement scores. Given enough time it appeared that the slow student could do as well as his swifter colleague.

The basic experimental research which had allowed the development of this new sort of educational technology was then discussed. This had relied not on statistical surveys but on the intensive study of individual organisms with a view to analysing the effective environmental factors and developing the ability to predict behaviour from a knowledge of the environment. It had been found that the contingencies of reinforcement had an extraordinary power to select and control behaviour. This was illustrated by a classroom experiment in which the class chose the behaviour, such as bowing, tracing a circle, or leaning over backwards, which they wished a pigeon to perform, and this was produced in a few minutes solely by governing the intermittent food reinforcement given it. At first the experimenter rewarded behaviour corresponding within liberal limits to that required; when the animal repeated this the reinforcement would be made conditional on a more exact correspondence to the prescribed behaviour. More complex reinforcement contingencies could not readily be produced by hand but required special apparatus. This set environments in which certain behaviours would develop. In one sense these devices were teaching machines.

One problem was whether learning could be produced if the animal never made an error; it had been shown experimentally that this was possible. In an experiment by Dr. H. Terrace the aim had been to train a pigeon to discriminate between a pattern of horizontal lines and one of vertical lines without ever having made the error of pecking at the vertical lines. Initially the animal was presented with a key in bright red illumination. It pecked at the key and was rewarded, and the illumination was simultaneously removed. When this sharp change has occurred the pigeon does not peck the key again at once. Before it can do so the key again becomes bright red, the pigeon pecks, and is again rewarded. The intervals between illumination of the key can then be gradually increased without the pigeon ever pecking at the key when dark. The next stage was gradually to add a greenish tinge to the dark key, and decrease the brightness difference, until eventually the pigeon was discriminating perfectly between a red and green of equal brightness. The horizontal lines were then superimposed on the reddened key and the vertical lines when it was green, and they were gradually made more salient while the colours were gradually 'vanished', until finally the desired discrimination was being made by an animal that had never made an error. This showed that trial and error was not essential for learning, and it produced a 'bonus': a pigeon trained in this way was completely neutral to the negative stimulus, it did not show the 'emotional' behaviour often found when an animal is confronted with a stimulus which it has frequently pecked without reward.

Some programmes have been produced in which the student makes errors and the programme then produces comments on them. But it seems this is not necessary; programmes can be devised in which very few errors need be made. Prof. Skinner's first attempt of this sort was made when his small daughter was given the task of learning a passage of poetry. He wrote it on the board and she read it through. She then left the room, he erased some letters, she returned and read it through again. After four or five readings there was nothing left on the board but she could still "read it through".

One of the most common criticisms of teaching machines was that they might be useful for rote learning, but that they could not teach a child to think. This was not the case. Actually in practice we did not usually teach thinking; students were presented with problems and we claimed credit for those who solved them. The others were left by the wayside. We most often taught verbally, and when we did not we tended to teach 'final products', for example, to teach driving in a motor-car or flying in a cockpit, real or simulated. If we analysed the process of thinking, and such achievements, we might find that there were basic skills necessary to them which could be taught one by one. A programme designed by Drs. Holland and Long to teach 'inductive thinking' was described. Programmes had been developed for teaching a number of non-verbal skills, and some of the more important developments of the future might lie in this area. Prof. Skinner described a device intended to cultivate a sense of rhythm. This required the child to tap in unison with a presented rhythm. At first very approximate agreement was acceptable, but the limits were gradually decreased until skilled performances were produced. Even professional musicians tested with this apparatus showed unexpected defects, such as a tendency to be just ahead or just behind the presented rhythm, or, very commonly, a tendency to speed up if their performance was good. Another device was being used to teach 'tone-deaf' children to discriminate pitch. Beginning with coarse discriminations they were gradually brought to the point where they could discriminate tones or semi-tones. Without devices such as these it was scarcely possible to provide the differential contingencies of reinforcement which would 'shape' such types of non-verbal behaviour. Eventually we may hope to be able to teach all the basic skills constituting adaptation to the environment, and so to enable man fully to exploit all his potential skills.

THE INSTITUTE OF PERSONNEL MANAGEMENT

A MANAGEMENT conference which, each year, attracts about 1,000 members from home and overseas must surely be a source of considerable attraction. Each year the conference of the Institute of Personnel Management attracts about one-fifth of its members, and the question could fairly be asked about the nature of the attraction. While the good fellowship would draw many to the conference, there is little doubt that the quality of speakers and the relevance of their subject-matter is the main draw. The content of the sectional meetings over the past five years could, indeed, well be compounded to form a useful 'Students' Refresher Guide to the Intricacies of Industrial Relations in Britain'.

This year's conference at Harrogate was held during October 4–6, and was no exception to the general rule. P. M. Uri, director of Lehman Bros., Paris, discussed the role of planning in economic growth and showed that, in those countries where there has been most rapid rate of growth—the U.S.S.R., Japan, Germany, Italy and France—the only truly common denominator has been a surplus of manpower. Planning has been carried out in different ways in different countries, with each one initiating new forms of long-term finance. In France, the planning organization is made up of a Commissariat Du Plan, consisting of three or four people supported by another small group of individuals who act as secretaries for different industrial committees. Apart from this small staff, there are about one or two thousand people around the small nucleus. They are organized in a certain number of committees by industries, plus an overall economic committee which is trying to see what kind of balance can be achieved between total investment, total resources and total sources of finance. The constitution of these committees is partly people from industry, partly people from the administration, partly independent experts, and partly members of the trade unions. This is considered the mainspring of democratic planning and how a plan can be implemented. Such a form of planning has played an important political part, because it has helped to create a conviction about important decisions which had to be reached on the morrow of the liberation. That was a time of utter economic destruction, and two hard decisions had to be reached. One was that the limitation of the working week to forty hours had to be abandoned, and the workers had to be prepared to work longer hours to speed up reconstruction. The second decision was to postpone reconstruction for housing purposes and give priority to the productive equipment of the country. Now the plan may play a different part.

Dr. N. W. D. Beese, works relations officer, Billingham, Imperial Chemical Industries, Ltd., examined the place of employee communications in the building up of good industrial relations. These have to be related to other forms of communication in industrial organizations. They are the day-to-day line management flow of information necessary for the operation of the organization and the exchange of information in joint consultative procedures such as works councils and efficiency committees. The line management and joint consultative channels of communication are first priorities and only when these have been properly organized and are working smoothly is it feasible to embark on an employee information service programme.

At a sectional meeting, some of the ramifications of the so-called wages drift were examined. This is probably a more important cause of inflation than increases in wages which are negotiated nationally. The drift occurs at factory- and office-level when earnings are increased by over-time, various kinds of incentive schemes and fringe benefits. According to K. J. Burton, formerly secretary of the British Employers' Confederation, wages drift is due to the fact that there are no appreciable unused reserves of labour, while there is insidious danger in the failure to recognize that the significance of basic wage-rates as the determinant of earnings and of total labour costs ranges considerably from industry to industry. False comparisons are often made during essential stages in collective bargaining. The remedy is not to weaken collective bargaining arrangements but to aim at greater co-ordination of variations within industries and localities.

Investigations by Dr. M. Jahoda, Nuffield Research Fellow at the Brunel College of Technology, into the relations between industry and technical colleges, showed that, while collaboration between industry and college functions by and large is in a satisfactory manner, there is also a number of general problems. The Hives Council's stipulation, for example, that responsibility be shared between colleges and industry permits in practice a variety of interpretations.

Since, from a company's point of view, education leading to a diploma in technology is expensive (the cost of one student for four years is estimated to be about £2,000), some companies feel that they carry, or should carry, major responsibility, certainly for the industrial periods. Where this view is also held by a college, divided responsibility alternating every six months between college and company is the inevitable consequence. Colleges cannot choose this interpretation, even if they wished to, because the diploma in technology is essentially an academic qualification; the Hives Council holds the college responsible for attesting that a student's industrial training has been adequate. This means that the college must exercise its responsibility in shaping the industrial training periods, preferably by joint agreement on their nature and content before a student begins his training. Shared responsibility is most easily achieved in student selection, which must find the approval of both partners; it is most difficult in arranging the actual practical work of the student.

A further difficulty arises from the fact that by far the most promising technique for integrating practical and theoretical knowledge in the student's mind is the tutorial system. Here, college staff visit the student at his place of work to discuss with him the manner in which his practical experience relates to his academic knowledge. There is still some confusion about the function of the college tutor, both among tutors and in industry. While visits to firms can serve public relations purposes, provide consultation opportunities on technical matters for the company or the tutor, or preferably both, the major object of these visits is to help individual students in achieving integrated knowledge. In the light of experience, the hope of achieving integration by arranging industrial work so that it is an immediate application of what the preceding college period presented theoretically must be regarded as naïve and impractical. The task of integration is made easier where companies appoint industrial tutors to students working for a diploma in technology, who remain a constant focus for immediate technical supervisors.

Jahoda had asked students how they liked their first industrial training period and how much they had learned from it. A combination of answers provides some yardstick for evaluating industrial training. By this yardstick, 64 per cent of students engaged in project work felt they had a good experience; 54 per cent in training schools, 38 per cent working on a technician-level, 31 per cent on a perambulatory course, and 18 per cent on a production worker-level valued the industrial period highly. In spite of the subjective nature of the judgment, these figures indicate some aspects of a successful industrial training period.

The question whether a personnel manager should advise general management on matters of organization structure and the style of operational management was examined by W. G. McClelland, Research Fellow in management studies at Balliol College, Oxford. McClelland indicated that the personnel manager has the responsibility of supplying satisfactory personnel, and, properly to discharge this responsibility, he must be able to analyse what they will be called on to do and what they will get out of it, and to make recommendations designed to ensure that the organization can compete successfully in the labour market. Since requirements and rewards are not merely matters of skill and pay, but are affected by organization structure and style of management, the case for the personnel manager's right to intervene would seem easy to establish. In the case of managerial personnel, most selection consultants insist on analysing the organization before embarking on recruitment and selection.

The personnel manager may also be called on to devise and apply tools of management—such as appraisal forms and training in communications techniques—required as the expression of an inappropriate management philosophy or to combat the consequences of an ineffective organization structure. He may be called on to deal with individuals cracking under the strain of an impossible situation, and may well feel that he would be better engaged as a public health officer devising means of prevention than a physician attempting cure.

On the other hand, he is at present ill-equipped to advise on these matters. How many personnel managers, to take the simplest example, would be prepared to say what span of control in a particular hierarchical organization minimized the sum of the disadvantages of too little attention to immediate subordinates on one hand, and too many steps between bottom and top on the other? From his safe haven of aptitude tests of proved reliability and validity, and accurately pin-pointed learning curves, the personnel manager steps here into a largely uncharted world in which line managers can—and certainly will—argue with him.

Better use of human resources was discussed by a research psychologist and a personnel director. Dr. J. Hopkins, of the National Institute of Industrial Psychology, suggested that any task can be made to vary in at least three ways: duration, scope of action and responsibility. Any of these variables can be increased or reduced and job enlargement can result when they are increased in ways which succeed in strengthening the meaningfulness, although there might be considerable increase in duration, scope or responsibility. It should, however, be considered how far job enlargement might be psychologically desirable. Evidence of people's reactions to their jobs is still scanty and anecdotal. Attitude surveys and similar types of inquiry have shown that highly repetitive and subdivided tasks—the opposite of job-enlarged tasks—are apparently almost as congenial to some people as they are abhorrent to others. Whatever the variety of people's comments about work, they consistently reveal preoccupations with only a comparatively limited number of themes. Among the most frequent are the pace of the job, the security or insecurity engendered by it, its variety, its inherent creativeness, its difficulty and its sociability. These themes indicate a response to at least three identifiable aspects of work: the amount of constraint the task imposes, the 'completeness' which it allows one to experience, and the degree of personal involvement which it demands. The status or value of an enlarged job as against a more subdivided one should be viewed in relation to these aspects of work. T. G. P. Rogers, of I.B.M., United Kingdom, Ltd., is in no doubt that Britain has not fully developed its human resources, and quoted an example from I.B.M. in the United States to show what can be done. Much of that Company's work has changed from electro-mechanical to electronic engineering. Redundancy is anathema to the Company. It had to make electronics technicians out of machinists and assemblers. They had to be taught algebra, electrical and electronic theory, and computer theory as a prelude to their practical training and their subsequent employment on final test operations on computer manufacturing. This was done in such a way as to enable the American management to conclude that a substantial percentage of what are called semi-skilled operators are capable of this change, regardless of their age.

The important factors are—good selection in assessing mental ability, aptitude, and motivation, and thorough full-time class-room and workshop training. What is required in British industry is a new attitude in management towards operator training—skills should be taught and not bought; and also in many cases a new shop-floor attitude—more self-confidence and less rigidity of outlook.

The two final speakers were asked to look at industrial relations in the future. One, Mr. Frank Cousins, general secretary of the Transport and General Workers' Union, expressed the strong opinion that the basis of all industrial troubles is insecurity and that economic planning will not be possible until there is a new approach to industrial relations. Trade unions want to be consulted about more than the incidence of redundancy, and which men will go. They wish to talk about the basic reasons for redundancy and whether the workers have any place in determining the policies which cause or do not cause this redundancy to arise. There is, also, far too much tendency to accept that "if a firm or a trade union has bright ideas about the future of industrial relations, it sounds nice but you cannot do it unless you can persuade everybody to do it first; you cannot, in fact, wait for the slowest". Other views put forward by Cousins were that "if we regard industrial relations as a steady process of agreement on consultation through operating an agreed but flexible structure, we have still failed", and that "we will not resolve the problems if we think we have a common purpose. We have two different purposes. We have to see how we are going to serve them and how to bring the consequent results together at some stage. We do not start off from the basic level. This could be one of the most significant contributions to economic planning, if we first accept that principle".

The other, Sir Roy Harrod, Nuffield reader in international economics in the University of Oxford, discussed the relations of economic planning and incomes policy. For a number of years after the Second World War inflationary tendencies were certainly due to "too much money chasing too few goods", and the right way to correct this situation was by monetary and fiscal policies. In more recent times it has appeared, both in Britain and in the United States, that there might be a tendency to inflation, in the sense of rising prices, even in periods when it was perfectly clear that the monetary demand for goods and services was not excessive, as made evident, for example, by the economy being slack or depressed. It seemed inescapable that a rise in prices might occur simply because increases of wages, salaries, etc., were being granted in excess of any increase in national productivity and give rise to 'cost-push' inflation.

In the recent period people have become self-conscious about the fact that, in the past seven years, Britain has shown a low growth-rate. The same is also true of the United States. If Britain is to make a change in her monetary and fiscal policies and try to achieve the growth-rate of which the country is capable, this brings into the forefront the question of how to prevent inflation of the kind that is caused by a 'cost-push'. There is the possibility that a policy of full growth will tend to accelerate claims for higher pay and make employers, who, under a growth policy, will be enjoying good profits, readier to give them. Hence the idea of a National Incomes Commission.

The idea of a policy of planning for economic growth entails problems of formidable dimensions. Such a policy cannot be implemented without goodwill on the labour side, and to get labour goodwill it will be needful to show that there is some method to secure restraint on the side of distributions of dividend also. But within the field of wages itself there are vast problems. There is the question of relation of wage increases to productivity increases, the question of the 'wages drift', the question of differentials between grades and between occupations, the proper function of wage increases in relation to the mobility of labour as between rising and declining industries, the problem of leap-frogging, the question of how a trade union leader can relate his thoughts to a national policy without sacrificing, or seeming to sacrifice, the interests of the particular group of workers which he is appointed to look after.

All these interconnected questions have to be looked at in an impartial way without prejudice. Public opinion in certain quarters has been too much inclined to suppose that labour is always to blame when overall wage increases have been excessive, without appreciating the complexity of the problem. On its side, labour may not always understand the relation between excessive wage increases and inflation. The idea of looking at this complex of problems in a systematic and impartial way presents a great challenge.

In his presidential address to the conference, Mr. Gerald Moxon of United Glass, Ltd., reminded members that the Institute would be celebrating its jubilee in 1963. Its influence over many years in building up good industrial relations deserves to be much more widely known and it is to be hoped that suitable opportunities will present themselves in the coming year. It is so long since the Institute appealed for money to extend its activities that the Jubilee Appeal should meet with considerable response.

T. H. Hawkins

BONE MARROW THERAPY AND CHEMICAL PROTECTION IN IRRADIATED PRIMATES

During August 15–18 an international symposium on "Bone Marrow Therapy and Chemical Protection in Irradiated Primates" was held in Rijswijk, the Netherlands. The symposium was sponsored by the Organization for Health Research of the Central Organization for Applied Scientific Research in the Netherlands (T.N.O.), and the Radiobiological Institute T.N.O. acted as host for the meetings. There were 32 participants from France, the Netherlands, the United Kingdom and the United States, of whom 22 presented papers. The main object of the symposium was to exchange ideas as to the causes of difficulties in the treatment of irradiated primates, including human patients, and to discuss future research plans to overcome these difficulties.

In the session on "Bone Marrow Transplantation in Monkeys", C. Ambrus (Roswell Park Memorial Institute, Buffalo) presented data on homologous and autologous bone marrow transplantation in rhesus monkeys. Autologous transplantation gave better survival than homologous, but a high percentage of the homologous-treated animals survived for as long as 24 months with evidence of secondary disease in only one animal. The data showed that there was a marked tendency toward reversal of the chimæric state in the homologous-treated monkeys.

R. J. Young (Radiobiological Laboratory, Austin, Texas) reported the results of homologous and autologous transplantation in twenty rhesus monkeys. Autologous transplantation afforded good survival, but there were no long-term homologous survivors. The data showed evidence of secondary disease in homologous animals in which the take of the graft was ascertained by red cell antigens.

The data presented by Mrs. F. Newsome (University of Tennessee Medical Units, Memphis) showed evidence that homologous marrow after 550 r was more effective 48 h after irradiation than at 30 h. She reported on heterologous transplants using cynomolgus donors and rhesus recipients with the electrophoretic patterns of hæmoglobin serving as an indication of the take. She concluded that the grafts took but were later rejected. The same results were seen in cynomolgus monkeys treated with homologous marrow. Autologous transplantation in 19 rhesus monkeys was reported by R. Schofield (Paterson Research Laboratories, Christie Hospital, Manchester) in which six animals survived for more than 30 days post irradiation. A large number of deaths was attributed to bacteræmia.

L. M. van Putten (Radiobiological Institute T.N.O., Rijswijk) reported that several methods used in mice to prevent or treat secondary disease apparently do not work in monkeys. Preliminary results, however, indicate that the use of fœtal liver shows some promise in this direction. A detailed report of the pathology of secondary disease in primates was presented by M. J. de Vries (Radiobiological Institute T.N.O., Rijswijk), in which he showed striking similarities between this process in monkeys and humans and pointed out their differences with reference to mice. The prominence of secondary disease in the monkeys treated by R. J. Young *et al.* and those treated by the Rijswijk group was in sharp contrast to the absence of this complication in Ambrus's cases and in cases reported previously by B. G. Crouch (U.S. Naval Radiological Defense Laboratory, San Francisco) and R. R. Overman (University of Tennessee Medical Units, Memphis) and this discrepancy gave rise to much discussion. It was also concluded that much more insight was needed into the reactions of primates on homologous bone marrow transplantation following midlethal and sublethal doses of radiation in view of the adverse results obtained by Mrs. F. Newsome.

There were two papers in a session on the "Immunological Activity of the Primate Fœtus". H. E. M. Kay (Royal Marsden Hospital, London) reviewed the knowledge of the development of the lymphatic tissues in the human embryo and presented the results of the administration of fœtal liver cells in cases of bone marrow aplasia. He also reported on preliminary results with fœtus to fœtus transplants in cases of Rh incompatibility with little evidence of a proliferation of the grafted cells. R. Bangham (National Institute for Medical Research, Mill Hill, London) described his methods of immunizing and sampling monkey fœtuses at various ages. He obtained evidence of immunological reactivity in embryos as early as 9 weeks gestation.

In the session on Monkey Colony Management, D. B. Gisler (Wright-Patterson AFB, Dayton, Ohio) presented details of a method for determining the age of monkeys from roentgen graphs by measuring skeletal growth and epiphyseal ossification. A. J. Riopelle (Yerkes Laboratory, Orange Park, Florida) discussed the breeding and raising of chimpanzees for biological research with particular reference to radiation studies. Problems of the sanitation of monkeys brought from their natural habitat into the laboratory situation were reviewed by D. van der Waay (Radiobiological Institute T.N.O., Rijswijk). He related experiences with rhesus, cynomolgus, pig-tail and African green monkeys and emphasized the problems connected with parasitism and bacterial infections.

N. Gengozian (Oak Ridge Institute of Nuclear Studies, Tennessee) described the care and handling of the pigmy marmoset for laboratory studies. In addition, he presented preliminary data on the LD_{50} for this species and data on various peripheral blood parameters following irradiation, which suggested that the marmoset is relatively more radiosensitive than other monkeys studied so far. A systemic dermatosis of uncertain ætiology in the rhesus monkey was described by M. J. de Vries (Radiobiological Institute T.N.O., Rijswijk). The disease has proved to be fatal in a number of cases and is postulated to be due to an interference with vitamin A utilization. Histological examination of tissues showed striking similarity to those seen in X-disease in cattle.

In the session on "Human Applications of Bone Marrow Transplantation", N. B. Kurnick (University of California, Veterans Administration Hospital, Long Beach) advocated the storage of bone marrow for all patients undergoing extensive radiotherapy. Re-infusion after the occurrence of aplasia is only occasionally needed, but may in those cases be of great benefit. Evidence was brought forward to show that in this respect man differs markedly from the mouse, since in this animal subtotal irradiation rarely causes dangerous aplasia. J. G. Humble (Westminster Hospital, London) gave an account of the application of autologous bone marrow suspensions in large volume radiotherapy. This improves the safety of the procedure and once the other risks of large volume therapy (radiation pneumonitis) have been identified, the technique opens new therapeutic possibilities. E. Loeb (Wadley Research Institute, Dallas) discussed the use of stored autologous bone marrow after radioisotopes in high doses in the palliative treatment of malignant tumours and attempts at homologous bone marrow treatment after total body irradiation for leukæmia. The techniques of storage were extensively discussed. J. Ambrus (Roswell Park Memorial Institute, Buffalo) similarly advocated the storage of hæmopoietic cells for patients undergoing chemotherapy or irradiation combined with chemotherapy and discussed in addition a number of experimental studies. G. Mathé (Hôpital Saint-Louis, Paris) summarized his experience with homologous bone marrow therapy in the treatment of radiation accidents and after therapeutic irradiation of patients with leukæmia. From his series of clinical data, man appears to be more radiosensitive than most experimental animals with the LD_{50} at approximately 400 r.

In the session on "Chemical Protection of Primates", J. R. Newsome (University of Tennessee Medical Units, Memphis) discussed the results of combined treatment with two different chemicals. The use of both aminoethylisothiuronum (AET) and para-aminopropiophenone (PAPP) had a markedly greater dose-reducing effect than did either compound alone. G. S. Melville (School of Aerospace Medicine, Brooks AFB, San Antonio) presented similar data combining AET with cysteine in rhesus monkeys. He also presented evidence to show that 'Nembutal' enhanced the dose-reducing capacity of these compounds. AET and cysteine were also combined in the protection of monkeys against 800 r by L. van Lancker (University of Wisconsin, Madison). He concluded that an effective X-ray dose reduction was obtained with these compounds and further that marked cellular regeneration of spleen and bone marrow were evident in protected animals by 30 days following irradiation.

The *Proceedings* of the symposium, including the discussions, became available on November 1 and can be obtained from the Radiobiological Institute T.N.O., 151 Lange Kleiweg, Rijswijk (ZH), Netherlands, and from Dr. R. R. Overman, University of Tennessee Medical Units, Memphis, Tennessee, U.S.A., at the price of 4 U.S. dollars.

D. W. VAN BEKKUM

MODIFICATION OF THE WEATHER

> "He who controls the weather controls the world."
>
> LEONARDO DA VINCI

SINCE the day when primitive man first became aware of, and was awed by, the forces of Nature he has sought to control them. Although throughout the centuries he has been successful to some extent in moulding his environment and harnessing the forces of Nature to his advantage, his success in controlling those natural phenomena which have marked the behaviour of the Earth, oceans and sky throughout time has been very limited, if not negligible.

With the rapid advances which have been made in the atmospheric sciences during the past two decades, it is not surprising that meteorologists have looked towards weather and climate control as a serious goal capable of attainment. Besides this desire is the natural wish of mankind to make his environment more comfortable and less hazardous. This is particularly true for the inhabitants of those parts of the world where the weather can cause loss of life or destruction of property or economic ruin, either by sudden catastrophic storms, or by slow, but just as disastrous, occurrences such as drought or floods which bring about crop failure or destruction and famine; and behind all this there are, of course, the military and political dreams of controlling the climate on a large scale. These thoughts may have existed for a long time; but it is only in the past fifteen years that any real attempt has been made to answer them, and only in the past three or four years has such an attempt been transformed into a major scientific programme. This programme is world wide. Within the global programme there exists a United States programme in weather modification, defined by natural needs for weather control. The responsibility for the national programme is held by the National Science Foundation, which has been directed to initiate and support a programme of study, research and evaluation in this field. The Foundation is advised by an Advisory Panel for Weather Modification*.

A great deal of the work which is being carried out under the heading of weather modification is concerned with basic studies in cloud physics. The work of Prof. B. J. Mason in Great Britain (*Nature*, 195, 647; 1962) is an example of the way in which the problem is being attacked. Fundamental laboratory research into the properties of condensation and freezing nuclei and cloud droplets and the mechanism of precipitation processes is essential before experiments in the atmosphere to stimulate precipitation artificially can be expected to give successful results as judged by accepted statistical evaluation tests. It is interesting to note that in the kaleidoscopic pattern of jargon the former much-used phrase of 'artificial precipitation' appears to have been replaced by the wider and more sober one of 'weather modification'. However, the more spectacular possibilities of rain-making seem, in part, to lie hidden behind the broad front of the programme of weather modification.

Much work on cloud seeding has been carried out not only in the United States but also in many other countries. Argentina, Australia, Canada, France, Israel, Italy, Japan, Mexico, South Africa and the U.S.S.R., among others, have all engaged in such experiments. But the conclusion cannot be escaped that success has been very limited indeed. All the results confirm that, although rain can at times be stimulated artificially, much more needs to be known about all the variables before cloud seeding can be carried out with predictable results.

Artificial precipitation is, of course, not the only form of weather modification. Cloud seeding has been used in an effort to evaluate the possible suppression of hail. Hail samples have been gathered from areas within and outside a seeded district to determine the amount of silver iodide, the seeding agent present, and to examine the effect the agent may have had in suppressing hail. Present theory is quoted as indicating that hail can be suppressed by cloud seeding. However, statistically the results of hail suppression projects are contradictory and confusing. It is certain that the physics of hail formation must be better understood before much further progress can be expected. In Switzerland, a Federal Commission for studying formation and prevention of hail was established in 1950 and at present operates three laboratories. A number of field experiments have been carried out. The experimental evidence shows that seeding with silver iodide has no extensive effect in terms of an overall suppression of hail. At the same time, it is not possible to determine from the available data whether the seeding has altered the extent and intensity of hail-fall. Generally speaking, the number of days on which hail falls is too small to justify a thorough statistical evaluation of the results according to the areas over which hail of varying intensities falls.

Other aspects of weather modification have also been considered. Some investigations have been made at New York University on the feasibility of the artificial modification of tropical storms; but even this involves the possible effects of cloud seeding. Wider objectives, such as the effect of modifying the air/sea boundary and the internal energy transformations, or altering the physical composition of the storm will also be studied. A U.S. Weather Bureau project will use standard cloud-seeding methods to release latent heat and so try to create asymmetries in the storm.

Another project is concerned with the absorptive power of a pure carbon cloud. It was found that for 3×10^5 particles of carbon per c.c. and a zenith distance of the Sun of 60°, 32 per cent of the Sun's radiation is absorbed in a cloud 100 m thick. Such a cloud would require 30 kg of carbon per km^2. The effects in the atmosphere are complex and depend on many variables such as the water vapour content above and below the cloud, the elevation of the Sun and other factors.

In Sweden plans were laid, but not carried out, to spread radiation-absorbing dust on snow-fields to accelerate melting in late spring. Experiments were conducted there to spread fatty alcohols on snow-fields in order to diminish their evaporation. Attempts have been made in several countries to reduce the natural evaporation from lakes and reservoirs by spreading and maintaining a suitable chemical film over the water surface.

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* National Science Foundation. Weather Modification—Third Annual Report for Fiscal Year ended June 30, 1961. Pp. vi+78. (Washington, D.C.: Government Printing Office, 1962.) 30 cents.

The third annual report on Weather Modification of the U.S. National Science Foundation shows that these problems are much in mind at the present time. It seems, however, from the existing results that progress will be slow. The next few years will see a consolidation of facts and basic ideas on the mechanism of the physical meteorological and climatological processes which govern the behaviour of our atmosphere, rather than any spectacular advance in man's attempts to modify the weather to any marked degree. Small local phenomena may indeed be brought into being, dissipated or modified. For the time being, meteorologists should not allow their imagination to run wild with the hope of changing things effectively on a wide scale. A. H. Gordon

THE LOW TEMPERATURE RESEARCH STATION, CAMBRIDGE

THE first International Congress of Food Science and Technology held in London during September directed attention to the ever-increasing interest in the nature of food materials and modern techniques of food production. The recent publication of the annual report for 1961[*] of the Low Temperature Research Station, Cambridge, is therefore timely and will, no doubt, be received with particular interest by those who visited the Research Station during the week of the Congress and attended sessions which dealt with papers on the progress of its activities.

An account of some of these activities, set against a background of the present state of each subject, forms the essential content of the report. Together with its accompanying brochure, *The Low Temperature Research Station, Cambridge*[†], the wide range of interests of the Station is well presented. A particularly noteworthy aspect is the substantial contribution to modern food handling and processing practices emerging from the results of its research work.

The preface describes the function of the Station as research into the principles of the preservation of foods. As would therefore be expected, considerable attention is directed to the newer methods of food preservation, such as radiation sterilization and freeze dehydration.

"Accelerated Freeze Drying of Meat" is the title of the second report, dealing with ultimate pH on the quality of freeze-dried meat. The results obtained should be of particular interest to those concerned with potential applications of accelerated freeze drying to the preservation of meat. This report is preceded by a detailed survey entitled "Anatomy and Physiology in Meat Research". Factors influencing quality are summarized under the headings of "Constitution" and "Condition".

The data presented on immersion freezing (air and liquid) of poultry are of immediate practical interest. A reassessment of heat extracted in freezing chicken should be found useful in equipment design. It will be of advantage to have further data with the publication of full experimental details.

Much of the pioneer work on liquid whole egg pasteurization was carried out by the late Dr. J. Brooks. This has now led to the successful development of the amylase test. The effectiveness of the test can be judged from the last paragraph of this account: "The results of a number of technical trials recently carried out indicated that the amylase test could be used to detect an error of 0·5° F (1° C) in the setting of the temperature control instrument of a commercial pasteurizer; serial results could be made available 1 h after taking the first sample. The amylase test has also been applied to samples of pasteurized whole egg of widely different origin and has correctly indicated the severity of the heating treatments used".

Under the title "Twelve Years of Radiation Preservation Research" progress in another branch of food research is briefly reviewed. Much interest has been shown to exist in this new method of food preservation and some of the results reported here show considerable promise. The study of radiation pasteurization of frozen whole egg carried out in collaboration with the Isotope Research Division of the Atomic Energy Research Establishment was reported at the Congress. This may prove to be of commercial value. The effects of irradiation of other foods, including pork sausage and fish, are also mentioned. The latter application deals with white fish species which have been found to respond well to treatment and, at the maximum tolerable dose, considerably extended the storage life at 0° C and 4° C.

The successful application of all methods of food preservation so far discussed depends on adequate packaging. Gas sampling in vacuum packs forms the main topic in the report entitled "Physical Problems in Packaging of Foodstuffs". Gas analysis is of great importance in assessing this type of package performance and a systematic study of the subject should be welcome.

The technique of gas chromatography has also been applied to the analysis of flavour. Work recently started on the flavour of meat and poultry and preliminary results indicate that here, too, the breakdown products of amino-acids can make a notable contribution to flavour. The study of flavour during irradiation should yield further results of considerable interest.

Two other reports, dealing with phospholipids and the classification of micro-organisms, complete a review of some of the activities during 1961.

The report contains a comprehensive list of recent publications. In addition, references to the literature are quoted at the end of most of the reports presented. A photograph depicting one of the rooms at the Meat Research Laboratory, Cherry Hinton, is included.

Other photographs appear in the accompanying brochure together with selected excerpts from the annual reports 1959–62. This booklet also includes an account of the work and achievements of Sir William B. Hardy, founder of the Low Temperature Research Station. R. A. P. Wertheim

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[*] Agricultural Research Station. Low Temperature Research Station, Cambridge. Annual Report, 1961. Pp. 40. (London: H.M. Stationery Office, 1962.) 2*s*. 6*d*. net.

[†] University of Cambridge and Agricultural Research Council. *The Low Temperature Research Station, Cambridge*. Pp. 48+3 plates. (London: H.M. Stationery Office, 1962.) 2*s*. 6*d*. net.

THE NATIONAL RESEARCH COUNCIL OF CANADA

A NEW format has been adopted for the forty-fifth annual report of the National Research Council of Canada, covering the year 1961–62, but the report is on the usual lines and includes the annual reports of the Medical Research Council and of Canadian Patents and Development, Ltd.[*]. During the year, 8·7 million dollars went in support of pure research in the universities, including 975 grants and 675 scholarships and fellowships, as well as a further 3·3 million dollars through the Medical Research Council. The scientific research staff now numbers 733 (including 163 postdoctorate fellows) with 997 technical personnel and 890 general service and administrative staff. A notable development of the year was the Government's decision to give financial assistance to research and development in Canadian industry, and in 1962–63 a special committee of the Council will make awards totalling 1 million dollars in support of long-term applied research in industry. Besides this, the Council is already responsible for wide programmes of objective basic research, as well as undertaking applied research under contract and operating technical information and documentary services. Support for university science has increasep from 3·6 million dollars in 1957 to 14·4 million dollars in 1962.

Brief summaries are included of the work done in the Division of Applied Biology in the Atlantic Regional Laboratory, Halifax, the Prairie Regional Laboratory, Saskatoon, the Divisions of Applied Chemistry, Pure Chemistry, Applied Physics, Pure Physics, Building Research and Mechanical Engineering, the National Aeronautical Establishment and the Radio and Electrical Engineering Division. Under the Medical Research Council, 96 programmes directed by senior scientists were supported on a three-year basis and 224 on a renewable annual basis, while 64 fellowships, totalling 236,642 dollars were held. Of cases coming to the Canadian Patents and Development, Ltd., during the year, 39 per cent originated in the laboratories of the National Research Council, 47 per cent from other Government agencies and departments and 14 per cent from universities and provincial research institutions. Besides 107 patent applications filed, 150 patents were issued in 13 countries.

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[*] The National Research Council of Canada. Forty-fifth Annual Report for 1961/1962, including the Annual Reports of the Medical Research Council, and Canadian Patents and Development, Ltd. Pp. 47. (NRC. No. 6815.) (Ottawa: National Research Council of Canada, 1962.)

PRESERVATION OF WILD LIFE AND ZOOS

IN her book *Orang Utan*[1] Mrs. Harrisson describes rearing baby orangs confiscated from poachers in Sarawak and taking them to European zoos. Having learnt something of their needs, she considers the possibility of training them to return to their natural jungle habitat. In a later paper, entitled "First Response to Freedom of Young Orang-Utans at Bako National Park, Sarawak"[2], she describes the first stages of an experiment in rehabilitation.

In the first three years orang babies are nearly as helpless as human babies; they need a mother-figure as much, and they will endlessly adapt their behaviour to fit the environment the 'parent' provides. The problem is to bring up a baby to a stage when it can cope alone with its jungle environment, without distorting its behaviour so much that it is unable to break free of human society when the time comes. Up to the time of writing the paper the problem had not been solved, and if the experiment is to be successful newly independent females must not only be self-sufficient, but so shy of their former nurses that they will not bring their own babies within easy shot of poachers.

An earlier paper of Mrs. Harrisson[3] and Dr. T. Harrisson's addenda to the book show how vital it is that these experiments should succeed. Simple arithmetic shows that if the present situation remains unchanged the orang-utan has about ten years left. An orang should live thirty or forty years: in zoos their average life is four years, and breeding in captivity is negligible, so to maintain the present world zoo population 60 replacements are required each year. To exhibit a baby costs the life of its mother, and the mother of another baby who will die in transit. Thus in ten years 2,400 animals are lost from a population which stands at present between two and five thousand and is probably already overweighted with adult males, which do not interest collectors.

It is highly questionable whether the import of orangs, or any other threatened species, should be allowed into civilized countries. There is no justification on scientific grounds because it is now cheaper and easier to transport the biologist to the ape than vice versa. The real irony of the situation is that these orangs acquired at so much cost are then kept in such a way as to make them useless as entertainment or for research. Probably the main function of this book will be to help to teach a basically kind-hearted but uncomprehending public that the sad-eyed hulks of apes in zoos bear no more resemblance to mentally healthy creatures than would a human being maintained in the same way. The clear account of the needs of the baby orang should prevent the demands on the pet market (and ultimately the poachers) which books of this type are apt to produce. (Compare, for example, the assortment of unsuitable felids that were bought as a result of *Born Free*). Orangs are said to be more stupid than chimpanzees, but this impression has arisen because they are expected to behave like the mainly terrestrial and imitative African apes. The orang is arboreal and must have trees to climb; it is an explorer rather than an imitator. Its requirements sound like a zoo director's nightmare. How do you provide thirty years' worth of exploration?—yet they should be considered as necessities, in the same way that a zoo does not acquire a giant panda until it has assured a supply of bamboo.

The expense, in space, materials, and time which is needed to provide adequate environments is one which very few zoos are willing to face. Before real improvements can be made it will be necessary for zoos to reconsider their whole function, because their present way of thinking belongs to the past century. Then it was valuable to collect together large numbers of closely related animals, a single specimen of each perhaps, since zoology was mainly a form of taxonomy: but completeness of the collection is still a criterion of excellence, as in a collection of postage stamps. Then the public could be educated and entertained just by seeing legendary foreign animals in the flesh: now that we have superb wildlife films people are surely becoming more sophisticated about animals, and realizing that cage-stupid isolated specimens are little better than stuffed animals, and that begging for food is the least entertaining behaviour that animals have in their repertoire.

The time has now come when for both entertainment and scientific value it would be more profitable for a zoo to specialize and concentrate on one species from any group—for the lay public, and indeed for most zoologists, the difference between, say, a chacma and an olive baboon is of little moment—but to use the space and resources saved to present that species as a flourishing breeding colony. In this way it might become possible to pay more than lip-service to the idea of breeding and maintaining vanishing species in captivity, which is the only excuse for having such animals as the orang in captivity at all. Co-operation between zoos could prevent overlap, so that each could become famous for its specialities and worth using the facility of modern transport to visit. Let us hope that this book will prod the imagination as well as the consciences of zoo directors, and help to make people dissatisfied with some of their present offerings—with the rare exception, the 'good' British zoos are not even up to the standards of the American and European.

For the orang itself, the most urgent need is for reserves in south-east Asia. These must be protected from poachers, of course; they must be large enough, because the population density of orangs seems to be about one per square mile; and the size of the area must be estimated in terms of useful orang habitat, which is forest at least eighty years old. A first park has been established in North Borneo, but on Mrs. Harrisson's estimate[4] the present boundaries include space for only fifteen adults. There is a conflict between the interests of the orang and those of timber merchants and traditional hunting rights; as usual, conservation is a political, not biological, problem. If nothing is done within the next few years, the conflict will cease to exist.

I found the arrangement of the book too chatty; orangs are interesting enough without padding (for example, a digression about turtle eggs) and I felt to be piecing together relevant information with difficulty. If, as must be the intention, this treatment attracts a wider audience, it is a worthwhile sacrifice. The photographs are delightful; I suspect that one of the orangs facing page 176 is a chimpanzee.

THELMA ROWELL

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[1] *Orang-Utan*. By Barbara Harrisson. Pp. 224+17 plates. (London: William Collins, Sons and Co., Ltd., 1962.) 25*s*. net.

[2] Harrisson, Barbara, *Sarawak Museum J.* (in the press).

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BOTANICAL EXPEDITION TO THE LIMESTONE AREAS OF ULU KELANTAN, MALAYA

By HSUAN KENG
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UNDER the sponsorship of the Unesco Science Co-operation Office in Djakarta, a botanical expedition to Ulu Kelantan, Malaya, was undertaken during July 24–August 17.

At a conference several years ago, Dr. E. J. H. Corner, former assistant director of the Singapore Botanic Gardens, directed attention to the fact that the present knowledge about the peculiar limestone flora in South-east Asia is very inadequate. This flora extends from south Thailand through Malaya to Borneo and Java.

The limestone hills in Malaya are widely distributed in the northern States. According to a recent report by Mr. J. R. Paton[1], they are concentrated in Ulu Kelantan. Furthermore, the limestone hills are less explored and less disturbed there. For these reasons, it was generally agreed that Ulu Kelantan was the most suitable place for the expedition.

Participants of this expedition included staff members from the Singapore Botanic Gardens, the Forest Research Institute of Malaya and the graduate students of the University of Singapore. The foreign participants came from neighbouring countries: Thailand, Indonesia, Hong Kong, and the Phillipines. Altogether the party consisted of sixteen members. The exploration was called a 'training expedition' because the participants were expected to gain knowledge and experience of botanical field work. Actually this was the third one of its kind sponsored by Unesco, the first being held in Java in 1960, the second in Sarawak in 1961.

The party spent three weeks in the field. By the end of this time the party had collected 691 (many with 4–6 duplicates) kinds of dried herbarium specimens and more than 200 kinds of living plants. The dried herbarium specimens, more than 3,200 sheets, are kept in the herbarium of the Singapore Botanical Gardens for future distribution to the leading herbaria of the World; the living plants, including a great variety of orchids and other plants, are being kept in the gardens for multiplication.

The only literature available about the Malayan Limestone flora is the paper written by Mr. M. R. Henderson[2]. In that paper, Mr. Henderson enumerated 745 species of seed plants which were found on the limestone hills throughout Malaya. Among these 745 species only 195, or approximately one-fourth, of them were confined to the limestone hills; the rest was found both on limestone and non-limestone hills.

From a preliminary examination of the present collections several interesting points emerge:

(1) Mr. Henderson listed 49 families which had not been found on Malayan limestone hills. However, among our collections from Ulu Kelantan, specimens belonging to Ranunculaceae, Caprifoliaceae, Hamamelidaceae and Bignoniaceae were found. These five were among Henderson's 49. Thus all of them are new records to the Malayan limestone flora. Incidentally, the first two families, Ranunculaceae and Caprifoliaceae, are represented in the collections by only single specimens of *Naravelia dasyoneura* Korth. and *Viburnum sambucinum* Bl. respectively. This means that they are extremely rare.

(2) Ferns and fern allies were excluded from Mr. Henderson's list. During this expedition, more than 90 species of ferns and fern allies were collected.

(3) Dipterocarpaceae, which are so characteristic of the tropical rain forests, are poorly developed in the forests on limestone hills[3]. According to Mr. Henderson's list, only three species, each representing a genus—*Vatica*, *Hopea* and *Pentacme*, were recorded. At the end of this expedition, 3 species of *Shorea* and 1 species of *Parashorea* were added to our knowledge of the limestone dipterocarp flora.

(4) Orchids are abundant, especially near the tops of limestone hills. About 100 species of orchids were collected during this expedition. A great variety of living specimens of orchids, plus ferns, begonias, aroids, gingers and others of ornamental value, were brought back. Most of them are growing well in the Singapore Botanic Gardens to-day. In this connexion, perhaps it is worth while to mention the fact that a large number of the beautiful herbaceous plants from the limestone hills have proved to be valuable garden plants in lowland Malaya. According to Mr. Tem Smitinand, a participant from Thailand, who identified all the orchid specimens, nearly one-third of orchids collected are new records of the Malayan limestone flora, and at least two of them are new species.

A list of the plant names of his collection will be compiled in due course.

[1] Paton, J. R., *Prof. Pap.* E-58.1.T. *Geolog. Surv., Malaya* (1962).

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[3] Richards, P. W., *The Tropical Rain Forest* (Camb. Univ. Press, 1952).

SUB-ACOUSTIC WAVES FROM RECENT NUCLEAR EXPLOSIONS

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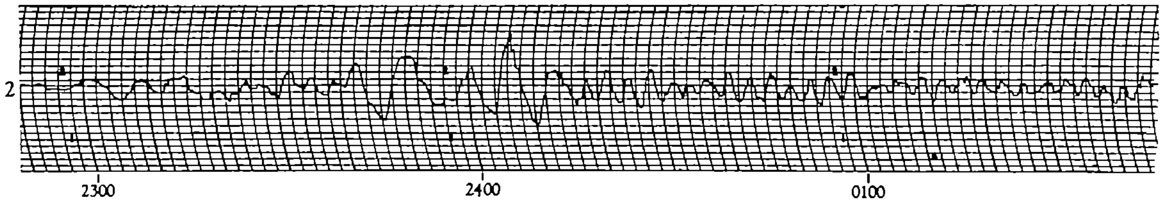
BETWEEN 2000 and 2100 G.M.T. on July 9, 1962, an unusual train of microbarographic waves was recorded in the Aberdeen area. The atmospheric pressure was otherwise uncommonly steady that evening; the normal degree of 'noise' in this area would have masked the wave-train, which had a peak amplitude of about 25 microbars (μb). Fig. 1 shows the appearance of the train, recorded on a microbarograph set to respond to periods between 10 sec and 5 min. The time of onset of the train is difficult to decide exactly; there is an indication of waves of period about 5 min and 8 μb amplitude arriving about 2000 G.M.T., followed at about 2020 G.M.T. by waves of period about 2·5 min and up to 25 μb amplitude. The tail of the train was lost in the noise by 2200 G.M.T.

We have previously seen wave-trains of this type only after violent explosions in the atmosphere; but then the initial longer period (4–6 min) waves have usually been of greater amplitude than the following 2–3 min-waves (see, for example, Jones[1]). Tentatively, we may examine the possibility that the July 9 wave-train originated in a nuclear explosion, perhaps with abnormal characteristics such as to



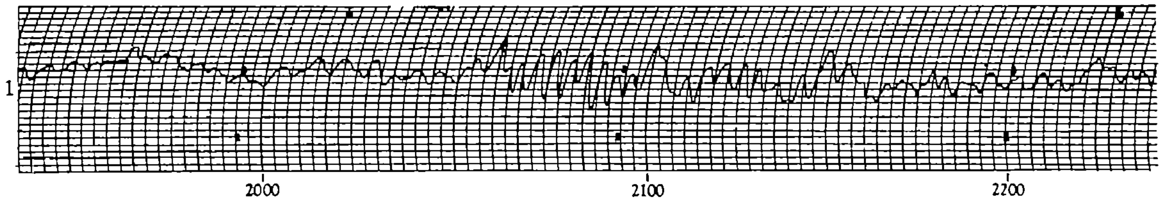


Fig. 1. Microbarograph record of July 9, 1962, at Aberdeen, showing wave-train appearing to have originated from 'Rainbow' explosion near Johnston Island at 0900 G.M.T. the same day. Numbered ordinates at 2000, 2100, 2200 G.M.T. Pressure scale, 1 division ~ 3 μb, for waves of period between 10 sec and 5 min

Fig. 2. Microbarograph record obtained on August 6 and 7, 1962, near Aberdeen of sub-acoustic wave-train from explosion near Novaya Zemlya on August 5, 1962. Wave-train was passing over Aberdeen for second time, after encircling Earth. Numbered ordinates at 2300 and 2400 G.M.T., August 6, 1962, and 0100 G.M.T., August 7, 1962. Pressure scale, one division ~ 15·5 μb, for waves of period between 15 sec and 7·5 min

moderate the initial violence of the disturbance. Earlier that day (0900 G.M.T.) the 'Rainbow' bomb[2] had been exploded at some hundreds of kilometres above sea-level near Johnston Island. The travel times for sub-acoustic waves arising from the explosion would give, on previous experience[3], expected times of arrival in Aberdeen of between 1912 and 1945 G.M.T. for waves of 5-min period, and between 1930 and 2020 G.M.T. for 2·5-min period. The times at which the waves were observed at Aberdeen are at the ends of these brackets, but the coincidence suggests the conclusion that the wave-train originated from the 'Rainbow' explosion.

It would thus appear that substantial energy was communicated from the explosion (stated to be in the megaton range) to the lower atmosphere, despite the fact that the explosion was nominally 'outside' the atmosphere. Presumably about half the explosive energy was radiated downwards, and the subsequent atmospheric disturbance was due to the partial absorption of this radiation. The comparative lateness of the wave-train may have been due in part to some feature of the process by which the pressure waves were developed.

The Russian explosion of August 5, 1962, gave rise, as did that of October 31, 1961 (ref. 1), to waves which could be traced on more than one circuit of Earth; the explosion is thought to have been of about 30 megaton TNT equivalent.

Times of passage at Aberdeen were as follows:

	G.M.T.		Peak amplitude
Direct wave	1148	5.8.62	> 500 μb
Antipodal wave	1734	6.8.62	150 μb
Direct wave (second transit)	2340	6.8.62	200 μb
Antipodal wave (second transit)	0542	8.8.62	80 μb
Direct wave (third transit)	1200	8.8.62	80 μb

These data give times of 35h 52m and 36h 20m for the completed direct circuits and 36h 08m for the completed antipodal circuit. These times are more uniform than those recorded here for the 1961 explosion.

Fig. 2 shows the record of the second passage of the direct wave, which had an unusually well-developed train lasting more than 2 h; there appears to have been strong dispersion. The record was obtained at the Loch of Skene, about seven miles west of Aberdeen, and our thanks are due to Lord Cowdray for allowing us the use of the Loch.

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LAKE VANDA: AN ANTARCTIC LAKE

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Lake Vanda as a Solar Energy Trap

LAKE VANDA is shown to be a natural example of the trapping and storing of solar energy by a salt water density gradient. The bottom of this Lake (218 ft.) is maintained at 25° C despite a mean annual air temperature of about −20° C, the solar heating being limited to the short antarctic summer.

The Lake (77° 35′ S., 161° 39′ E.) is permanently covered with about 12 ft. of ice and is 5 miles long and 1 mile wide. It occupies a depression in the lowest part of the Wright Dry Valley, Victoria Land, Antarctica. In the summer of 1960–61 an American biological party discovered that its bottom waters were warm and highly saline[1].

We examined the physics and chemistry of the Lake and the geology of the surrounding area during the first half of December 1961. This communication deals with the heat balance of the Lake.

Twelve holes were drilled at 1,000-ft. intervals along two lines, one along the length of the Lake and the other across its widest part. Water temperatures were measured to ± 0·05° C at 5-ft. intervals, using a copper–constantan thermocouple and a potentiometer. The thermocouple was checked against a reversing thermometer of the type used in oceanography. Density and chemical composition were measured at 5-ft. intervals through one hole in the central part of the Lake. The lower region of the Lake below 160 ft. is strongly density-stratified saline water which we consider to be non-convective. The *in situ* density increases from 1·007 g/cm³ at 160 ft. to 1·100 g/cm³ at 215 ft.

The temperature gradient in the soft mud at the Lake bottom was measured near the centre of the Lake by driving in a geothermal gradient probe. This probe consisted of 76 copper–constantan couples connected in series and mounted on a wooden rod with their junctions 25 cm apart. The probe was calibrated by placing it at the 170-ft. level in the Lake where the gradient was known from the thermal profile measurements. This calibration agreed with that calculated theoretically to within 10 per cent. 2 ft. below the bottom of the Lake the temperature was found to be decreasing downward with a gradient of 0·04° C per foot. Heat is thus being conducted downwards from the Lake. Assuming the thermal conductivity of the mud salt water mixture at the bottom of the Lake to be similar to that of water, the annual flow of heat would be about 50 cal/cm²/yr.

Bolometer measurements of the solar energy flux were made through five holes under a variety of cloud conditions and at depths up to 50 ft. below water-level.

The heat balance of the Lake presents some interesting problems. The temperature gradient at the 165-ft. level is 0·37° C/ft. This means that the non-convective lower part of the Lake is losing heat at 550 cal/cm²/yr. by conduction upwards. To this must be added the 50 cal/cm²/yr. that is being lost downward by conduction through the bottom of the Lake.

There are five possible heat sources: (1) biological activity within or at the base of the Lake; (2) chemical heating; (3) hot springs in the bottom of the Lake;

Table 1

Temperature versus depth profiles for lower part of Lake Vanda (°C)

Depth below water-level (ft.)	*1	2	2 N	2 S	3	4	*In situ* density (g/cm³) hole 2.
160	12·4	12·4	12·6	12·5	12·5	12·5	1·008
165	14·3	14·2	14·5	14·3	14·2	14·3	1·014
170	16·1	16·1	16·3	16·1	16·3	16·1	1·024
175	17·7	17·8	18·0	17·9	17·3	17·8	1·034
180	19·3	19·4	19·5	19·4	19·4	19·3	1·044
185	20·8	20·8	20·9	20·8	20·8	20·8	1·054
190	22·1	22·1	22·3		22·1	22·2	1·064
195	23·3	23·3	23·4		23·4	23·4	1·073
200	24·2	24·1	24·3		24·2	24·3	1·080
205	24·8	24·7	24·8		24·8	24·8	1·087
210		25·2	25·2		25·2	25·1	1·091
215		25·4	25·6				1·096
	Bottom 208 ft. 25·2° C	Bottom 216 ft. 25·6° C	Bottom 218 ft. 25·7° C	Bottom 187 ft. 21·4° C	Bottom 212 ft. 25·4° C	Bottom 213 ft. 25·1° C	

* Positions of holes 1, 2, 3, 4 are on an east–west line at 1,000-ft. intervals. 2 N and 2 S are at positions 1,000 ft. north and 1,000 ft. south respectively of position 2.

(4) abnormally high geothermal gradient under the Lake ; (5) radiant energy from the Sun penetrating into the Lake and being absorbed.

According to Zobell *et al.*[2], biological activity would not produce more than an extremely small part of the heat required even under the most favourable conditions.

Coring of the bottom yielded no solid soluble salts. The bottom waters of the Lake contain in solution calcium chloride with smaller amounts of sodium and potassium chlorides. Even if these salts did occur on the bottom of the Lake, consideration of their heats of solution[3] rules out any appreciable heating from this source. There is no evidence for any other chemical reactions that are producing appreciable heat.

There is no geological evidence for hot springs in the area surrounding the Lake. The temperature-gradient is positive at all depths in all profiles measured. In the lower half of the Lake the temperature versus depth profile is remarkably similar (± 0·1° C) over an area at least 3,000 ft. by 2,000 ft. (Table 1), whereas hot-spring areas are characteristically non-uniform in lakes.

The possibility of a high geothermal gradient is ruled out, since the temperature-gradient probe showed that heat is being conducted out through the bottom of the Lake. Other evidence is that the temperature gradient decreases from 160 ft. downward. This is not a salinity effect since the thermal conductivity of water decreases as the salt concentration increases[4], and implies that the flow of heat increases as one goes upwards.

Thus we are left with radiant energy from the Sun as the only possible source of heat. No data are available on the total annual radiant solar energy flux at Lake Vanda, but it is known[5] that the solar energy flux at Scott Base, 80 miles due east of Lake Vanda, is 91,000 cal/cm²/yr. The fraction of the incident solar energy which penetrates the 12 ft. of ice was determined with a bolometer. With a cloudless sky, 5 per cent, and with a cloudy sky, 8 per cent of the radiant energy flux enters the water below the ice. Measurements in the upper 50 ft. of the Lake showed that 45 ft. of water reduced this energy flux to a half. By adopting a 6 per cent value at the bottom of the ice and assuming that the water maintains the same attenuation value, the quantity of energy reaching the 165 ft.-level would be 550 cal/cm²/yr., which is in reasonable agreement with the 600 cal/cm²/yr. calculated above as the loss of heat from the Lake water below 165 ft.

As additional evidence of a solar heating mechanism, the temperature gradient below 160 ft. has been calculated using the above data and is compared with the observed data in Table 2.

Table 2

Depth below water-level (ft.)	Colour of water	Per cent of incident energy	Total energy flux (cal/cm²/yr.)	Calculated temperature gradient (°C/ft.)	Measured temperature gradient (°C/ft.)
165	colourless	0·6	550	0·33	0·37
170	,,	0·55	500	0·30	0·35
175	,,	0·51	465	0·28	0·33
180	,,	0·47	430	0·25	0·30
185	,,	0·44	400	0·23	0·27
190	,,	0·41	375	0·22	0·26
195	brown	0·38	350	0·20	0·20
200	,,	0·35	320	0·18	0·14
205	,,	0·32	290	0·16	0·10

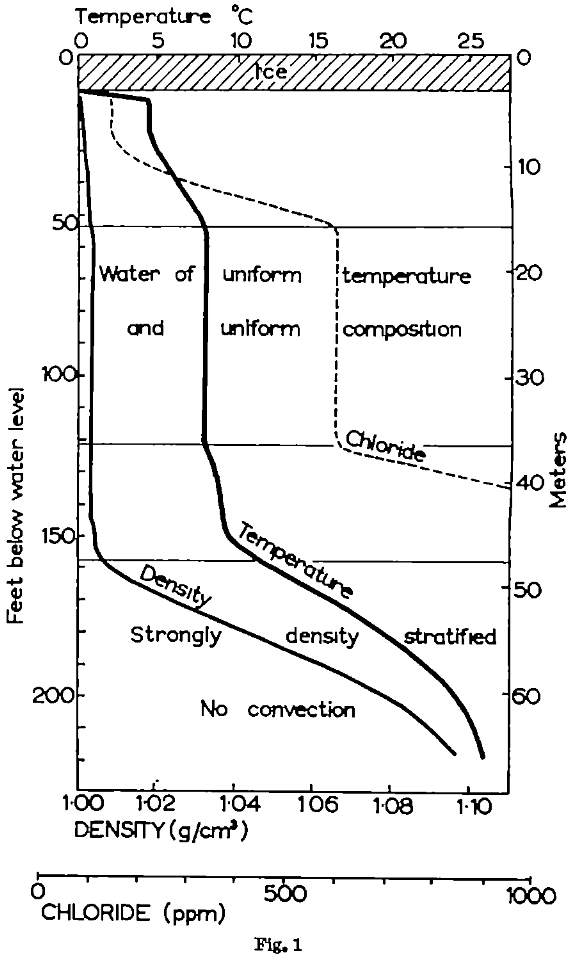

Fig. 1

It is seen that above 190 ft. the temperature-gradient versus depth profile is as would be expected from a solar heating mechanism. At 195 ft. the temperature-gradient falls off more rapidly than is predicted from theory, but it was observed while sampling that the water below 195 ft. is brown in colour.

It is interesting to speculate on the construction of similar installations for the trapping and storing of solar energy in the more temperate latitudes. The heat could then be used for the heating of buildings or for evaporation processes such as salt production from sea-water. Some experimentation along these lines has been carried out in Israel[6].

Ecology of Lake Vanda

During a recent investigation of the chemistry and heat balance of Lake Vanda, *Phormidium* and other blue-green algae were accidentally collected on a nylon line. The algae were collected only in a strongly convective layer. We wish to report this interesting ecological situation and also the novel manner by which these algae were collected.

Armitage and House[1], during a limnological survey in the summer of 1960–61, found that the water at the bottom of the Lake was at 22° C and strongly saline. They obtained only one organism, a *Chlorella*-like alga, from the filtration of more than 100 gallons of Lake water.

Our work in the summer of 1961–62 (previous section) made it likely that the bottom water is warmed by solar radiation. The bottom water is prevented from convecting by its strong density stratification.

The main layers in the Lake are shown by Fig. 1. The top layer is the 12 ft. of ice. The waters from 12 ft. to 55 ft. and from 125 ft. to 160 ft. have a salt gradient and are weakly density stratified. From 55 ft. to 125 ft. the temperature and chemical composition are uniform and this part is considered to be most strongly convective.

While making measurements on the heat balance of the Lake equipment was frequently suspended from nylon monofilaments. If these lines were allowed to remain in position for 24 h a red material was found to accumulate on that part of the line that had been between 55 ft. and 125 ft. This material froze as it came into the air and accumulated at the eyelet of the winding reel. A sample of this material was examined by the biological staff at McMurdo base and was identified as being principally *Phormidium* with a few *Chroocuccus*, and a few *Anabaena*. One live rotifer was present.

It appears that these algae live in a strongly convective layer isolated from the surface of the Lake (mean annual temperature, 20° C) by 50 ft. of colder less saline water and by 12 ft. of ice. Their environment is an almost uniform 7·8° C, their nutrient medium almost isotonic, with 600 p.p.m. Cl', 120 p.p.m. Na^+, 37 p.p.m. K^+, 14 m.equiv./l. Ca^{++}, 3 m.equiv./l. Mg^{++} and 1·3 m.equiv./l. HCO_3'; its available energy from sunlight is about 1,000 cal/cm^2/yr., mostly in the blue part of the spectrum and all during the summer months.

We thank David Mason, John Bunt and Dr. C. R. Goldman for identifying the micro-organisms, Mr. C. J. Banwell, and Mr. I. C. Donaldson and other members of the staff of the Department of Scientific and Industrial Research for helpful advice and for the loan of equipment; also Dr. Angino of the University of Kansas, the U.S. Navy and the U.S.A.R.P. personnel without whose co-operation the expedition would not have been possible.

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STRUCTURE OF LYSOZYME

A Fourier Map of the Electron Density at 6 Å Resolution obtained by X-ray Diffraction

The enzyme lysozyme, discovered by Fleming in 1922[1], is responsible for "the power of rapidly dissolving certain bacteria which is possessed by many animal and vegetable tissues and secretions, and to a very marked degree by egg-white". Intensive study by many workers[2–5] has shown that the enzyme attacks a mucopolysaccharide component of bacterial cell walls, liberating acetyl-amino sugars derived from glucosamine and muramic acid. The reaction appears to involve the breaking of a β-glycosidic linkage.

Chemical analysis of hen egg-white lysozyme has shown that the molecule consists of a single polypeptide chain of about 129 amino-acid residues, cross-linked by four disulphide bridges. The amino-acid sequence is known in some detail but the positions of the disulphide bridges have not yet been determined[6,7].

We have now begun a detailed X-ray crystallographic investigation of the structure of hen egg-white lysozyme which we hope will lead to a knowledge of the molecular configuration; this would be of especial interest in connexion with the mechanism of cell lysis and the structure of cell walls. The first result, which is described here, is a three-dimensional electron-density map at 6 Å resolution of lysozyme in the tetragonal crystal form.

X-ray methods. Earlier crystallographic investigations[8–11] have shown that egg-white lysozyme can be crystallized in several crystal forms as the salts of mineral acids. Crystals of lysozyme chloride grown at *p*H 4·7 (ref. 8) are tetragonal with cell dimensions $a = b = 79{\cdot}1$, $c = 37{\cdot}9$ Å and space group $P4_12_12$ or $P4_32_12$. Each unit cell contains eight lysozyme molecules (one per asymmetric unit), molecular weight about 14,600, together with 1 M sodium chloride solution which contributes about 33·5 per cent of the weight of the crystal[10].

The structure has been determined at 6 Å resolution by the method of isomorphous replacement which was used successfully in the study of myoglobin[12,13] and hæmoglobin[14]. Several types of heavy-atom derivatives have been prepared[15] of which three have been used in the present investigation, those containing: (1) mercuri-iodide; (2) chloro-palladite, $PdCl_4$;

Table 1. HEAVY-ATOM PARAMETERS

			Mercuri-iodide			
	$PdCl_4$	MHTS	Crystal 1*		Crystal 2†	
X	0·147	0·218	0·131	0·178	0·125	0·178
Y	0·841	0·620	0·869	0·822	0·875	0·822
Z	0·963	0·054	0·250	0·250	0·250	0·250
N	72	47	74	74	72	72
B	52	−18	38	38	111	111
E	57	66	66		55	
R	35%	49%	42%		39%	
R'	10·7%	11·6%	11·6%		12·2%	

N, occupancy of heavy-atom site—electrons; B, isotropic temperature-factor constant—A^2; E, average difference between observed and calculated heavy-atom changes of centric reflexions—electrons; R, 'reliability index' for observed and calculated heavy-atom changes of centric reflexions; R', Kraut agreement indexes (ref. 25) for all reflexions.

* Centric 0,1,2*kl* reflexions only.
† *hk*0 reflexions only.

(3) *ortho*-mercurihydroxytoluene *para*-sulphonic acid (MHTS).

The positions of each of the heavy atoms were found independently from the centrosymmetric projections by using Patterson and Fourier difference syntheses in the standard way. Determination of the relative positions of the heavy-atoms was facilitated by the fact that in this space-group the projections on (001), (100) and (110) are all centrosymmetric. As a result 173 of the 393 reflexions from planes with spacings ⩾6 Å have heavy-atom contributions exactly in or exactly out of phase with the protein contributions to the structure factors. Hart's method[16] was used with these centric reflexions to refine the heavy-atom parameters, the final values of which are shown in Table 1. The absolute scale of intensities is approximate.

The MHTS and $PdCl_4$ are at single sites in general positions. The mercuri–iodide complex, however, is in a special position on a two-fold rotation axis and at this resolution the group was found to be best represented by two isotropic 'atoms' lying on the axis at a distance of 5·6 Å apart. Use of the mercuri–iodide derivative was further complicated by the fact that the heavy-atom content diminished during exposure to X-rays. Measurements from the two crystals of this derivative were therefore treated separately (with appropriate weighting) and their site occupancies were made a function of exposure time in the phase calculations. There were no significant indications that the mercuri–iodide migrated to another discrete site.

Three-dimensional data for two crystals of each type were measured by means of the linear diffractometer[17] and all the data processing and phase calculations were made on a Ferranti *Mercury* computer with specially developed programmes. The phases of the reflexions were determined systematically by the phase-probability method[18,19]. The differences in intensity between reflexions *hkl* and *khl* from the heavy-atom derivatives, which are due to anomalous dispersion, were taken into account in these phase calculations[20] and were also used to distinguish between the enantiomorphous space groups. This analysis has shown that the space group is $P4_32_12$, that is, that the four-fold screw axis is left handed.

The quality of the phase determination is indicated by the measure of agreement between the angles indicated by the different heavy-atom derivatives taken separately. For the acentric reflexions the mean 'figure of merit'[18,19] is 0·79, a value which compares favourably with that obtained in the study of hæmoglobin[14]. The mean 'figure of merit' for the centric reflexions, on the other hand, is 0·95, so that the overall value is 0·86. A check on the sign determinations for the centric reflexions is obtained by direct comparison of the three separate sign predictions. These are compared in Table 2, again with satisfactory results.

Table 2. SIGN DETERMINATION FOR CENTRIC REFLEXIONS

Heavy atom	$PdCl_4$	MHTS	Mercuri-iodide
Predictions agree with 'best' signs (ref. 18)	144	129	102
Predictions disagree significantly*	6	10	12

* A prediction is classed as significant when the calculated heavy-atom contribution ⩾ E (cf. Table 1).

Electron-density distribution. The electron-density distribution was calculated on *Mercury* with structure amplitudes weighted by the figures of merit so as to give the 'best' Fourier[18]. The map has been plotted on a stack of 'Perspex' sheets in the usual way, and a photograph of it is shown in Fig. 1.

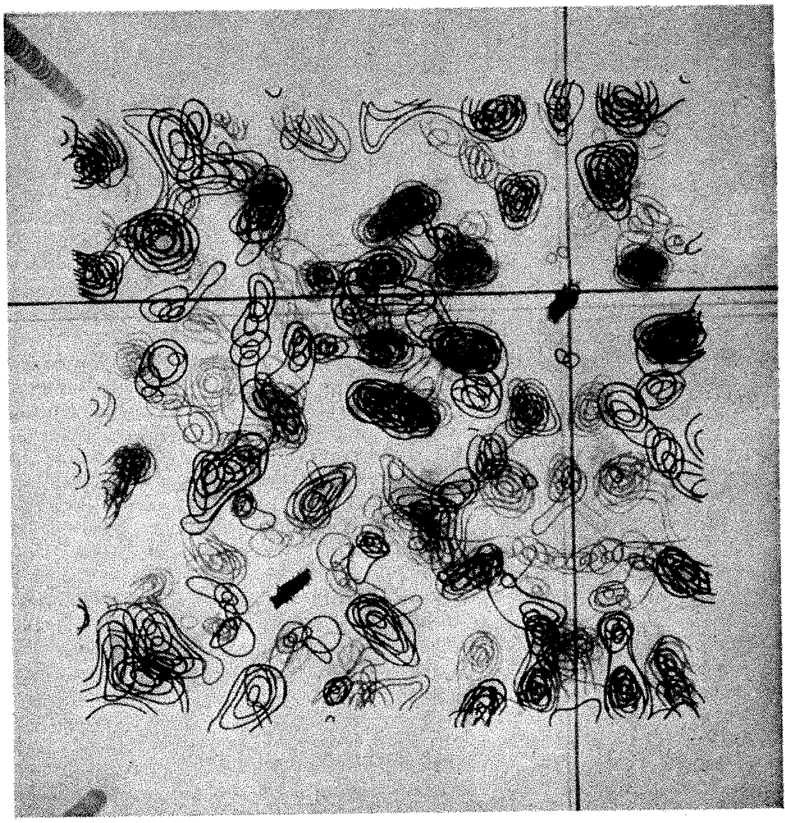

Fig. 1. Electron density distribution in lysozyme, view from above the stack of 'Perspex' sheets, that is, parallel to *c*. The horizontal and vertical lines represent the two-fold rotation axes and intersect the two-fold screw axis, upper right of centre. The four-fold screw axis is at the lower left of centre. The contour interval is 0·07 electron/Å^3, the lowest heavy contour being at 0·6 electron/Å^3. The absolute scale is approximate

Our first object in studying this map was to determine the boundary of a single molecule. Comparison of the unit-cells of various crystal forms of lysozyme[10] suggests that in tegragonal lysozyme the molecule occupies the full length of the c-axis and, on average, one-eighth of the area of the ab-plane. On this basis, the map of Fig. 1 includes the whole of the c-axis and a sufficient area of each section to ensure that one whole molecule is included in addition to parts of neighbouring molecules. Only contours indicating where the electron density is greater than average are included.

Some featureless regions of average electron density (0·4 electron/Å^{-3}), probably occupied by liquid between molecules, are immediately apparent. The most marked of these is around the two-fold screw axis parallel to c. This axis is intersected at intervals of 9·5 Å by two-fold rotation axes, and packing considerations, therefore, make it impossible for substantial portions of the molecules to penetrate into the neighbourhood. It is encouraging to find it so free from detail. Similarly, the immediate vicinity of the four-fold screw axis also is void of significant features and must be a region of intermolecular space. The two-fold rotation axes help to determine the boundary, particularly where relatively high density approaches or intersects them as it does at two places. Such regions must represent close contact or bridges between adjacent molecules.

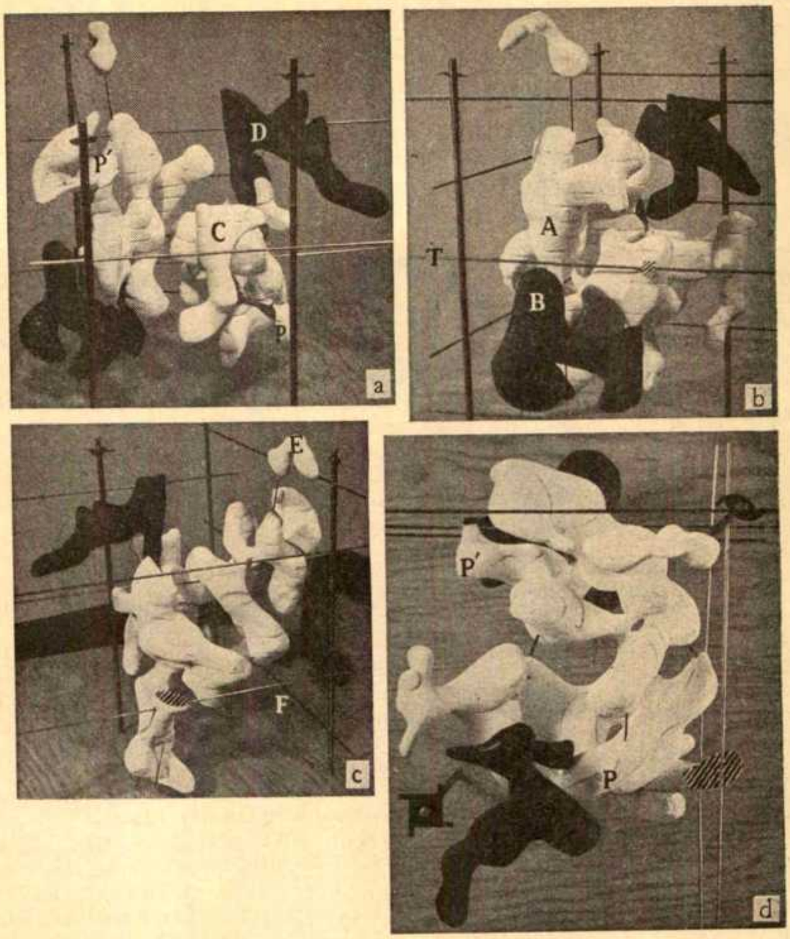

Fig. 2. Several views of a model of the regions in which the electron density exceeds about 0·53 electron/Å^3. The vertical rods indicate the two-fold and four-fold screw axes parallel to c. The thinner horizontal rods are two-fold rotation axes. At two places, shown hatched, rotation axes pass through continuous regions of density. The white regions alone make up the most compact asymmetric unit of the structure. In Fig. 2b, the grey section (B) is alternative to the region (A) to which it is related by the two-fold axis T. In Fig. 2a, the grey section (D) is alternative to the white (C), related by the four-fold screw axis. The grey piece (F) in Fig. 2c is alternative to the white E, to which it is related by a unit cell translation along c. The scale is indicated by the framework of symmetry elements, adjacent parallel two-fold axes being 18·95 Å apart

Some regions remain, however, in which the boundary of a single molecule cannot be decided unequivocally. The most serious difficulties are illustrated by the model shown in Fig. 2, which represents the volume occupied by density greater than about 0·53 electron/Å^3. One asymmetric unit is shown in white with additional pieces in grey to show alternative shapes. The white pieces together make up the most compact asymmetric unit, which is roughly ellipsoidal in shape with axes 52 × 32 × 26 Å. This asymmetric unit may also represent a single molecule, but we cannot be certain that it does and the alternatives include at least one major variation. Fig. 2a and d show that there is a region of low density dividing the model roughly into two halves. These parts could in fact be assembled differently, following the crystal symmetry, so as to form a dumb-bell-shaped molecule connected at PP'. This seems rather an improbable shape, but the possibility cannot be entirely ignored.

Our second object is to determine so far as possible the course of the polypeptide chain and the positions of the disulphide bridges, that is, the tertiary structure, and to correlate our result with what is known of the primary structure[6,7]. This has proved to be difficult if not impossible at this stage. In comparison with the maps of myoglobin[12] and hæmoglobin[14] at this resolution it is apparent that our map of lysozyme has a much smaller proportion of clear-cut rod-like features representing helical configurations of the polypeptide chain. This is not surprising since optical-rotatory-dispersion measurements[21] and other X-ray evidence[22] suggest that only 30–40 per cent of the polypeptide chain in lysozyme is in the form of α-helix as compared with 77 per cent in myoglobin. Tracing the course of the chain, which was not completely straightforward in myoglobin[12], is therefore much more difficult here since non-helical portions of chain appear at much lower density and are often not easily discernible. The task is further complicated by the presence of the disulphide bridges, which are expected to have about the same electron density as α-helix at this resolution and which are bound to make the chain appear branched. In fact there are some branches in the density which are suggestive of disulphide bridges and there is sufficient continuity to set close limits on the possible configurations of the polypeptide chain. We consider, however, that it would be misleading to discuss these possibilities further or to suggest a tertiary structure until we have determined the molecular boundary with more certainty, by increasing the resolution of our analysis and by comparing our results with those obtained by other workers.

An X-ray crystallographic study of tetragonal lysozyme chloride crystals containing complex ions of niobium and tantalum is in progress at the California Institute of Technology[23] and through the courtesy of Prof. Corey *et al.* we have been able to compare their results with our own. In this comparison allowance must be made for the facts that: (1) the niobium-containing crystals are not quite isomorphous with ours, at least the orientation of the lysozyme molecules has been changed by the inclusion of the heavy-atom group; (2) the Californian analysis is at 5 Å rather than 6 Å resolution and is based in effect on one isomorphous heavy-atom derivative; (3) the map (Fig. 1 in the following article) has been plotted in the space group $P4_12_12$. When allowance is made for these differences the two maps are found to be in satisfactory agreement with many features in common.

An examination of triclinic lysozyme nitrate is in progress at the University of Illinois and through the courtesy of Drs. Dickerson and Steinrauf[24] we are also comparing their map with ours. The results of both these comparisons will be described in detail elsewhere.

We thank Sir Lawrence Bragg and Prof. R. King for their interest and encouragement; the Director of the University of London Computer Unit for computing facilities on *Mercury*; and Mrs. W. Browne, Mrs. A. Hartley, and Miss D. Glass for their assistance with data collection and computation. We also thank the U.S. National Institutes of Health for financial assistance.

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An X-ray Investigation of Lysozyme Chloride Crystals containing Complex Ions of Niobium and Tantalum : Three-dimensional Fourier Plot obtained from Data extending to a Minimum Spacing of 5 Å

THE first X-ray investigations of egg-white lysozyme chloride were made by Palmer[1,2] in 1948. He reported that both wet and air-dried crystals are tetragonal, space group $P4_12_1$ or $P4_32_1$, with eight molecules per unit cell, one per asymmetric unit; $a = 79{\cdot}1$, $c = 37{\cdot}9$ Å (wet); $a = 71{\cdot}2$, $c = 31{\cdot}4$ Å (dry). A later investigation[3] of the air-dried crystals provided no information about the structure of the molecule. More recently, we have been engaged in an examination of the structure of lysozyme chloride by use of isomorphous series of crystals containing heavy ions; a preliminary report of this work is in print[4]. An investigation of lysozyme chloride is also in progress in the Davy Faraday Laboratory of the Royal Institution[5], and crystals of triclinic lysozyme nitrate are being investigated at the University of Illinois[6,7].

So far, our work has been based on crystals containing the complex ions of tantalum and niobium $Ta_6Cl_{12}^{++}$ and $Nb_6Cl_{12}^{++}$. These crystals (hereafter referred to as LTa and LNb) are highly isomorphous as shown by careful measurements of their unit cell dimensions, which, however, differ significantly (1–3 per cent) from those of lysozyme chloride alone. Calculations based on accurate measurements of the densities of these crystals, and confirmed by chemical analyses, indicate that they contain eight complex ions per unit cell, or one per lysozyme molecule. Successive two-dimensional and three-dimensional difference Patterson maps calculated from data extending to a minimum spacing of 5 Å served to determine the positions of the centres of the complex ions. Calculations based on these co-ordinates were used in the adjustment of the values of F_{obs} to the absolute scale. For the 186 centrosymmetric $hk0$ and $h0l$ reflexions corresponding to a minimum spacing of 5 Å, the discrepancy factor R is 0·26 where:

$$R = \frac{\Sigma \big|\, |\Delta F|_{\text{obs}} - |\Delta F|_{\text{calc}} \,\big|}{\Sigma |\Delta F|_{\text{obs}}}$$

and:

$$|\Delta F|_{\text{obs}} = |F_{\text{LTa}} \pm F_{\text{LNb}}|$$

$$|\Delta F|_{\text{calc}} = |F_{\text{Ta}}| - |F_{\text{Nb}}|$$

Bearing in mind that the terms ΔF_{obs} are differences between observed quantities, the small value of this factor gives strong support to the conclusions that the crystals are indeed isomorphous and that the centres of the ions have been correctly located. Corresponding attempts to adjust the scale of the lysozyme hydrochloride data to those of the crystals

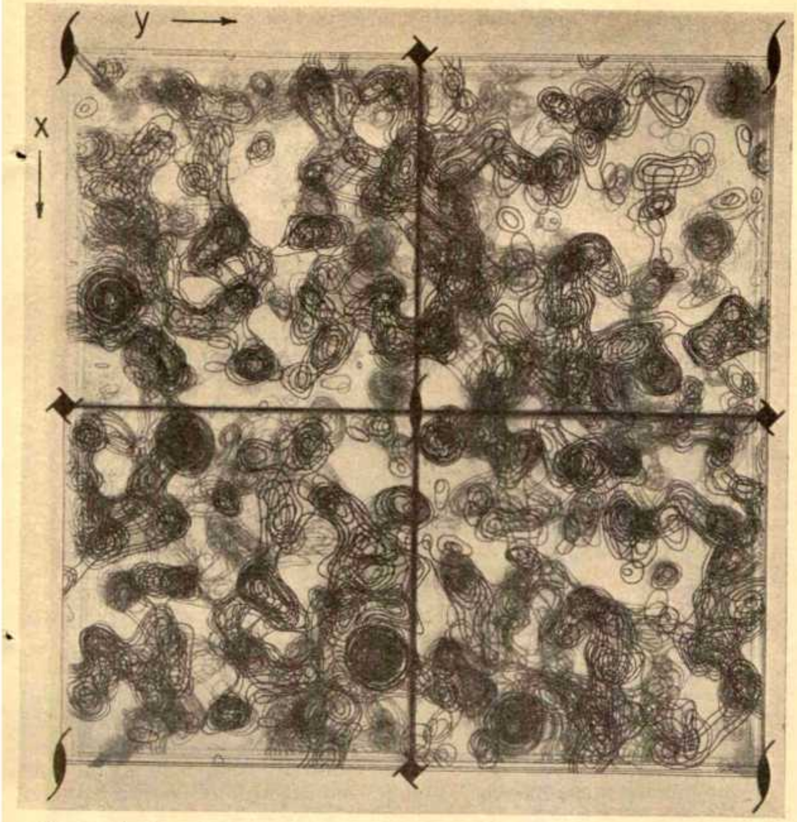

Fig. 1. A view of a portion ($z=0$ to 1/2) of the three-dimensional weighted Fourier diagram of lysozyme–Ta_6Cl_{14} calculated with the phase angles of the Ta_6 octahedron. Contours are drawn at equal but arbitrary intervals. The top layer corresponds to $z=0{\cdot}50$

containing the complex ions were unsuccessful, indicating that the complex ions cause a significant spatial alteration in the protein molecule. Two-dimensional Fourier projections[4] of electron density on to 010 and 001 of LTa crystals failed to provide information about the protein molecule.

We have now extended this investigation by the calculation of two three-dimensional Fourier syntheses of electron density based on 625 *hkl* reflexions ($d_{\min} = 5$ Å) from crystals of LTa and LNb. In the complex ions the atoms of tantalum and niobium are arranged at the six corners of a regular octahedron[8], and it has been shown[9] that, for reflexions extending to a minimum spacing of 5 Å, the combined scattering by these atoms is practically independent of the orientation of the octahedron. An arbitrary orientation was therefore assumed for purposes of calculation. The scattering powers of tantalum and niobium, which were taken from the *International Tables*[10], were corrected for the real part of the anomalous dispersion[11]. An isotropic temperature factor $B = 30{\cdot}6$ was also applied. The positions of the complex ions were used to calculate the quantities:

$$\Delta F_c = F_c(\mathrm{Ta}) - F_c(\mathrm{Nb})$$

and:

$$A_c(\mathrm{Ta\text{–}Nb}),\ B_c(\mathrm{Ta\text{–}Nb})$$

which represent the amplitudes and phase angles, respectively, of the vector differences between the contributions of the tantalum and niobium atoms.

In our first three-dimensional Fourier synthesis, the quantities $A_c(\mathrm{Ta\text{–}Nb})$ and $B_c(\mathrm{Ta\text{–}Nb})$ which, of course, were also equivalent to the phase angles of the $Ta_6Cl_{12}^{++}$ contribution, were applied directly to the scaled absolute values $|F_0(\mathrm{LTa})|$ of the amplitudes of reflexions from crystals of LTa. Thus, in these calculations:

$$A_0(\mathrm{LTa}) = \frac{A_c(\mathrm{Ta\text{–}Nb})}{|\Delta F_c|} \times |F_0(\mathrm{LTa})|$$

and:

$$B_0(\mathrm{LTa}) = \frac{B_c(\mathrm{Ta\text{–}Nb})}{|\Delta F_c|} \times |F_0(\mathrm{LTa})|$$

The 625 *hkl* reflexions with minimum spacing of 5 Å included 104 *hk*0, 82 *h*0*l*, and 62 *hhl* for which the phase angles are 0, $\pm\pi/4$, $\pm\pi/2$, $\pm 3\pi/4$, or π, and hence could be quite unambiguously assigned by inspection of the intensity data.

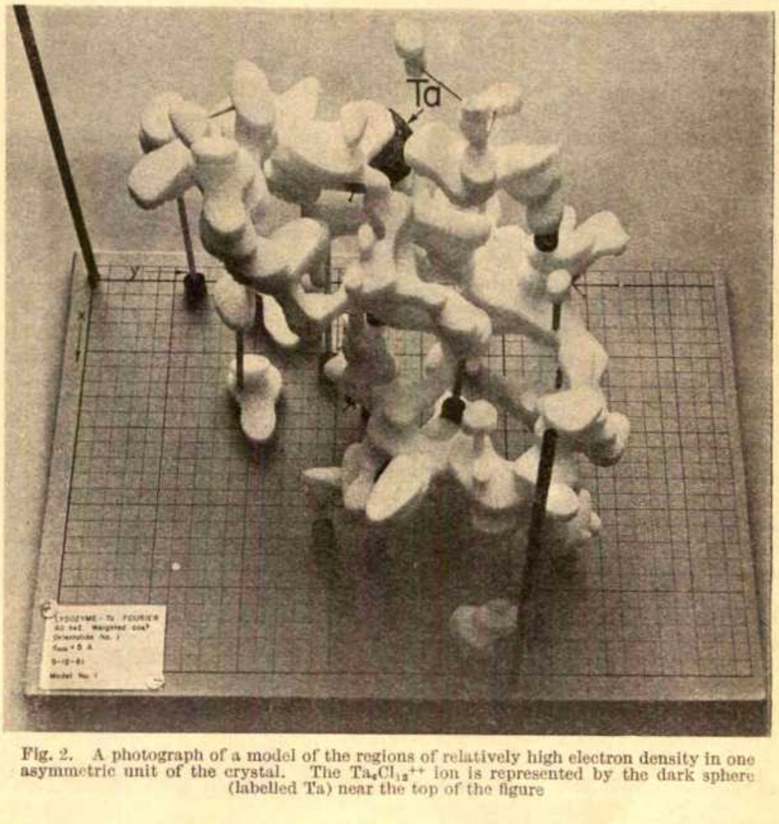

Fig. 2. A photograph of a model of the regions of relatively high electron density in one asymmetric unit of the crystal. The $Ta_6Cl_{12}^{++}$ ion is represented by the dark sphere (labelled Ta) near the top of the figure

For the remaining 377 reflexions, or 60 per cent of the data, the choice of phase angle rested between two ambiguous values. It is to this last class of reflexions that we have applied the phase angles which were derived from the positions of the heavy atoms and were therefore intermediate between the ambiguous values.

In spite of these approximations in the phase angles, the plot of this Fourier contained interesting features which appear to have some significance. The $Ta_6Cl_{12}^{++}$ ion was roughly spherical, and the contours throughout the remaining portions of the plot showed continuous regions of high density which suggested portions of a polypeptide chain, possibly in the α-helix configuration.

The second three-dimensional Fourier was calculated in an attempt to reduce the effects of the errors in phase angles present in the first Fourier. In this calculation the values of A_0(LTa) and B_0(LTa) were weighted with a factor $\cos^3 \varphi$ in which φ is the difference between the phase angle of the heavy-ion contribution and the ambiguous phase angles of the total lysozyme–$Ta_6Cl_{12}^{++}$ complex. From the terms A_c(Ta–Nb), B_c(Ta–Nb), and $|\Delta F_c|$ defined here, and the observational results from the two types of crystals, $|F_0(\text{LTa})|$ and $|F_0(\text{LNb})|$, the ambiguous phase angles of the reflexions from LTa and LNb crystals were calculated; from these the values of φ and $\cos^3 \varphi$ were computed. The effect of this weighting factor, $\cos^3 \varphi$, is to compensate in some degree for the errors inherent in the first Fourier by giving relatively high weight to those reflexions for which the true phase angle differs little from the assumed phase angle and causing the weight to fall off rapidly as this difference increases.

In the calculation of both Fourier syntheses the F_{000} terms were omitted, and in the plots the contours were drawn at equal but arbitrary intervals. The plot of the weighted Fourier (Fig. 1) is conspicuously clearer than that of the unweighted one. The $Ta_6Cl_{12}^{++}$ ion is very close to spherical, although slightly flattened in the direction of the c axis. The continuous regions of high density in other portions of the plot are also better defined, and more suggestive of coiling or folding of polypeptide chains than are those in the unweighted plot. A photograph of a portion (z = 0 to 1/2) of the plot of the weighted three-dimensional Fourier is shown in Fig. 1. The positions of the complex ions are clearly shown as spherical groups of contours near the four-fold screw axes at the bottom and left side of the figure.

Fig. 2 is a photograph of a 'Styrofoam' model of the regions of relatively high density in one asymmetric unit selected with a view to represent the volume perhaps occupied by a single molecule of lysozyme. The position of the $Ta_6Cl_{12}^{++}$ ion is shown by the dark sphere, Ta, near the top of the figure. Although the presence of continuous regions of high electron density is strongly suggestive of a polypeptide chain in a folded configuration, the analysis must be carried much further before this interpretation can be considered to be established.

We thank Mr. Robert E. Long and Mr. Harold D. Nathan for assistance with the calculations and to Miss Lillian Casler for the preparation of the three-dimensional Fourier models.

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A 6 Å Model of Triclinic Lysozyme

WE have recently calculated in this laboratory a three-dimensional structure of triclinic lysozyme nitrate to a resolution of 6 Å, using three isomorphous heavy atom derivatives: $HgI_4^{=}$, $HgBr_4^{=}$ and $PtCl_6^{=}$. The space group is $P1$ with one molecule per unit cell, and there were found to be five, six and six heavy atom occupancy sites per molecule respectively for this crystal form. The mean figure of merit from the centroid phase determination is 0·85, and the mean lack-of-closure error of the phase triangles or 'R factor' as defined by Kraut[1] is 6–7 per cent for each derivative.

This is the first time that the multiple isomorphous replacement method has been tried with structures having no centrosymmetric projections and with multiple-site derivatives of this nature. Two questions of fundamental importance have been raised about such an analysis: (*a*) To what extent can one be sure of obtaining the correct heavy atom positions from three-dimensional $(\Delta F)^2$-type approximate Patterson functions in the absence of centrosymmetric projections to serve as a check ?

(*b*) With the large number of sites per molecule and hence with a large number of parametric degrees of freedom, can one exclude the possibility of falling into a false structure which would still produce reasonable phase circles and a high figure of merit ? As part of a programme to test these points, work is now in progress which will lead to a redetermination of the structure using the most favourable new derivatives selected from a screening of about two dozen. It is hoped that this work will resolve questions concerning both the structure and the validity of the methods used.

A complete account of this structure will be presented at a later date.

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MORPHOLOGY AND DEVELOPMENT OF RESPIRATORY SYNCYTIAL VIRUS IN CELL CULTURES

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RESPIRATORY syncytial (RS) virus was first described under the name of 'chimpanzee coryza agent' by Morris *et al.*[1] in 1956. Later, it was isolated from infants with severe respiratory infections[2], and its importance in the ætiology of human respiratory illnesses is firmly established[3,4]. The virus is ether-sensitive and its size has been estimated as roughly 90–130 mμ[2]. It multiplies in cultured cells causing the formation of syncytial masses with eosinophilic cytoplasmic inclusions, and recently it was suggested that this virus may be included among the larger myxoviruses[5]. This view derives some support from observations we have made on the fine structure of infected epithelial cells.

HeLa cell cultures were maintained and used for virus propagation as previously described[6]. Tube cultures containing 50,000–100,000 cells were inoculated with approximately $10^{4 \cdot 5} TCD_{50}$ of the Long strain of RS virus and incubated at 37° C without rotation. Visible cytopathic changes followed the pattern of earlier descriptions: multinucleate cells were detectable after 24 h, and by 48 h the syncytia were large and numerous. The cell sheets then rapidly disintegrated. Cultures inoculated 46 h previously were selected, with uninoculated controls, for cytological study by light and electron microscopy. Coverslip monolayers were prepared for phase-contrast microscopy by the method of OsO_4-fixation and ethanol reduction[7], and were mounted in water. Feulgen and periodic acid–Schiff techniques were carried out after osmium-fixation, and Bouin-fixed cultures were stained with hæmatoxylin and eosin.

Phase-contrast microscopy showed a substantial area of the inoculated monolayers consisting of large syncytia, each with 30 or more nuclei deployed in annular fashion or in clumps towards the centre of the cell mass. There were many multinucleate forms of smaller sizes and also a number of persisting individual cells with only one nucleus. The single cells were usually spindle shaped and provided with elaborately branching cytoplasmic processes. Rounded cytoplasmic inclusions, measuring up to 10μ across but of only moderate optical density, were present in great numbers throughout the cultures. After fixation in Bouin's fluid the spindle cells were mostly retracted, and typical shrinkage spaces had formed around the inclusions. The latter stained with eosin but were Feulgen and periodic acid–Schiff-negative.

For electron microscopy cultures were fixed with Palade's 1 per cent OsO_4 solution at *p*H 7·4, and embedded as loose pellets in methacrylate or in 'Vestopal *W*'. Ultra-thin sections were stained with uranyl acetate or lead hydroxide and examined with Philips *EM* 75*B* and Siemens *UM* 100 electron microscopes. Abnormalities of fine structure found regularly in electron micrographs of the inoculated cultures can be summarized under three headings: (*a*) the cell wall; (*b*) cytoplasmic inclusions; (*c*) the cell nuclei.

The cell wall. Spindle cells and the smaller syncytia presented an abnormal degree of folding and irregularity of the cell membrane. Sections revealed numerous extracellular particles which were virus-like in their size, morphology and electron density (Fig. 1). Close inspection of the particle aggregates demonstrated not only circular profiles but many that were elongated. A typical round form is shown in Fig. 2*a*. It consists of a dense central core about 20 mμ in diameter enclosed within a zone of relatively low density; this is surrounded by a dense outer ring some 15 mμ in thickness, giving a total particle diameter of about 65 mμ. Sometimes the core had an irregular outline or appeared as two or three separate parts (Fig. 2*b*). Certain profiles gave the impression of a diffuse layer external to the dense ring.

The most elongated profiles were clearly filamentous structures cut longitudinally, and the others were attributable to oblique sectioning of similar forms (Figs. 1 and 2*c*). Filaments up to 2·5μ in length could be measured; but maximum lengths could not be determined by inspection of random sections.

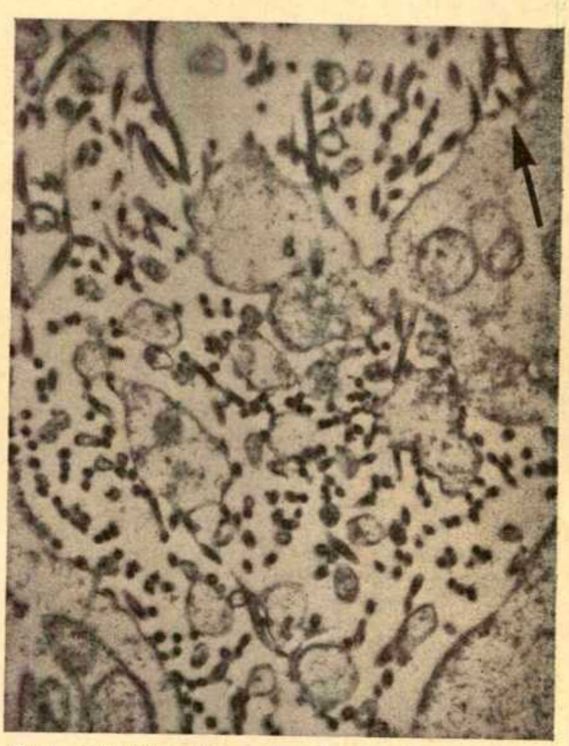

Fig. 1. Virus-like particles at the cell surface 46 h after inoculation. Some elongated profiles (arrow) are continuous with the cell membrane. (× 14,000)

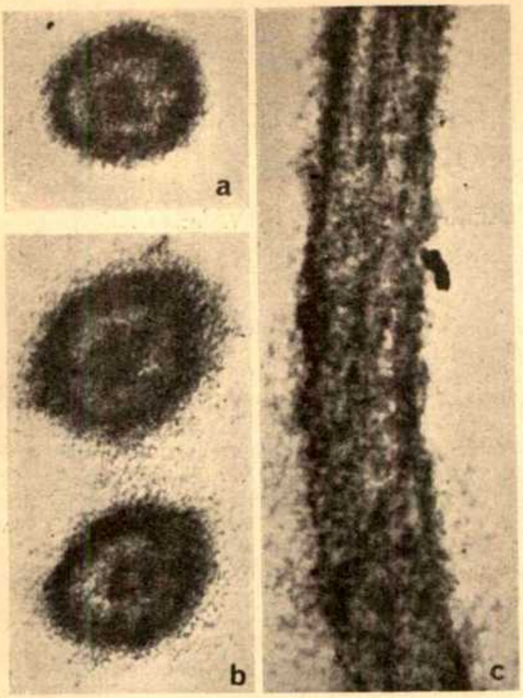

Fig. 2. Sectioned particles at high magnification: *a* and *b*, circular profiles; *c*, a filament. (× 300,000)

Some had a vesicular swelling at, or near to, their distal extremities. Proximally, continuity of the filaments with the host cell membrane was frequently revealed. There was no difficulty in distinguishing the filaments from microvilli. Having a uniform width of between 60 and 70 mμ, they were bounded by a layer of exceptionally high density. Each contained a central thread-like element, usually forming a dense core about 20 mμ thick but at some points appearing to consist of separate strands or even to be tubular. Transection of filaments would naturally account for at least some of the circular profiles in the micrographs; indeed, we are forced to conclude that truly spherical particles amounted, at the most, to a minority of those actually seen.

The number of associated virus-like particles noticeably diminished with increasing size of the syncytia examined, the largest examples of which had smooth and particle-free surface membranes.

Cytoplasmic inclusions. These consisted of masses of abnormal material up to several microns in diameter, with no limiting membrane. Their distribution approximated to that of the eosinophilic bodies seen by light microscopy, but at the fine structural level they were evidently of two types. One was invariably paranuclear and comprised a fine fibrillar matrix, while the other type was more widespread, more sharply demarcated from the surrounding cytoplasm, and composed of dense material of an amorphous or granular nature (Fig. 3). Mononuclear spindle cells had only occasional inclusions, of the fibrillar type, whereas the syncytia contained a profusion of both types of inclusion. Virus-like particles were never seen in the inclusions or elsewhere within the cells.

The cell nuclei. Large and bizarre nucleoli were a feature of the inoculated cultures. Well-defined dense areas in the nucleoplasm amounting to veritable inclusions were also present, but these occurred only in the largest syncytia (Fig. 4). That the inclusions were not merely manifestations of nuclear degeneration was evident from their uniformity and the intact condition of the nuclei concerned. The inclusions were always in contact with some part of the nuclear envelope, yet at the same time related closely to the nucleolus. At high magnifications they were seen to consist of a network similar to that of the fibrillar cytoplasmic inclusions. Patches of this nature were also located in small cavities within the nucleoli, suggesting a possible nucleolar origin for the inclusion material.

It would appear from these results that RS virus, at least in some circumstances, exists in the form of filaments measuring about 65 mμ across and developing at the host cell surface in the manner of myxoviruses. Supporting evidence was obtained by electron microscopy of disrupted cells, using the phosphotungstate negative contrast method of Horne and Nagington[8]. Filaments were observed measuring 50 mμ across and up to 2μ in length; they possessed an outer layer of projecting 'spikes' similar to, but rather longer than, those characterizing the myxoviruses. The diameter of RS virus particles as measured by electron microscopy is somewhat less than the original estimate (90–130 mμ) derived from sucrose density sedimentation data[2]. In dealing with a population that is largely filamentous in form it is inevitably difficult, in sections, to identify truly spherical particles with certainty; thus the occurrence of RS virus in the form of both filaments and spheres, as with certain strains of influenza virus, is not excluded by the present observations.

The cell cultures exhibited a progression of cytological alterations, from occasional normal cells to the largest syncytia, on the basis of which the following cytopathic sequence is proposed: (1) New virus particles emerge from the surface of individual spindle cells, inside which the only obvious changes are nucleolar enlargement and occasional fibrillar cyto-

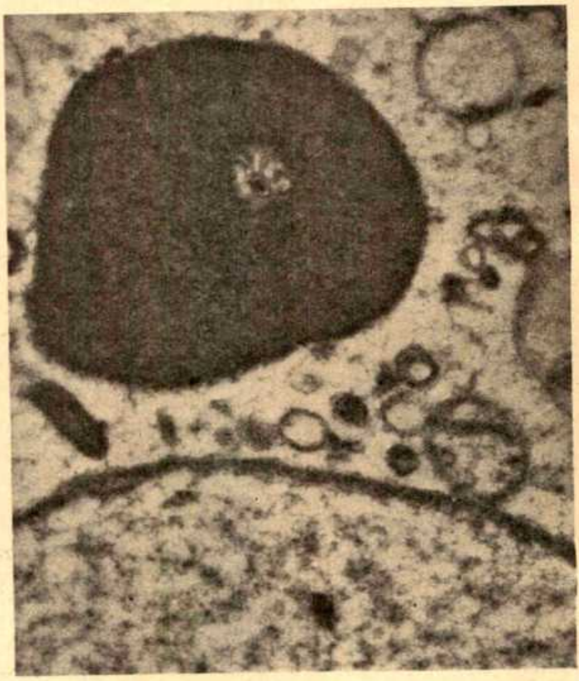

Fig. 3. A cytoplasmic inclusion of the dense type, near to a nucleus in a syncytial cell mass. (× 25,000)

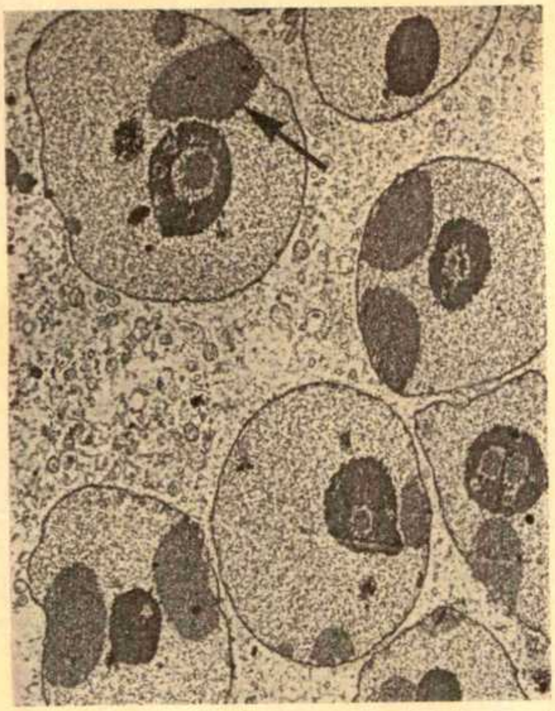

Fig. 4. Survey micrograph showing intranuclear inclusions (arrow) in a large syncytium. (× 3,000)

plasmic inclusions. (2) Incorporation of the cells into small syncytia is followed at first by continued production of virus particles and an increase of cytoplasmic inclusions. (3) Syncytial enlargement is accompanied by cessation of particle production, disruption of cytoplasmic fine structure, and the appearance of intranuclear inclusions.

Fluorescent antibody investigations suggest that at least some of the cytoplasmic inclusions contain viral antigen, but fluorescence was not observed in the host cell nuclei[5,9]. The present findings exclude the occurrence of organized virus particles in the cytoplasmic and nuclear inclusions, the significance of which is still uncertain. We favour the view that formation of discrete inclusions, and also the tendency to syncytium formation, are effects consequent on, rather than leading to, the synthesis of new virus. It is of some interest that the intranuclear inclusions were not discernible by light microscopy, in spite of their large size.

With regard to classification, the morphology of RS virus and its mechanisms of maturation and liberation seem to resemble more closely those of myxoviruses[10–12] than of any other group. Nevertheless, thin sections of influenza virus filaments give no sign of internal structure[10] whereas the filaments seen here had a well-developed core. Also, RS virus lacks the properties of hæmadsorption and hæmagglutination[2,3], and fails to multiply in the hen's egg[2]; and no serological relationship with known myxoviruses has so far been discovered[13]. Chemical analysis of RS virus has yet to be done. In view of these considerations, we are inclined to reserve judgment on the taxonomic issue, while recognizing the convenience of regarding this virus provisionally as an atypical member of the myxovirus group.

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TRANSFER OF SUBSTANCES BETWEEN THE BLOOD AND CENTRAL NERVOUS SYSTEM IN VERTEBRATE AND INVERTEBRATE ANIMALS

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THE nature of the physiological processes involved in the exchanges of substances between the blood and the tissues of the brain has been the subject of some controversy ever since the original observation by Ehrlich[1] that certain aniline dyes stained all the tissues of the body except those of the central nervous system. The site of the blood-brain barrier has been variously identified with the neuroglia, with the 'ground substance' of cerebral tissues and with the capillary endothelium[2–4]. According to this latter concept the similarity of the rates of penetration into cerebral tissues with those demonstrated for single cells has been postulated to result from some special characteristics of the cerebral capillaries which have been supposed to be functionally more analogous to the cell plasma membrane than to those of capillaries in other parts of the body[5].

The direct measurement of transfer processes and the identification of rate-limiting factors is rendered extremely difficult by the complicated structure and organization of the mammalian brain. The purpose of this communication is, therefore, to summarize some results which have been obtained in experiments on the relatively simple arthropod central nervous system and to consider them in relation to the more complex physiological organization of the mammalian brain. The nervous systems of insects and other arthropods differ from those of the vertebrate animals in possessing no internal blood circulation so that, apart from the oxygen carried by tracheoles, all the exchanges of nutrient and excretory substances must take place across the peripheral fibrous and cellular nerve sheath. This superficial position for the exchanges of substances between the insect central nervous system and the hæmolymph makes the study of its permeability properties relatively easy. Furthermore, the diffusion processes occurring within the tissues of the insect central nervous system are likely to be more obvious than those in the mammalian brain as the width of some insect ganglia are measured in thousands of microns as compared with the 20μ or so which represents the maximum distance of any of the cerebral tissues from their associated capillaries.

In the insect central nervous system it has been found that the exchanges of sugars labelled with carbon-14, sodium-24 and potassium-42 occurred relatively rapidly with the hæmolymph[6,7]. In addition, it was shown that the passage across the fibrous and cellular sheath was not a rate-limiting factor for an appreciable proportion of the exchanges of sodium-24 occurring in the cockroach terminal abdominal ganglion[8,9]. The efflux of the slowly exchanging sodium fraction, which was found to be reduced in the presence of metabolic inhibitors or in the absence of potassium ions, was identified with an active extrusion of these ions from the cellular components of the central nervous system[10]. The rapidly exchanging ion fraction was identified with that occurring within the extracellular spaces of the nervous system. This finding was strengthened by the observation that an appreciable portion of the nerve cord water was accessible to inulin molecules labelled with carbon-14 (refs. 10 and 11). The inulin space, of 18·2 per cent, was approximately equivalent to the rapidly exchanging fraction of tritiated water which accounted for 21·6 per cent of the nerve cord water.

By contrast with the mammalian brain the apparent extracellular space in the insect central nervous system demonstrated by the use of labelled ions and molecules was found to approximate to that revealed by electron microscopy. In the cockroach terminal abdominal ganglion the extracellular system was found to consist of an extensive system of fine ramifying channels (about 200 Å in width) throughout the ganglion, together with an irregular series of large cavities (the 'glial lacunar system' of Wigglesworth[12]) towards the periphery of the structure[13,14]. The presence of the glial lacunæ appeared to contribute to the much larger extracellular system demonstrated by electron microscopy in the insect ganglion, as compared with the mammalian brain in which the very *fine* channels only account for some 4 per cent of the total space[15,16].

The extracellular fluid of the cockroach abdominal ganglia has been shown to have a composition which differs from that of the hæmolymph in possessing an excess of cations together with a relatively low level of chloride ions due to the presence of a Donnan equilibrium across the nerve sheath[11,17]. The free anion groups producing the Donnan distribution have been tentatively identified with those of an acid mucopolysaccharide which has been recently demonstrated in the extracellular spaces of cockroach ganglia[18]. The mucopolysaccharide component of cartilage ground substance is known to exclude negatively changed dye molecules such as trypan blue due to the presence of the free anion groups[19]. By analogy with this condition the demonstrated exclusion of trypan blue from the cockroach central nervous system[12] was postulated to be due to the free anion groups associated with the extracellular mucopolysaccharide rather than to any significant properties of the nerve sheath as a diffusion barrier[11].

It is instructive to extend these observations on the arthropod central system to the more complex state of affairs in the mammalian brain. Thus by analogy with the invertebrate nervous system it might be expected that the mammalian cerebral capillary endothelium would be relatively permeable, perhaps being similar to those in other parts of the body. This supposition would be contrary to the hypothesis that the transfer across the capillary wall is a rate-limiting process. This concept is, however, based on very little direct physiological evidence and largely derives from the fact that this structure is morphologically the superficial boundary between the blood and cerebral tissues. An explanation for the apparent slow penetration of water-soluble substances into cerebral tissues can be obtained from a consideration of the fine structure of the extracellular system in the brain. The very limited volume of the extracellular channels demonstrated by electron microscopy, taken into consideration with the very small distances involved for the diffusion from the capillary endothelium to the various cerebral cells, might imply that the extracellular fluid could, as in the arthropod, achieve a relatively rapid equilibrium with the blood plasma, as has been suggested by Edström[20]. Such a small, rapidly exchanging extracellular fraction would be very difficult to demonstrate experimentally and could account for the very small amount of sucrose and other water-soluble substances which are said to enter brain tissues[21-25]. According to this hypothesis the slow penetration into the brain tissues would be due to the relative impermeability of the plasma membranes of the cerebral cells. Thus the close similarity commented on by Krogh[5] for the penetration into the tissues of the brain might not be due to any special properties of the capillaries but to the fact that the measured exchanges are, in fact, largely those taking place across the membranes of the cells which form about 96 per cent of the brain volume.

In the arthropod central nervous system de-sheathing involves a significant increase in the rapidly exchanging ion fractions. This increase was shown to be associated with an enlargement of the measured inulin space and was supposed to result from an enlargement of extracellular spaces or to cellular changes in this preparation[11]. A somewhat similar state of affairs would seem to exist in the mammalian brain where an increased accessibility of sucrose, thiocyanate and iodide has been measured in isolated pieces of brain, or during repeated replacement of the cerebrospinal fluid with Krebs–Ringer solution, as compared with the intravenously infused organ[24,26]. The analogy with the arthropod central nervous system thus supports the concept that the failure of such water-soluble substances to penetrate cerebral tissues in appreciable amounts is due to very restricted extracellular spaces[20], which are increased in abnormal conditions, rather than to the presence of a discrete relatively impermeable blood–brain barrier.

By analogy with the arthropod central nervous system it might also be tentatively postulated that one factor causing the exclusion of various negative dyes from the mammalian brain might be the presence of free anion groups in the restricted extracellular spaces. This postulate would also serve to explain the relatively rapid entry of basic dyes into the cerebral tissues. Mucopolysaccharides, which might be expected to possess free anion groups, have been shown to produce particularly intense staining of the internal elastic lamella of the large arterial and capillary walls[27,28]. Such a distribution could conceivably explain the relatively slow penetration of trypan blue (which possesses four negative charges) obtained on intravenous as compared with intrathecal administration. Any discontinuous distribution of substances containing free anion groups in the general extracellular spaces might thus allow some permeation of the dye molecules following intrathecal administration. These postulates bear some resemblance to the earlier theories which attempted to identify the blood–brain barrier with the 'ground substance' of cerebral tissues.

It has been pointed out that the very restricted extracellular system in the mammalian brain, described by electronmicroscopists as consisting essentially of 200 Å channels between adjacent cells, has been difficult to reconcile with the known minimum metabolic requirements of active nerve cells[8]. In particular, it might be imagined that the amount of sodium ions immediately available in such a restricted extracellular fluid might not be sufficient to sustain prolonged nervous activity. Such difficulties in the interpretation of normal nerve function in the brain are more explicable on the basis of a capillary endothelium which is relatively permeable to cations. Furthermore, if the organization of the extracellular system is similar to that in the arthropod central nervous tissues then it could be postulated that any indiffusible free anion groups might maintain a significant excess of cations which would thus tend to function as a reservoir of sodium ions.

I thank Drs. C. Crone, J. Dobbing, P. R. Lewis and F. E. Russell for discussion of some of the topics mentioned in this article.

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CORRELATION OF DIFFERENT EXCITATORY AND INHIBITORY INFLUENCES ON CELLS IN THE NUCLEUS GRACILIS OF THE CAT

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THE nucleus gracilis is the first synaptic region encountered by primary afferent fibres from the hinder half of the body in an important somæsthetic projection system. Earlier investigations in this Department[1,2] have shown that there is a marked functional differentiation along the rostro–caudal axis of this nucleus with respect to the cutaneous receptive fields of its cells, the presence or absence of afferent inhibition by cutaneous stimuli outside these receptive fields ('surround' inhibition), and the extent of rostrally directed (lemniscothalamic) projection. We can now amplify this earlier analysis and correlate it with the differential effects of stimulation of the cerebral cortex (contralateral area *SI*). External records were made by conventional methods from single cells in cats anæsthetized with sodium pentobarbitone.

We have continuously verified the observation of Gordon and Paine[1] (see also McComas's work on the rat[3]) that cells in the middle 3 mm of the long axis have a markedly smaller mean receptive field than those in the rostral 4 mm or those lying more caudal. We find this is true whether one considers cells specifically sensitive to bending hairs (*HS* cells) or cells sensitive to light touch or continuous pressure on the skin (*TP* cells) which are far less sensitive to bending hairs; though in each part of the long axis *TP* cells have a much larger mean receptive field than *HS* cells. This is also true if for each category of cells one considers separately those lying proximally and those lying distally on the hind-limb, the former having in each part of the nucleus larger mean receptive fields than the latter.

Cells showing afferent inhibition are common in the middle region of nucleus gracilis[1]. We have confirmed the observation of Perl *et al.*[4] that afferent inhibition is seen in *HS* cells but not in *TP* cells. We have found it in 95 per cent (of 50) *HS* cells tested in this part of the nucleus. It is also almost invariably found in cells sensitive to light brushing of the pads, and in cells responding to gentle pressure at the base of a claw. It can be demonstrated by various means; but the most sensitive method we have used is shown by Fig. 1*A* and *D*: this method

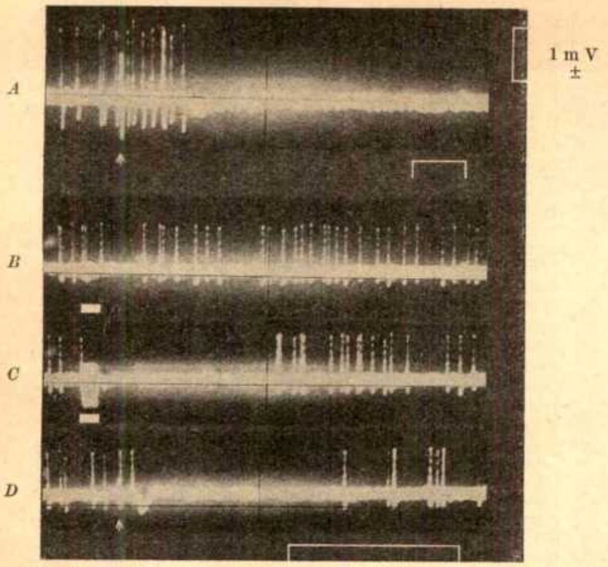

Fig. 1. *A*, inhibition of spontaneously discharging *HS* cell in middle region of the nucleus gracilis by electrical stimulus (single shock) applied to skin outside the receptive field. 25 sweeps, superimposed on storage oscilloscope. Arrow marks time of conditioning shock. Latency of complete inhibition 12 msec (note small negative wave at about this time from excitation of other cells by stimulus). Time calibration 10 msec.
B and *C*, inhibition of another *HS* cell in middle region of nucleus by brief train of shocks to contralateral cerebral cortex (*SI*), demonstrated by same method as in *A*. In *B* the stimulus is just at threshold strength: in *C* it is four times threshold. Timing and duration of cortical stimulus indicated by solid white bars. *D*, afferent inhibition in same cell as in *B* and *C*. Single shock applied to skin outside receptive field; timing shown by arrow. Time calibration for *B*, *C* and *D*, 100 msec. Voltage calibration applies to all records

can give an accurate measurement of its latency, which is of the same order as that for excitation of the cell from its receptive field, and of its duration, which increases with the strength of conditioning stimulus (up to several hundreds of msec).

Cells which do not show afferent inhibition can be shown to be facilitated by electrical stimuli outside their receptive fields (afferent facilitation). We have found this effect in all *TP* cells tested throughout the nucleus (confirming Perl *et al.*[4]), and also in those *HS* cells without afferent inhibition, most of which lay rostrally, where such inhibition is less common than in the middle region.

Electrical stimulation of the cortical sensorimotor area is known to excite or inhibit single cells in the nucleus gracilis[5-7]. We have found, on stimulation of the contralateral cortex (*SI* 'hind-limb area'), that excitation and inhibition are exerted selectively. Cortical inhibition, in the cells tested from the rostral and middle parts of the nucleus, was confined to cells (*HS* and 'pad' cells) showing afferent inhibition also (Fig. 1*B*, *C*, *D*). When elicited by a single cortical shock or brief train of shocks, latencies for inhibition in different cells ranged from 9 to 20 msec, the effect lasting up to about 100 msec, depending on strength of cortical stimulus. We have seen summation of effect between cortical and peripheral inhibitory actions, both of which may be presynaptic in nature[8]. Cortical excitation was seen only in cells in which afferent inhibition was absent, such of these cells as were tested showing afferent facilitation also. Latencies for excitation by a maximal cortical shock ranged from 5 to 10 msec (cf. ref. 7). All the *TP* cells tested, throughout the nucleus, were excited from the cortex (Fig. 2*A*). Cortical excitatory influences tend to dominate in the rostral part of the nucleus.

Antidromic excitation of cells by stimulating the contralateral medial lemniscus[2] shows that virtually all *HS* (and 'pad' and 'claw') cells with afferent inhibition project rostrally along this path. By contrast, only half the *TP* cells in the middle, and very few in the rostral part of the nucleus seem to project in this way; but lemniscal stimuli commonly cause 'trans-synaptic'[2] excitation of rostral *TP* cells, at a latency compatible with a path mediated by the thalamus and cortex (Fig. 2*B*). Some cells, not apparently projecting in the lemniscus, but excited by cortical stimulation, may be interneurones concerned in mediating cortical effects within the nucleus.

It is known that stimulation in the medial lemniscus can also cause inhibition of cells in the nucleus gracilis[1]: we have found this effect to be common, and easily elicited by brief trains of shocks to the contralateral but not to the ipsilateral lemniscus. It was observed in both *HS* and in *TP* cells. Such an effect may arise as a transcortical effect initiated through lemniscus and thalamus, but the latency of

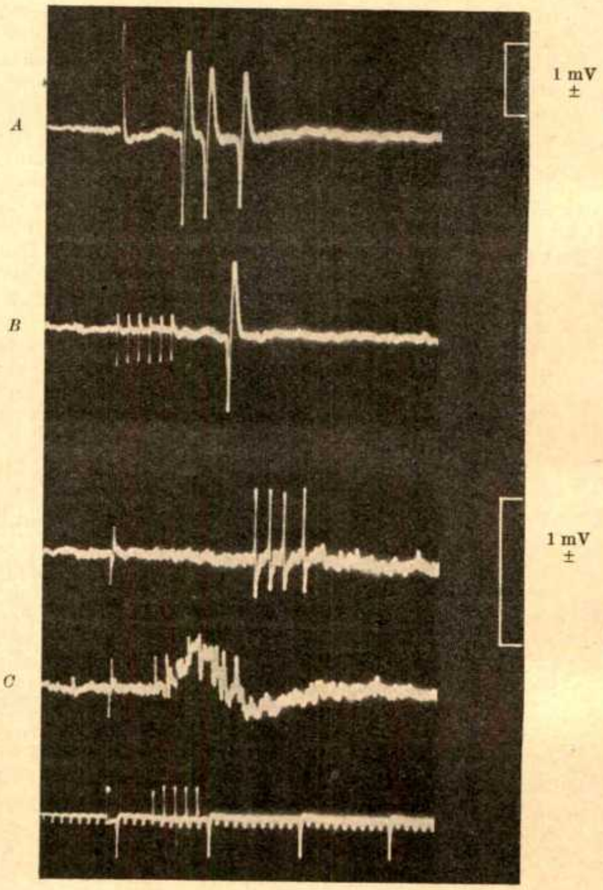

Fig. 2. *A*, excitation of typical *TP* cell in middle region of the nucleus gracilis by single shock to contralateral cerebral cortex (*SI*), of strength 10 times threshold to show minimal latency of response.
B, excitation of same cell as in *A* by brief train of shocks to contralateral medial lemniscus, of strength twice threshold to show minimal latency
C, inhibition of another *TP* cell (also excited from cortex) by brief train of shocks to contralateral medial lemniscus. Upper trace: control showing response to testing shock in skin receptive field. Lower trace: response to test shock totally inhibited by lemniscal stimulus, which itself evokes antidromic discharge in other cells. Onset of inhibition shown from other records to be 5 msec from beginning of lemniscal stimulus
Below, time calibration for all records, 1 and 10 msec; and stimulus marks for *C*. Timing of stimuli in *A* and *B* seen on sweeps. Upper voltage calibration refers to *A* and *B*, lower to *C*

the inhibition is commonly too short (as little as 5 msec) for such a path, and furthermore it is seen after wide excision of the sensorimotor cortex. It could arise through stimulation of aberrant corticofugal fibres passing through the lemniscus[9], though we have produced it by stimulation in the ventrobasal complex of the thalamus, where such fibres are not known. Its occurrence in *TP* cells which are excited from the cortex (Fig. 2*C*) renders either of these explanations unlikely for such cells, and again suggests the existence of a type of inhibition unconnected with the other inhibitory systems described: it is possibly a recurrent inhibition operated through axon collaterals (cf. the 'Renshaw system' of motoneurones) and initiated in our experiments by antidromic activation.

We consider that the close correlation which we find between cortical inhibition and 'surround' inhibition on one hand, and between cortical excitation and 'surround' facilitation on the other, suggests a general principle of some importance in the transmission of sensory information. While it is still unknown in what combinations or sequences these cortical influences are normally exerted, it appears from a consideration of such of these cells as project rostrally in the medial lemniscus, some excited and some inhibited by cortical activity, that such activity cannot be directed towards general suppression of sensory transmission at this level. Cortical modification must be selective when it occurs, one of its targets possibly being the level of spatial contrast achieved by the afferent inhibitory system.

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CORRELATED ORIENTATION SOUNDS AND EAR MOVEMENTS OF HORSESHOE BATS

MOST of the bats which rely on echo-location have relatively large and complex external ears compared with those of other mammals. Some of the Microchiroptera, especially the family Vespertilionidae, hold their ears in a virtually fixed position relative to the head when they are active, while others move them in a variety of ways. One of the most striking of the various patterns of ear movements are those of the horseshoe bats (Rhinolophidae) described in considerable detail by Schneider and Möhres[1]. These bats also differ from those of other families in emitting their orientation sounds in the form of relatively long pulses which have a constant frequency for the greater part of their duration, followed by a downward frequency sweep at the end of each pulse[2]. Since sound travels through air at 34·4 cm/msec, a typical *Rhinolophus* pulse lasting 30 msec overlaps its own echo if the object is within about 5 m. In other Microchiroptera the orientation sounds are usually much shorter (0·3–10 msec). With a 1 msec pulse, overlaps occurs only at distances less than 17·2 cm.

Of the various types of ear movements adopted by the European greater horseshoe bat, *Rhinolophus ferrum-equinum*, the alternating ones associated with the scrutiny of objects at close range are of especial interest. As pointed out by Schneider and Möhres[1], when one of these bats is hanging quietly from a convenient toe-hold, the greatest activity of the external ears occurs when the bat is alert and is emitting numerous pulses of orientation sound. If a small object is slowly brought within 1–2 ft. of its head, the animal commonly begins rapid, alternating ear movements as it turns to face the approaching object. While the right ear is moving forward, the left pinna moves in the opposite direction, and the whole sequence is repeated so rapidly that it can only barely be seen even in good light.

Horseshoe bats, like other Microchiroptera so far examined, emit a 'buzz' of rapidly repeated and shortened orientation sounds when echo-locating objects at close range. Direct observation suggested that the rapidly alternating ear movements were closely correlated with the buzz. But the short pulses are emitted at repetition-rates up to 50 or more per sec, though only for a small fraction of a sec at a time, and the unaided eye cannot determine just how closely pulses and ear movements are correlated. Although Schneider and Möhres[1] report that the alternating, out-of-phase ear movements in the greater horseshoe bat have a maximum frequency of 5–6 c/sec, our preliminary observations suggested much more rapid movements. We have therefore taken motion pictures and synchronized tape-recordings of this species, some at 768 frames/sec by techniques described elsewhere[3] and others at 64 frames/sec with conventional equipment. Several cases of ear alternation were selected on the basis of photographic clarity. These appear to be typical of the majority of instances in which the bat is hanging from a toe-hold that allows a free 'view' below and horizontally and in which its interest was aroused by a small stick or forceps moved slowly to 30–50 cm below its head. Under these conditions the buzzes consisted of 6–10 pulses, each of which was 9·5–22 msec long, at repetition-rates of 28–88 pulses/sec.

There was indeed a close, though not perfect, correlation between the emission of the short pulses and the alternating movements of the ears. It was our distinct impression that the closest correlation occurred when the bat was most interested in the stick or forceps. The most closely correlated of the three cases photographed at 768 frames/sec was used to prepare the graphic comparison of ear movements and orientation sounds shown in Fig. 1. The time of arrival of the orientation sound waves at the microphone is plotted above the corresponding ear movements. Since the microphone was roughly twice as far from the bat as the forceps on which its attention appeared to be concentrated, the upper part of Fig. 1 shows the time when echo waves from

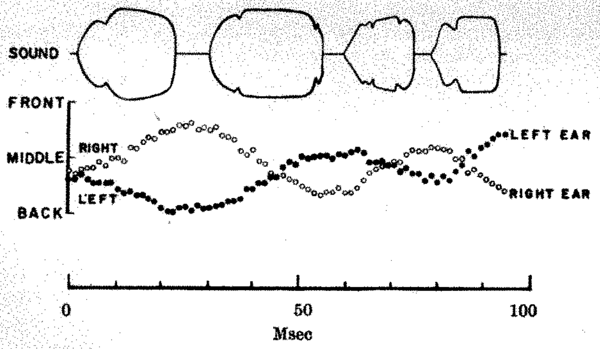

Fig. 1. Correlation between ear position and time of arrival of orientation of sound waves. Ear position was measured from a reference point on the bat to the tip of the ear for each frame in this part of the sequence. This is a detail of Fig. 2*a* and shows the approximately sinusoidal nature of the ear movements

the forceps reached the bat's ears, subject to an uncertainty of about 1 msec. It is clear from Fig. 1 that, in this case, the ears were being moved almost synchronously with the orientation sounds. In other cases, however, the ear movements and sound patterns were not so closely correlated. In Fig. 2 the whole of three sequences is plotted in less detail. As can be judged from the scatter of the points in Fig. 1, there was enough uncertainty in the frame-by-frame measurements from the 16-mm film that only the main outline of the ear movements could be determined. Nevertheless, Fig. 2 shows typical examples of shifting phase relations between extremes of ear position and sound emission.

A few additional high-speed motion pictures showed a *Rhinolophus* while landing. One of these involved a buzz consisting of 11 pulses in 152 msec, and in two others, bursts of 15 pulses in 205 msec, corresponding to pulse repetition rates of 72 and 73 pulses/sec, respectively. In one of the three cases the ears were visible in the motion pictures for part of the buzz during which there was a 1 : 1 correlation between pulses and ear movements similar to that shown in Fig. 1. The pulses in the buzzes which accompanied landing manœuvres were very similar to those recorded when a horseshoe bat flies close to small vertical strings[2]. These 'landing buzzes' occurred when the bat was within 10 cm of its landing place, so that there must have been almost total overlap between the emitted pulse and the echo from the surface on which the bat was about to land.

Under other conditions, including flight through relatively unencumbered rooms, the orientation sounds are as long as 70 msec, have relatively low repetition-rates, and may occur independently of the ear movements, which are much slower. It is probably of considerable significance that, when a horseshoe bat has the greatest need for accurate information about some object at close range, it uses its shortest duration pulses and emits each of these while one ear is moving forward and the other backward.

The importance of these ear movements to the bat is indicated by their consistency and by Schneider and Möhres's discovery that if horseshoe bats are deprived of the use of the muscles that move the pinnae relative to the head, they compensate by nodding the head vigorously. A variety of hypotheses can be advanced to explain how ear movements might facilitate echo-location. Pye[4] has proposed one such theory, and a number of others are suggested by analogy to various radar systems. But we have no information about the physical acoustics of the external ear of *Rhinolophus* at rest or in motion, or its sensitivity to sounds arriving from various directions. Neurophysiological studies on bats of the family Rhinolophidae might well be as informative as those on the Vespertilionidae by Grinnell[5]. In the absence of such information for the horseshoe bats, it seems to us unprofitable to speculate in this communication about the precise function of these pulse-correlated ear movements.

We thank Dr. W. H. Thorpe for making it possible for one of us (D. R. G.) to examine these bats at the Madingley Field Station of the Department of Zoology, University of Cambridge. We also thank Roger Ransome and other zoologists who helped us obtain the horseshoe bats. Financial support was provided by a contract between the Office of Naval Research and Harvard University.

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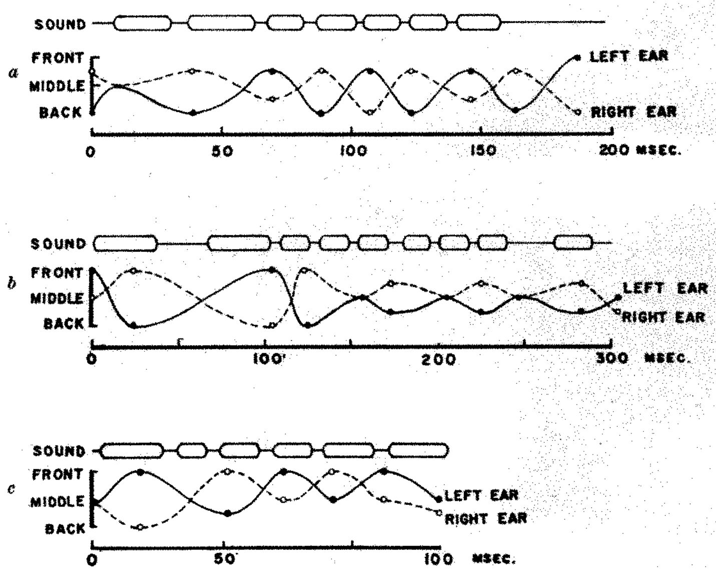

Fig. 2. Arrival time of orientation pulses relative to extremes of ear movement. Ear position was estimated relative to the head of the bat. Case *b* shows a common type of buzz consisting of an initial long pulse followed by a series of shorter ones

VERY rapid alternating movements of the two ears of *Rhinolophus ferrum-equinum* were first described by Möhres[1], and the importance of these movements to echo-location was demonstrated by Schneider and Möhres[2]. Recently, Griffin *et al.* (preceding communication) have shown that in general each echo-location sound-pulse is accompanied by a forward movement of one ear and a backward one of the other; that is, complete cycles of ear movement occur at half the pulse repetition-rate. This relation may easily be observed by eye in bats which are awake but inactive and producing pulses at very low rates. Observations at higher rates in active bats have been made from high-speed film and by other methods which have yielded similar results to those of Griffin *et al.*

The rate of ear movement in flight was measured from a ciné film kindly made available by the Granada Television–Zoological Society Film Unit at the London Zoo. The film (taken at 1,000–1,500 frames/sec with a 10 msec time-marker along one edge) showed greater horseshoe bats taking off and flying in to land. Rapid alternating movements of the two ears were clearly demonstrated and in several sequences could easily be measured. The time taken for a pair of forward and backward movements (one cycle) by each ear varied from 1/16 sec to 1/25 sec, with one instance of 1/28 sec. Peak-to-peak amplitudes for the tip of the ear often appeared to exceed 1 cm but the movements also involved variable complex turning components and changes in shape of the pinna, as well as movements of the head. No sequence was long enough to follow more than a few cycles of movement and no information about the bat's sounds was available.

In subsequent laboratory experiments ear movement was measured by an acoustic Doppler echo-location system operating at 95 kc/s. Echoes were amplified by a radio receiver, heterodyned to zero centre-frequency and recorded on magnetic tape or by an oscilloscope and camera. The transmitter and receiver were masked by paper tubes, convergent at their open ends, in order to limit the records to one ear at a time. In some experiments the echoes were heterodyned to 5 kc/s for subsequent examination with a sound-spectrum analyser which distinguished between forward and backward movements. Orientation sounds produced by the bat, at 84 kc/s, were heterodyned by a second radio receiver to 1 kc/s and recorded on a second channel of the oscilloscope or tape-recorder. The two ultrasonic microphones were placed so that the timing error between channels was under 1 msec and no cross-talk was experienced.

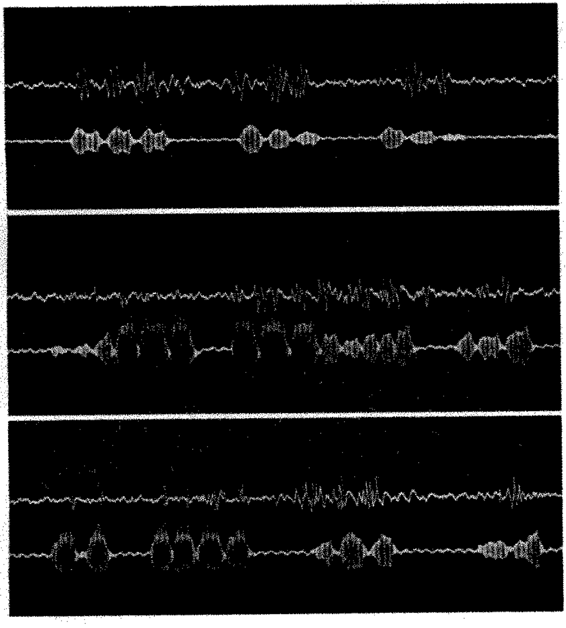

Fig. 3. Three samples from recordings of ear movements and echo-location pulses of *Rhinolophus ferrumequinum* (reading from left to right). Upper traces, the output from the Doppler movement detector; lower traces, the bat's pulses with a time-marking ripple of 100 c/s. Amplitude changes in both traces are caused by the bat turning its head

This method permits long continuous recordings (Fig. 3) the evaluation of which is extremely simple. The velocity and amplitude of the ear movement are given respectively by the frequency and the number of cycles of the output of the Doppler system. Amplitudes measured in this way agree closely with those obtained from the ciné film. Amplitude of the Doppler output is only related to the intensity of echoes received from the ear.

The bats were held in the hand. They vigorously investigated their surroundings or objects brought into their vicinity and always gave particularly strong responses to nearby movements. They were not disturbed by the Doppler sensing signal and always appeared to ignore it. Under these conditions the animals emitted probing pulses in discrete groups at a steady rate of 6·0–8·3 groups/sec which seemed to correspond to the respiration-rate. In familiar surroundings each group contained only two pulses, each of 30–50 msec duration, or a single pulse of 40–60 msec. In unfamiliar conditions or when a new object was presented for inspection, as many as seven or eight pulses were produced in each group, the pulse length reducing to as little as 10 msec and the 'instantaneous rate' rising to 60–80 pulses/sec.

Activity of the pinna was always associated with the emission of pulse groups, as may be clearly demonstrated by replaying the tape-recordings at reduced speed into an oscilloscope or loudspeaker. Correlation between individual movements and pulses was somewhat variable as found by Griffin *et al.* Numerical correspondence was generally good and was seldom much in error even at pulse-rates greater than 50/sec (25 cycles of movement/sec). But at all speeds the temporal relation between sound and movement varied rapidly. The greatest velocity may be reached at any time from the beginning to the end of a pulse or even a little outside it. Whether this is functionally significant or demonstrates imperfect control by the bat cannot be decided until the role of the movements in echo-location is established.

A brief investigation of the muscles responsible for ear movements was undertaken. A fine concentric electromyograph electrode, made from a No. 30 hypodermic needle (300μ outside diameter), was used to explore the scalp of an active bat. The presence of this electrode often suppressed ear movements but some recordings were obtained. Short bursts of potential spikes, having a relation to ultrasound pulses similar to that of the ear movements, were recorded from the region of the superficial cervico-auricularis muscle[2] and were believed to originate in that muscle. Simultaneous recording of muscle potentials and Doppler echoes was not attempted.

The speed of contraction of the superficial cervico-auricularis muscle when loaded with the pinna was tested by direct electrical stimulation. The bats were deeply anæsthetized with 'Nembutal' in alcohol and were warmed to a constant rectal temperature of 37° C. The scalp was reflected on one side to expose the muscle, which was stimulated by negative 1-msec square-waves through a Ringer-wick electrode. Movement was observed by eye and by attaching the pinna to a light-weight liquid potentiometer for oscilloscope display. Tetanic summation was absent at all rates of stimulation up to 25/sec but developed progressively above 30/sec. There was still a considerable amplitude of vibratory ear movement at 70/sec and the alternating component disappeared completely at about 100/sec. The gastrocnemius muscle of a laboratory mouse was nearly as fast as this, but only when its normal load was removed by severing the tendon. This shows that the auricular muscles are capable of moving the pinna by separate contractions within the normal speed range of the active bat.

Further work is needed to decide the function of these remarkable movements. Little is known of their finer detail and nothing of their nervous control. However, the present results are compatible with the hypothesis[3] that *Rhinolophus* is able to use the Doppler shifts which the moving ears impose on returning echoes of its own orientation sounds.

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RESPONSES TO SOUND IN PYRALID MOTHS

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SOUNDS of high audible or ultrasonic frequency have been shown to affect the flight patterns of certain grass-dwelling pyralid moths[1]. In laboratory experiments the pyralid species *Anagasta kuehniella* (Zell.), a flour moth; *Ostrinia nubilalis* (Hbn.), the European corn borer, and *Galleria mellonella* (L.), the wax moth, were affected by the same type of sounds. The behavioural responses are variable: tethered moths may stop flying or start to fly; moths at rest may move their antennæ or wings in a particular way each time they are stimulated or they may show no overt response; immobilized moths often contract their abdominal musculature in such a way as to protrude the tympanal organs, which are situated on the ventral surface of the first abdominal segment. Because of the economic importance of members of this group of moths, an investigation of any factors that affect their behaviour is desirable. The tympanal organs of pyralid moths are anatomically distinct from those of other groups of Lepidoptera, which may indicate that they evolved independently[2], so that the comparative aspects of the investigation are also of interest.

Moths were decapitated and immobilized, ventral side upward, on a cork platform. Electrical activity was recorded differentially between a glass-capillary microelectrode and a steel microelectrode placed on either side of the tympanal nerve; the tip of the abdomen was earthed through electrode jelly. Signals were fed into a condenser-coupled amplifier and displayed on an oscilloscope.

Electrical responses were recorded from *O. nubilalis* and *G. mellonella*. Fig. 1 shows examples of the nerve impulses recorded from one specimen of *O. nubilalis* in response to clicks, a Galton whistle with its main

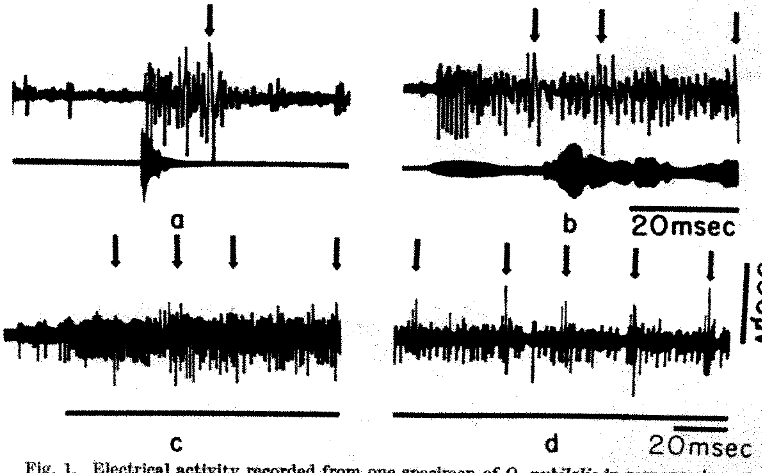

Fig. 1. Electrical activity recorded from one specimen of *O. nubilalis* in response to *a*, a click produced by two coins; *b*, a Galton whistle with its main output at 22 kc/s; *c* and *d*, a pure tone with a frequency of 32 kc/s. The lower trace in *a* and *b* is the response of a condenser microphone near the preparation; the horizontal line in *c* and *d* indicates the duration of the stimulus. The arrows indicate responses of nearby muscle fibres that are distorted by the recording system. The amplitude of the nerve impulses is indicated by the vertical calibration line

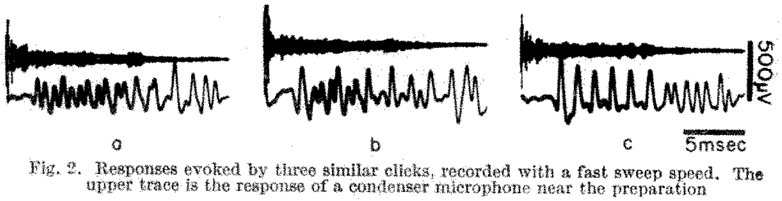

Fig. 2. Responses evoked by three similar clicks, recorded with a fast sweep speed. The upper trace is the response of a condenser microphone near the preparation

output at 22 kc/s and a sine wave with a frequency of 32 kc/s delivered by a condenser transducer, respectively.

The four successful preparations of *O. nubilalis* showed an irregular resting discharge in the nerve, although experiments were conducted in a sound-proof room, in which the ambient sound-level was reduced by about 60 db. When the electrical activity was made audible it became clear that the resting discharge was reduced in frequency immediately after stimulation so that it seemed probable that the discharge was indeed produced by acoustically sensitive cells. The tympanal organs of *O. nubilalis* and *G. mellonella* responded to sound-levels of about 30 db (with reference to a sound pressure of 0·0002 dyne cm^{-2}) generated by the whistle and also responded to the sound produced by clicking two coins together 30–40 ft. away from the moth (a peak sound-level of approximately 40 db at the preparation).

The frequency of nerve impulses in response to a continuous tone is reduced with time. This can be seen in Figs. 1*c* and *d*, which show the response at the onset (Fig. 1*c*) and after 5 sec (Fig. 1*d*) of stimulation. The frequency of responses greater than 50 μV in amplitude dropped from 990/sec during the first 100 msec to 480/sec in the same time, after 5 sec. The preparations did not respond to pure tones below 18 kc/s at the largest sound pressures that could be generated (a sound-level of about 90 db). Preparations did respond to the Galton whistle when it was set at a frequency of 8 kc/s, perhaps because of the harmonic content of the sound it produced. At this setting, 15 per cent of the output of the whistle consisted of second and third harmonic distortion.

The response of preparations made from *O. nubilalis* to the click produced by coins is shown with a faster sweep in Fig. 2. It is apparent from these records that at least two sensilla were responding to the sound. Although the sensilla are connected to the same region of the tympanum and are enclosed in the same capsule of connective tissue, the two sets of responses were independent. The two units that can be distinguished clearly fire alternately in Fig. 2*a* and in varying degrees of synchronization in Fig. 2*b* and *c*. If the six large responses of Fig. 2*c* represent two units firing synchronously, there then appears to be a third acoustic unit with a small response firing alternately with the two larger units; this is consistent with the results of histological investigation[3] that demonstrated the presence of four chordotonal sensilla in the tympanal organ.

The sensitivity of the pyralid organs to high audible or ultrasonic frequencies is about the same as that of the corresponding organs of noctuid moths which have been investigated by Roeder and Treat[4]. The latency of the repsonse to clicks is also similar (3·5–4 msec), and initial synchronous firing of two fibres was observed in the tympanal nerves of both groups (compare Fig. 1*b* with Fig. 3*c* of Roeder and Treat[4]). The chordotonal sensilla of all the tympanal organs which have been investigated share one important property: the responses which they generate in the auditory nerve fibres are independent of one another and bear no clear relation to the frequency of the sound. This is almost certainly related to the fact that the nerve fibres they supply are unable to conduct impulses at rates higher than a few thousand per sec. Differences in physiological mechanism must exist between this type of sensillum and, for example, the chordotonal sensilla of the mosquito antenna that generate nerve impulses in strict relationship with the low-frequency sound stimulus[5].

The large responses that were observed in the tympanal nerve of pyralid moths showed adaptation in response to a continuous tone. However, this was not so rapid as the adaptation observed in noctuid, or in arctiid and notodontid moths[4,6]. There is evidence of a non-acoustic sensillum associated with the tympanal organ in those three groups, but no electrical evidence for the presence of such a cell was obtained in any of the preparations made from pyralid moths.

Perhaps the most striking feature of the pyralid tympanal organ is that it is apparently insensitive to range of audible frequencies between about 2 and 18 kc/s, which can be detected by the tympanal organs of other insects that have been examined.

There is now a good deal of evidence in support of the hypothesis that the tympanal organs of Lepidoptera are of use in the detection of echo-sounding bats. It appears that the tympanal organs of pyralid moths could also have this function.

The experiments described here led up to a field test in the summer of 1962 to determine whether sounds would influence the behaviour of *O. nubilalis* to such an extent that some reduction of their natural population in cultivated maize could be obtained. A sound resembling that produced by a single bat was broadcast over experimental plots from mid-June until the maize was mature. The sound reduced the number of larvæ in the stems and ears of about 1,000 plants by between 50 and 60 per cent.

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CELLULAR DIFFERENTIATION IN STALKED BACTERIA

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THE genus *Caulobacter* was created in 1935 by Henrici and Johnson[1] to accommodate a distinctive, but previously unrecognized, group of stalked unicellular bacteria. Henrici and Johnson encountered these organisms in the course of their studies on fresh-water bacteria which were conducted by the slide culture technique[2]. When slides are immersed in water for a week or two, the stalked cells of *Caulobacter* adhere to the glass and can be readily recognized on microscopic examination. Although Henrici and Johnson did not cultivate these bacteria, one representative of the group had been isolated as early as 1905[3], and later workers[4-6] have obtained three additional strains in pure culture. The isolation and maintenance of these bacteria is in fact simple; they are strict aerobes, and grow readily on a dilute peptone-yeast extract medium. From water samples and other natural sources we have obtained about forty strains in pure culture and are now conducting a systematic study of the group. The developmental cycle which is characteristic of *Caulobacter* has been elucidated in the course of this work.

Two different kinds of cells occur in any *Caulobacter* culture: swarmer cells and stalked cells. Both types are small rods of similar shape and dimensions; in some strains the cells are straight, in others distinctly curved. The swarmer bears a single polar flagellum and is grossly indistinguishable from a simple unicellular bacterium of the *Pseudomonas* or *Vibrio* type, except for the presence of a secreted substance occasionally observable at the base of the flagellum. This substance confers on the cell the property of adhesiveness to solid objects and will be referred to as the holdfast material.

The second type of cell bears a stalk at one pole in in place of a flagellum and is, therefore, not motile. The stalk is much thinner than the cell and can barely be resolved by the light microscope; the interpretation of its structure and relation to the cell became possible only as a result of observations with the electron microscope. As shown by Houwink[4,5,7], the stalk is not a secreted structure, but an outgrowth of the cell itself; its tubular wall is continuous with the cell wall, and encloses a very thin cytoplasmic core, continuous with the cytoplasmic region of the cell. The only secreted element is the holdfast material which, after the stalk has developed, is present at the distal end of the stalk.

Cells often occur in rosettes which adhere to one another by means of a common mass of holdfast material. Both types of cell and a rosette are shown in Fig. 1.

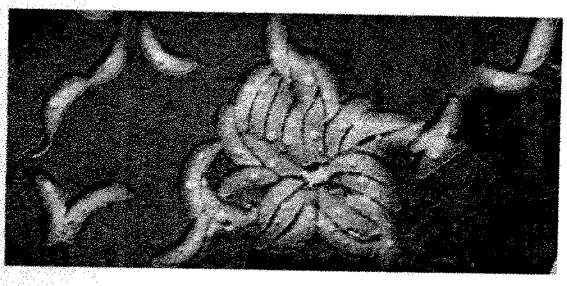

Fig. 1. Swarmers, a dividing stalked cell and a rosette of *Caulobacter*. Electron micrograph, shadowed with uranium

As shown by several workers[1,4,5,7], a division of a stalked cell gives rise to two unlike sister cells: a basal stalked cell and an apical swarmer. Existing accounts imply that the swarmer undergoes the transition to a stalked cell as a consequence of attachment to a solid substrate; according to this interpretation, stalk formation is a facultative event in the life-cycle, conditioned by environmental factors. We have found that this is not the case; on the contrary, stalk formation is an obligatory stage in the development of every swarmer, attached or unattached, and always precedes the first division of the swarmer.

The two kinds of cells in a culture of *Caulobacter* can be physically separated from one another by centrifugation. Swarmers will form a well-packed pellet in a centrifugal field in which stalked cells sediment as a looser, supernatant layer, apparently because the stalks prevent close packing. Consequently, it is possible to prepare almost homogeneous suspensions of each cell type, and to study their subsequent growth. In a liquid culture inoculated with swarmers, nearly all the members of the population develop stalks before there is any increase in viable count; the population then doubles within a period that is shorter than the mean generation time of swarmer cells, and during this doubling the percentage of cells which have stalks falls to about half its preceding maximal value (Fig. 2). In a liquid culture inoculated with stalked cells, the first division occurs significantly earlier than in a culture inoculated with swarmers.

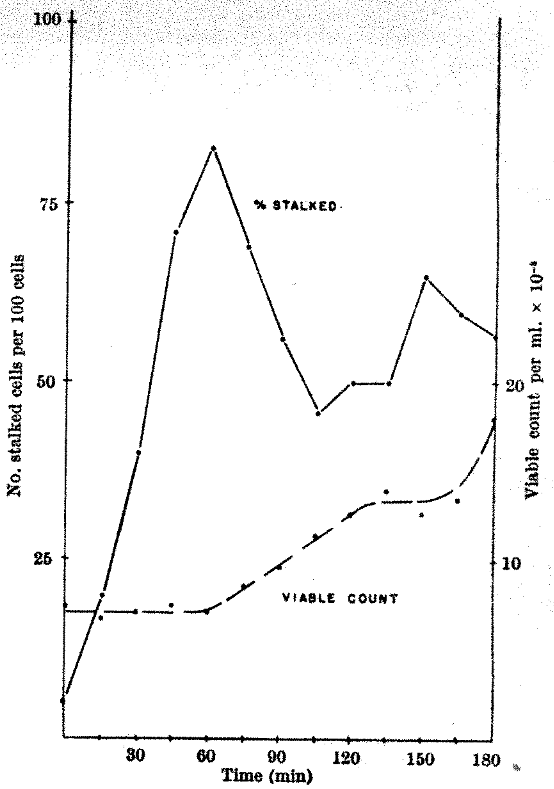

Fig. 2. Stalk development and cell division in an agitated culture inoculated with swarmer cells

We have also followed the growth-cycle by continuous microscopic observations on single clones in microcultures. When a stalked cell divides, giving rise to two unlike sister cells, division of the stalked sibling always precedes division of the swarmer sibling. However, after the swarmer has formed a stalk and divided once, its subsequent division times

Table 1. DIVISION TIMES OF SWARMER AND STALKED CELLS AS DETERMINED BY DIRECT OBSERVATION OF MICROCULTURES

Clone number	Total number of divisions observed	Average division time (min) Swarmer cells	Stalked cells
A	18	115 (7)	90 (11)
B	11	128 (4)	96 (7)
C	9	109 (3)	97 (6)
D	11	124 (4)	91 (7)
E	9	124 (3)	90 (6)
F	12	120 (5)	95 (7)

Cells were inoculated into the microculture and observed continuously for several hours. In this experiment, one field was observed and each cell mapped; in this way, several individual clones could be observed under identical conditions. Observation was terminated when the field became too crowded for accurate tracking of each cell's progeny. The number of observations of each type of division is given parenthetically after the average division time.

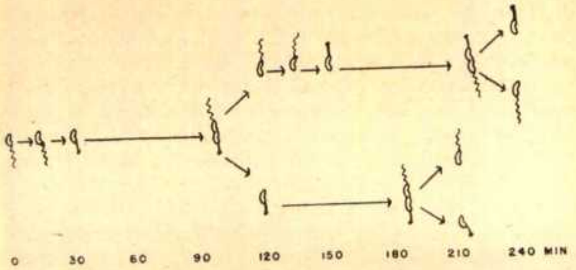

Fig. 3. Stalk development and cell division within a clone

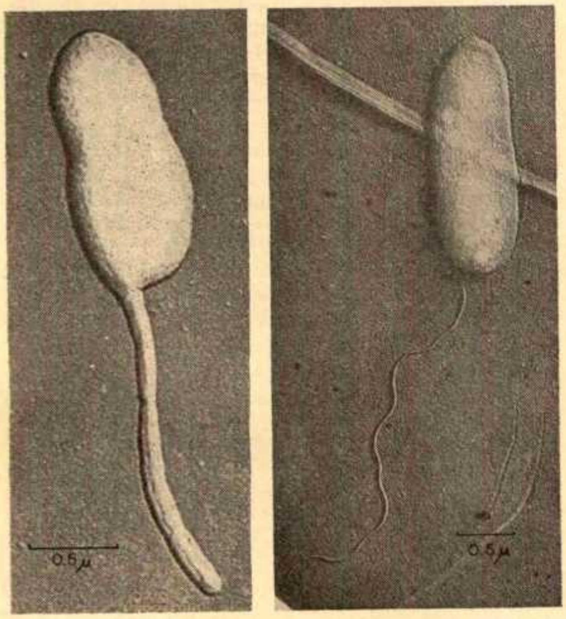

Fig. 4. Swarmer cell and stalked cell showing excentral location of flagellum and stalk in this strain. Electron micrograph, shadowed with uranium

are comparable with those of its original stalked sibling. Some typical data are shown in Table 1, and represented diagrammatically in Fig. 3. In effect, the difference between the division time of a swarmer and of a stalked cell represents the time required for the growth of the stalk. In the particular strain that we have used for detailed studies, it can be calculated that stalk formation takes 25–30 min under conditions where the division of a stalked cell requires 90–95 min.

The observations described here show that the capacity for stalk formation is a genetic property of the swarmer cell that is invariably expressed during its development. A single swarmer cell can develop only one stalk; and the stalk, like the flagellum, always arises at one pole of the cell. Although mature stalked cells are typically non-motile and devoid of flagella, the flagellum persists for a short time during the outgrowth of the stalk. As first observed by Houwink[7], the flagellum is attached to the distal end of the developing stalk; clearly, therefore, outgrowth of the stalk proceeds from the flagellated pole of the cell.

In most strains, both flagellum and stalk arise centrally at the pole of the cell. However, some of the strains in our collection are characterized by stalks which arise excentrally from the cell apex; in such strains, electron micrographs show that the flagellum is likewise attached excentrally to the cell (Fig. 4). One may, therefore, suppose that the cytoplasmic determinant of stalk formation is very closely related to the basal granule of the flagellum, and perhaps identical with it. This interpretation of the relationship between flagellum and stalk is distinctly different from that offered by Zavarzin[8], who contends that the flagellum becomes 'ensheathed' and is thus converted into the structure recognized as a stalk.

If the hypothesis that the determinants of flagellum and stalk are related is correct, the capacity for stalk formation might be lost as a consequence of a mutation which abolished the capacity for the formation of a flagellum. It is possible to recognize bacterial mutants in which flagellar function has been lost, since they form compact colonies in semi-solid agar, whereas colonies arising from motile cells are more diffuse[9]. Using this technique, we have isolated a series of non-motile *Caulobacter* mutants from ultraviolet irradiated populations. In one class of mutants, the swarmer cells have short flagellar stumps demonstrable by electron microscopy, and must consequently have retained the basal granule. A second class forms no flagella. All mutants so far obtained exhibit normal stalk formation, which shows that the mechanism of stalk formation proposed by Zavarzin cannot be correct. These observations do not support the postulate that the determinants for the formation of flagellum and stalk are closely related. However, they do not exclude it, since loss or alteration of the flagellum does not necessarily reflect a loss of the cytoplasmic components of the flagellar apparatus. Despite the failure of this test, the attempt to isolate stalkless mutants through indirect selection on the basis of immotility appears to merit further exploration.

In addition to its capacity for stalk development, the flagellated pole of a *Caulobacter* swarmer has another specific property: adhesiveness. By virtue of this property, the swarmer can undergo polarized attachment to a variety of solid objects: glass, cellu-

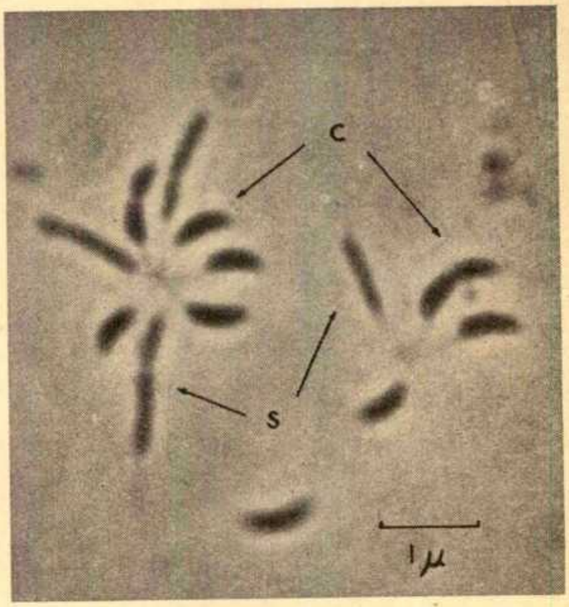

Fig. 5. Rosettes comprised of cells of two different morphological types of *Caulobacter*. *C*, curved; *S*, straight. Phase-contrast photomicrograph

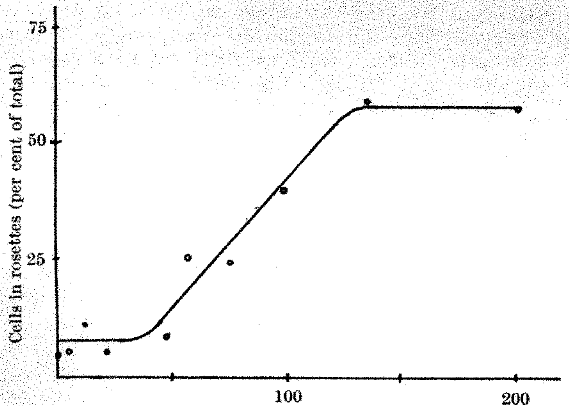

Fig. 6. Relation between initial turbidity and rosette formation, measured after 2 h incubation of agitated cultures of swarmer cells

lose fibres, other *Caulobacter* cells, and the cells of other micro-organisms[4,6,7,10,11]. Owing to the identical site of flagellum and stalk formation, development of the stalk takes place at the adherent pole of the cell. However, adhesion is not necessary for stalk formation; a cell which fails to make an effective contact as a swarmer proceeds to normal development in the unattached state. In an agitated culture where the population density is low, most cells develop stalks in the unattached state.

In a dense suspension of *Caulobacter* swarmers, the most common type of effective contact is contact with another swarmer. To be effective, however, such contact must be made between flagellated poles: a swarmer cannot adhere to any other portion of the cell surface of another swarmer, and has not been seen to adhere to a stalked cell. As a consequence of these restrictions, effective collisions result in the formation of clusters of swarmers, attached to one another by the flagellated poles. The subsequent development of stalks by the cells in such clusters gives rise to the rosettes which are such a characteristic feature of these organisms in pure culture. Polar adhesion is not a strain-specific character, as shown by the fact that when swarmers of two structurally distinguishable strains are mixed at high density, mixed rosettes develop (Fig. 5).

Adhesion of the swarmers is not initiated at 4° C, and, therefore, suspensions of isolated swarmers can be prepared at any desired density by differential centrifugation of chilled cultures and resuspension of the swarmers in a pre-chilled medium. When such a suspension is warmed to room temperature, clusters of swarmers are formed within a few minutes, provided that the cell density is high. The fraction of the swarmers that adhere to one another and thereby give rise to rosettes is proportional to the initial density of the suspension (Fig. 6).

These findings show that the swarmer cell of *Caulobacter*, although grossly indistinguishable from the vegetative cell of a typical pseudomonad, is endowed with a unique potentiality for differentiation. The flagellated pole of a *Caulobacter* cell has two specific properties—adhesiveness, and capacity to develop a stalk—which are not possessed by the flagellated pole of a *Pseudomonas* or *Vibrio*.

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METHOD OF PREPARING HISTAMINE SENSITIZING FACTOR

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BORDETELLA pertussis contains a substance capable of sensitizing mice to histamine[1,2]. Most workers who have examined this histamine sensitization phenomenon have used whole, killed *B. pertussis* cells[1–4], some have used cell-free culture supernatants or extracts from these organisms[5–8], and recently Niwa[9] has published a method of purifying the histamine sensitizing factor from culture supernatants. It has been shown[10,11] that cell wall preparations contain most of the histamine sensitizing factor activity when cells are collected before lysis occurs. In this preliminary report a simple method is described for preparing histamine sensitizing factor from whole bacterial cells grown in liquid medium.

Procedures and results. *B. pertussis* cells grown in liquid medium[12] were collected by centrifugation, re-suspended in saline to a concentration of 1,000 billion cells per ml. and extracted twice with 3 volumes of acetone at room temperature. (The cells were kindly supplied to us through the courtesy of Dr. Lee F. Schuchardt of Merck, Sharp and Dohme, Inc., West Point, Pennsylvania.) The cells were dried by applying suction through the cell paste contained in a Buchner funnel and then stored at 2°–5° C in a firmly stoppered bottle. From these acetone-dried cells histamine sensitizing factor was extracted in the following manner: 15 g of cells were added to 500 ml. of saline and thoroughly re-suspended by means of a tissue homogenizer. The cells were disrupted in the pressure cell of Ribi *et al.*[13] at 40,000 lb./in.^{2}. Cold nitrogen gas was used instead of carbon dioxide to prevent the cell paste from heating during this procedure. An additional 500 ml. of saline were added to the disrupted cells and then the *p*H was adjusted to 8·5 with 1 N sodium hydroxide. The suspension was kept cold and stirred constantly during addition of base and for 1–2 h thereafter. The suspension was centrifuged at 13,000 r.p.m. (27,000*g*) in a Servall refrigerated centrifuge and the clear, amber-coloured supernatant was decanted and its *p*H adjusted to 7·4 with 0·1 N hydrochloric acid. The sediment was re-suspended in saline. Both supernatant and sediment were dialysed for 4 days against 4 eight-litre changes of cold distilled water and then lyophilized. Most of the histamine sensitizing factor activity was found in the supernatant (Table 1). The activity of dried saline extract was found to be about 10–30μ per mouse. The saline extract contains various antigens as shown by agar diffusion studies with hyper-immune *B. pertussis* antiserum. At least three distinct bands were obtained in Ouchterlony diffusion tests with a hyper-immune rabbit serum. The acid-precipitated saline extract contained 14·6 per cent nitrogen, 4·5 per cent hexose, 1·8 per cent phosphorus, 28·4 per cent fatty acid esters + fatty acid amides and 0·4 per cent hexosamine.

Table 1. HISTAMINE SENSITIZING ACTIVITY OF *B. pertussis* EXTRACTS

Preparation	Dose per mouse 90 μg	30 μg	10 μg
Whole cells	8/17*	8/20	4/20
Acetone dried cells	9/20	9/20	1/20
Saline extracted cells	12/20	3/20	3/20
Saline extract	15/20	18/20	3/20
Untreated controls		0/18	

$*\ \dfrac{\text{No. of mice dying}}{\text{Total No. of mice tested}}$

Various methods have been used in an effort to purify the histamine sensitizing factor further. The most promising technique so far has been that of starch-block electrophoresis[14]. With lyophilized saline extract as starting material, the following procedure has been used with some success.

A starch block (potato purified starch, J. T. Baker Chemical Co.) was prepared in phosphate buffer (*p*H 7·2 and 0·024 ionic strength). 70 mg of saline extract were dissolved in 4·0 ml. of buffer and 3·5 ml. of this solution were mixed with 4 g of dry starch and placed in a 1 × 6 × 1-cm well cut out in the starch block. Electrophoresis was carried out in the cold (2°–5° C) for 21 h at 8–10 m.amp and 400 V. At the end of the run the starch block was cut into 2-cm pieces and each was extracted with 15 ml. phosphate buffer. The extract from each fraction was analysed for tyrosine by the Folin–Ciocalteau method and for histamine sensitizing factor activity in mice. The tyrosine peaks obtained (Fig. 1) indicate that saline extract contained at least 6 substances and that the histamine sensitizing factor (Fig. 2) is found in a fraction which moves slowly towards the cathode at the *p*H used. This material gives a typical nucleic acid absorption in the Beckman *DU* spectrophotometer probably due to contamination with a substance which moves just ahead of the histamine sensitizing factor and has a strong nucleic acid or nucleoprotein absorption. Investigations are now being carried out to establish the exact chemical nature of histamine sensitizing factor, as well as its role on protection of mice to infection with *B. pertussis*.

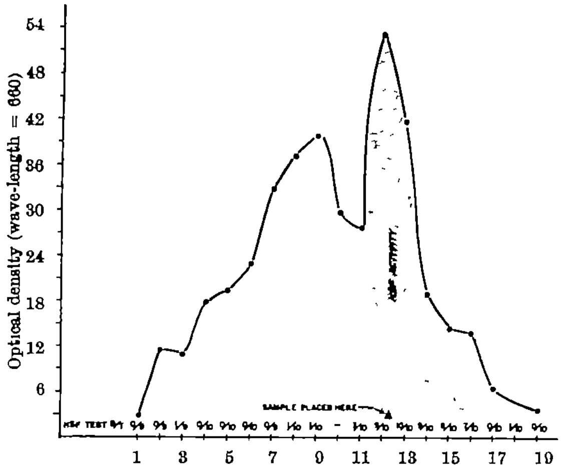

Fig. 1. Each starch block fraction was tested for the presence of tyrosine with Folin–Ciocalteau reagent and for its capacity to sensitize mice to histamine. The results of the mouse test at the bottom of graph are given as number of deaths over total number of mice tested

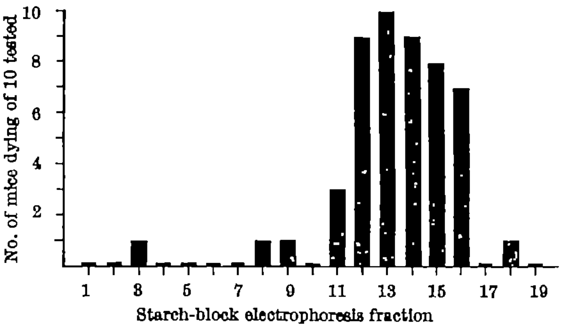

Fig. 2. Histamine sensitizing factor activity of individual fractions from starch-block electrophoresis

As found by Niwa[9], histamine sensitizing factor is more soluble at a *p*H higher than 7. Histamine sensitizing factor activity can be completely precipitated out of solutions at *p*H 4·8 and, as found previously with whole cells by Kind[2] and in extracts by Niwa[9], it is destroyed by heating at 75° C for 0·5 h. The substance adsorbs to paper, to Seitz and candle filters and to some extent to sintered glass filters. Perhaps for this reason yields obtained by curtain electrophoresis were low. Fractionation in diethylaminoethyl cellulose (DEAE) columns, as evident from Niwa's results[9], has resulted in low yields. Niwa's best preparations were obtained by the simple procedure of dialysis and precipitation at a *p*H of 6·2.

It is of interest and possibly significant that in preliminary experiments we have found the mouse protective activity of *B. pertussis* only in the microbial fractions containing histamine sensitizing factor activity. These findings will be reported elsewhere.

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LETTERS TO THE EDITOR

PHYSICS

A Time-resolved Spark as a Source for Atomic Absorption Analysis

A HIGH-PRECISION, time-resolved spark which is controlled electronically[1] has been used as a source for the atomic absorption determination of copper, with promising results.

From time-resolved spark investigations the following facts have been ascertained: (*a*) The field strengths and ion concentrations in the spark channel decrease with time. In a spark with a 0·01 farad condenser charged to 20 kV and a discharge duration of about 45 μsec, the ion concentration 10 μsec after initiation of the spark is about 10^{18} ions/cm^3. After approximately 50 μsec the ion concentration has decreased to 10^{17} ions/cm^3. The field strength under those spark conditions changes from about 600 kV/cm to 200 kV/cm (ref. 2) over the same period. (*b*) Pressure broadening appears to be at a maximum during and shortly after the breakdown of the spark gap[3]. (*c*) Self-reversal of resonance lines increases with time. For certain lines, however, the line profiles improve with time[4]. (*d*) For a spark discharge in a point to plane system, with a duration of about 9 μsec, some arc-like and spark lines continue to radiate after the termination of the electrical current[1]. (*e*) An appreciable improvement in line to background intensity ratios of resonance lines may be achieved by increasing the inductance in the spark discharge circuit[5].

The light source used for atomic absorption spectroscopy should give atomic spectral lines with narrow bandwidths[6]. Considering these requirements, it was realized that a spark might be used as a source for atomic absorption analysis if the radiation, after the termination of the electrical current, is used for absorption.

Table 1. EXCITATION CONDITIONS

Type of spark: electronically controlled high-precision spark

Peak voltage	17 kV
Capacitance	6,000 μfarad.
Inductance	residual (5–6μ henry)
Control gap electrodes	tungsten wtih 2 per cent thorium
Control electrode gap	3·6 mm
Analytical electrodes	point to plane top, ¼″ spherical bottom, 2¼″ diameter aluminium disk containing about 0·3 per cent copper
Analytical electrode gap	6·0 mm
Duration of a single discharge	9·3 μsec
Prespark period	10 sec
Exposure period	50 sec
Time-resolved portion	9–11 μsec

If an accurately timed spark with the conditions listed in Table 1 is used, and the radiation restricted to 9–11 μsec after initiation of the spark, the following is further achieved: (1) Doppler-broadening is reduced to a minimum with minimum inductance in the discharge circuit. In this way thermal radiation is restricted. (2) Stark broadening is reduced by at least one order of magnitude accepting the results of Bardócz[2]. (3) Self-reversal of the resonance line is reduced to a low value by using a low copper concentration in an aluminium alloy. (4) Reasonable line to background ratio is obtained[1]. This ratio can be improved by adding inductance for the circuit, but due to the increased thermal excitation, Doppler broadening will increase the line-widths.

To investigate the use of the spark as a source for atomic absorption analysis, the light from the spark was passed through a low-temperature flame into which copper solutions were sprayed. The intensities of the spectral lines were measured with a medium Hilger quartz spectrograph fitted with a direct-reading head[7]. In Table 2 some results of the absorption measurements are given.

Table 2. ATOMIC ABSORPTION RESULTS OBTAINED ON COPPER SOLUTIONS WHEN A SPARK IS USED AS A LIGHT SOURCE

Solution	Line pair $\frac{3247\ \text{Cu I}}{2816\ \text{Al II}}$	$\frac{3247\ \text{Cu I}}{3066\ \text{Al}}$	$\frac{3247\ \text{Cu I}}{\text{background*}}$
Water	0·786, 0·783†	1·29, 1·26	1·61, 1·65
80 p.p.m. Cu	0·758, 0·756	1·26, 1·26	1·59, 1·63
500 p.p.m. Cu	0·663, 0·660	1·10, 1·13	1·40, 1·45

* Background measured in the region of 4000 Å.
† Results for two exposures are given.

As is to be expected, the method is appreciably less sensitive than conventional methods using a hollow cathode lamp as a source, because of the wider spectral lines. However, the results have shown that a spark may be useful as an atomic light source for certain applications. Further research is being conducted to investigate: the possibility of using complex alloys or powders for multi-element analysis; which elements can be determined simultaneously; what spark conditions will give the most satisfactory results; what concentrations of the alloying elements can be used.

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Electrical Behaviour of Egg Albumen Solutions at Ultra-High Frequencies

FROM previous work[1,2] it is known that egg albumen, in common with other protein solutions, exhibits dielectric dispersion at megacycle frequencies and microwave frequencies due to the relaxation of the protein and water molecules, respectively. The measurements recorded here, however, indicate that there is another small dispersion region occurring at tens and hundreds of megacycles between the two main regions which is similar to the effect reported by Schwan[3] for the case of hæmoglobin.

An 8 per cent solution of egg albumen was prepared by dissolving crystalline egg albumen in conductivity water, the albumen being supplied by Armour, Ltd., with less than 1 per cent ash and 5 per cent moisture. The pH of the solution was 4·86 and the ionic conductivity 2·43 × 10^{-4} ohm^{-1} cm^{-1} at 1,000 c/s and 25° C.

The real (ε') and imaginary (ε'') parts of the dielectric constants were measured at six different frequencies using previously described apparatus and techniques[4] with recent modifications[5], and the dipolar contribution to ε'' was obtained by subtracting the correction due to ionic conductivity. The dielectric decrement (δ) and the absorption increment ($\Delta\varepsilon''$) were then calculated at each frequency by finding the difference between ε' and ε'' and the corresponding values for pure water, and then dividing these differences by the concentration. All measurements were made at 25° C in order that the results could be compared directly with those of Oncley and Buchanan *et al.*[1] made at lower and higher frequencies, respectively.

The results are shown in Fig. 1, which illustrates significant variations of δ and $\Delta\varepsilon''$ with frequency, despite the large experimental error which arises from taking a small difference between two large quantities. By extrapolating his megacycle measurements to high frequencies Oncley obtained $\delta = 0{\cdot}7$ and, conversely, by extrapolation of microwave measurements to lower frequencies Buchanan obtained $\delta = 0{\cdot}95$. These two results taken in conjunction also indicate subsidiary dispersion, a conclusion consistent with the present work.

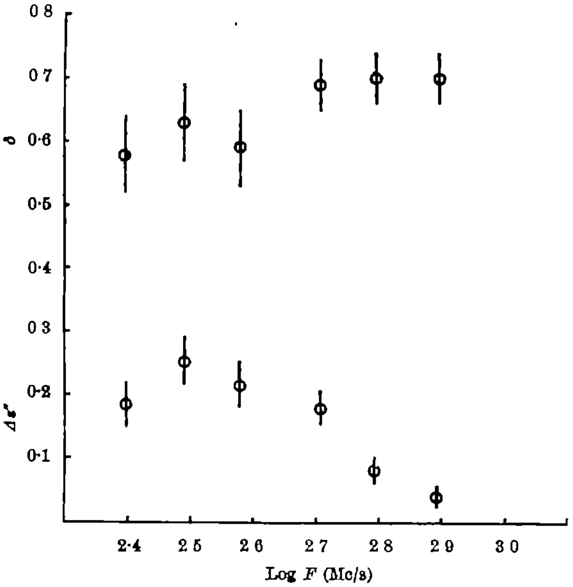

Fig. 1. Variation of dielectric decrement and absorption increment with frequency

In his measurements on hæmoglobin, Schwan[3] noticed similar subsidiary dispersion and suggested that this was consistent with a range of relaxation frequencies extending from 40 Mc/s to 3,000 Mc/s. In the present work, however, it seems unwise to draw detailed conclusions about the characteristics of the dispersion except to point out that there appears to be a suggestion of a maximum in $\Delta\varepsilon''$ at about 300 Mc/s.

It is interesting to consider the significance of this dispersion at a molecular level. In previous measurements[6] which I made on human serum albumen a high value of $\Delta\varepsilon''$ was recorded at one frequency (580 Mc/s) and it was suggested that this could be due to incorrectly assuming that the ionic conductivity was the same at 580 Mc/s as at 1,000 c/s. Such an explanation, however, could not account for the present results since $\Delta\varepsilon''$ is seen to increase with decreasing frequency. Thus it seems likely that a relaxation mechanism is involved and possibilities would include the rotation of individual amino-acid groups within the molecule and rotation of the bound water. It is hoped to investigate these possibilities further by extending the work to cover several proteins over a wide frequency and temperature range.

I thank Mr. J. M. Richards for preparation and measurement of the pH of the egg albumen solutions.

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GEOPHYSICS

Selective Volatilization from Tektites

THE possibility of selective volatilization of certain elements from tektites during the fusion process is of importance for the interpretation of their chemical composition. Lovering[1] has pointed out that if tektites were formed by the impact of large meteorites, ultra-high temperatures must have prevailed over large areas of the site, thus permitting appreciable selective volatilization, and, further, that if significant volatilization has occurred, tektites could be derived from certain acid igneous differentiates but not from normal argillaceous sediments.

On the other hand, Cohen[2] has observed a depletion of germanium (a volatile element) in tektites, and states that this must represent an original low germanium content in the parent material, since germanium is not commonly lost from silicate melts in glass tanks. If, however, an ultra-high fusion temperature of tektites cannot be precluded, the glass tank analogy may not be appropriate since the viscosity of the melt decreases approximately exponentially and the diffusion coefficient increases approximately exponentially with temperature[3].

The length of time to which tektites could have been exposed to temperatures greater than 2,000° K is limited to a maximum of a few minutes, as shown by the presence of lechatelierite particles and the alkali elements[4,5]. It may be thought that this evidence precludes an ultra-high fusion temperature for tektites. That this is not the case may be seen from a simple calculation of cooling times.

The approximate equation for the radiative cooling of a spherical black-body can be derived from the Stefan–Boltzmann radiation law as[6]:

$$t = \frac{r\rho c}{9\sigma}\left[T(t)^{-3} - T(0)^{-3}\right]$$

where r is the radius, c the specific heat, σ the Stefan–Boltzmann constant, ρ the density, $T(0)$ the assumed fusion temperature, and $T(t)$ is the temperature of the

Table 1. RATE OF COOLING OF TEKTITES

$T(t^\circ)$ K	Cooling time (sec) from 10,000° K to $T(t^\circ)$ K $r = 1$ cm	 $r = 5$ cm
5,000	0·29	1·43
3,000	1 48	7·40
2,000	5 05	25·2
1,000	40 8	204
500	326·4	1,632

body after t sec. Solving this for the time required for a tektite of 1 cm radius and of 5 cm radius using the values $\rho = 2{\cdot}4$ g/cm and $c = 0{\cdot}21$ cal/g-mol. deg. (value from Grant quoted in ref. 7) gives the results shown in Table 1. Calculations by Prof. J. C. Jaeger (personal communication) show that if the effect of the finite thermal conductivity is taken into account, the figures in Table 1 are not significantly altered. Further, since the molten material is ejected from the impact site at velocities of several kilometres per second, heat shielding would not be expected to affect these figures significantly. The original fusion temperature was arbitrarily taken as 10,000° K for the purposes of calculation, and no implication as to the actual prevailing temperature is intended. In fact, this temperature was chosen because it is probably greater than the vaporization point of tektites. Thus the figures in Table 1 indicate that the rate of cooling from any temperature at which tektites could exist is so rapid that the presence of lechatelierite particles and the alkali elements does not preclude an ultra-high temperature origin for tektites.

It should be noted, however, that even the vaporization temperature of tektites does not necessarily put an upper limit on the temperature of their formation since they could be flash-melted well above this point without actually vaporizing. In the d.c. arc, operating at temperatures of 3,000°–8,000° C, minutes are required, rather than seconds, completely to volatilize milligram quantities of silicates. The data in Table 1 indicate that tektites weighing less than 200 g would have achieved a temperature of 3,000° K in less than 5 sec regardless of the original fusion temperature.

Although the extent of volatilization losses depends on the fusion temperature one assumes, it is not necessary to assume that vaporization temperatures have been achieved. It is well known to spectrographers that significant losses of the alkali elements occur within seconds of striking the d.c. arc at temperatures of 3,000° C[8].

These calculations do not, of course, give any evidence that tektites have in fact been subjected to ultra-high temperatures; but they indicate that such a possibility, with the concomitant implication of significant selective volatilization, must be seriously considered. Evidence that selective volatilization from tektites has occurred will be presented elsewhere.

We thank Prof. J. C. Jaeger for helpful discussions.

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CHEMISTRY

Bimolecular Substitution in Octahedral Transition Metal Ions, and the Concept of Amphiphilicity

THE problem of assigning mechanisms to certain octahedral substitution reactions has been complicated by two principal ambiguities. If the reacting complex has acidic protons on the non-participating groups, second-order kinetics resulting from substitution by basic anions may indicate either an S_N2 or an S_N1CB mechanism, and, in general, it is difficult to distinguish the two. With non-basic substituting anions or systems without extractable protons, first-order kinetics may indicate either an S_N1 mechanism or substitution involving preliminary bimolecular solvent intervention. The compound *cis-bis*-pyridino-*bis*-*o*-phenanthroline ruthenium II perchlorate, *cis*-$[\mathrm{Ru\ phen_2\ py_2}](\mathrm{ClO_4})_2$, has no extractable protons, and therefore should give a unique answer to the first ambiguity; and it was hoped that dry solvent acetone would not engage in preliminary bimolecular substitution.

The following reactions were carried out under kinetically controlled conditions in dry acetone: *cis*-$[\mathrm{Ru\ phen_2\ py_2}]^{2+} + X^- \rightarrow$ *cis*-$[\mathrm{Ru\ phen_2\ py}\ X]^+ + \mathrm{py}$, where X = Cl, Br, I, NCS (nitrogen bonding), N_3, and NO_2. Using dry *o*-dichlorobenzene, the same substitutions were carried out, together with those of the extra two anions, OH^- and CN^-, but in less detail than the acetone reactions. In all cases the complex was introduced as the perchlorate and the anions as their tetraphenyl arsonium salts. Although ion-pair formation is likely between the positive and negative species in these solvents, there are good reasons for believing that this phenomenon does not greatly qualify the general conclusions in regard to the mechanisms which will be put forward here.

In acetone the anions Cl^-, Br^-, I^-, and NCS^- reacted by a first-order process; the rate depended only on the concentration of the complex and was independent of the nature and concentration of the anion. Moreover, for these four anions a mass-law retardation and ionic-strength acceleration were observed. The N_3^- and NO_2^- anions substituted at a rate which was dependent on the concentration of the complex and also on the nature and concentration of the anion; the NO_2^- anion substituted considerably faster than the N_3^- anion, the reaction of which in turn was much faster than the first-order reactions. At 100° C, the relative ratios (Cl^-, Br^-, I^-, NCS^-): N_3^- : NO_2^- of the rates was 1 : 4 : 9, and, furthermore, the activation energy of the NO_2^- reaction was 7 kcal less than that of the Cl^-, Br^-, I^-, and NCS^- reactions. A similar pattern was observed in *o*-dichlorobenzene, except that the OH^- anion substituted by a first-order process and at the same rate as the anions Cl^-, Br^-, I^-, and NCS^-, but CN^- substituted fastest of all the anions (including N_3^- and NO_2^-) by a second-order process. If optically active complex was used all the substitutions (independent of order) proceeded with complete retention of configuration.

These results are interpreted as follows: substitution by anions which proceed by a first-order process are unimolecular, and the second-order reactions are bimolecular. The anions therefore divide themselves mechanistically into two categories, and it is notable that only those anions (N_3^-, NO_2^-, and CN^-), which have potentially vacant p-orbitals for π-bonding,

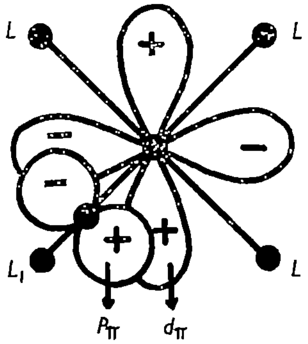

Fig. 1

engage in bimolecular substitution. The anions (Cl^-, Br^-, I^-, NCS^-, and OH^-), having no such orbitals sufficiently low-lying in energy, substitute by a unimolecular process. It seems plausible to conclude that vacated $p\pi$ orbitals of the incoming group are involved in the substitution process, and that the greater stability afforded to the incoming group by π-bonding is necessary to carry the bimolecular reactions through to completion.

A group which engages in a substitution reaction of the foregoing type, because it simultaneously donates and accepts electrons, may be called 'amphiphilic', and a bimolecular amphiphilic substitution reaction may be symbolized as $S_A{}^2$. The nature of these bimolecular substitution reactions may be envisaged as follows. The incoming group attacks an octahedral edge and donates its lone pair of electrons to the highly positive ruthenium atom; at the same time a vacated $p\pi$ orbital on the donor atom of the incoming group accepts electrons from the filled $d\varepsilon$ subshell of the ruthenium. A plausible mode of donor π-orbital overlap is that illustrated in the diagram, in which the four ligands (L) are at the corners of the square, and two others (not shown) are above and below the plane.

The incoming ligand is situated at an octahedral edge and the $p\pi$ orbital overlaps laterally with the $d\varepsilon$ orbital the longitudinal projection of which is towards the outgoing ligand L_1.

In the system, *cis*-[Ru phen$_2$ py$_2$]$^{2+}$, it is likely that the pyridino groups are stabilized, to some extent, by $d\pi$—$p\pi$— double bonds. Experiment shows that simple σ-donors are ineffective as bimolecular substituting agents, but that groups which are both σ-donors and π-acceptors are effective. This seems to imply that, in the foregoing system, both ρ- and π-bonding is required to release the π-bonded pyridino groups from the complex by a bimolecular mechanism. In the bimolecular transition state of the foregoing reactions, the incoming and outgoing groups must share a $d\pi$ orbital, and thus, relative to the ground-state, the incoming group will be stabilized and the outgoing group be destabilized by the partial transference of the $d\pi$ bond to the incoming group. Therefore π-bonding in the transition state promotes both the bond making and the bond breaking components of this bimolecular mechanism.

In analysing the amphiphilic order of a series of reagents several electronic factors must be discussed, relating to the metal and ligand orbitals available. Probably the best single experimental criterion which embraces, to some extent, all these factors is the *trans*-effect in platinum II complexes. It is proposed that for a given charge series the amphiphilicity will follow the *trans*-effect in platinum II complexes. Amphiphiles with the strongest *trans*-effect will have the highest amphiphilic power. Arsines, phosphines and sulphides might be considered potentially amphiphilic reagents using empty $d\pi$ orbitals in the transition (and final) states. There are clearly others; but it is notable that the reagents used here do, in fact, follow the *trans*-effect series, that is, $CN^- > NO_2^- > N_3^- > NCS^-$, I^-, Br^-, Cl^-, OH^-.

I thank Prof. D. P. Craig, Sir Christopher Ingold and (the late) Prof. F. P. Dwyer for helpful discussion.

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Absorption Correction in the Viscometry of Dilute Solutions of High Polymers

In a private communication, Drs. S. Gundiah and S. L. Kapur (of the National Chemical Laboratories, Poona, India) have pointed out to us that the derivation of the principal equation in our earlier communication[1] is in error, and that there is, because of this, a term missing from the equation. We acknowledge the error and are grateful to them for detecting it; but we wish to make it clear that the missing term should be negligible not only for the system we used as an example, but also for all systems of the type we specified, namely, "dilute solutions of high polymers". In this communication, then, we provide (*a*) a correction for the record and (*b*) additional evidence that the method we proposed is both sound and useful, and applicable to a wide variety of systems.

(*a*) The third paragraph of the earlier communication should be replaced by the following:

From this it follows that, in sufficiently dilute solutions:

$$\frac{t_{sp}}{c} = \left(1 + \frac{4a}{R}\right)[\eta] + k'\left(1 + \frac{4a}{R}\right)[\eta]^2 c + \frac{4a}{R} \cdot \frac{1}{c} \qquad (2)$$

and

$$\frac{\ln t_r}{c} = [\eta] - (\tfrac{1}{2} - k')\,[\eta]^2 c + \frac{4a}{R} \cdot \frac{1}{c} \qquad (3)$$

for in such solutions:

$$\frac{\ln t_r}{c} = \frac{\ln\,(1 + t_{sp})}{c} = \frac{t_{sp}}{c} - \frac{\tfrac{1}{2}\,(t_{sp})^2}{c} + \ldots$$

Subtracting (3) from (2), we obtain:

$$\frac{t_{sp}}{c} - \frac{\ln t_r}{c} = \frac{4a}{R}\,[\eta] + \left(\frac{1}{2} + k' \cdot \frac{4a}{R}\right)[\eta]^2 c \qquad (4)$$

In this equation, the term $4a/R\ .\ 1/c$ (the most significant of the absorption terms in equations (2) and (3)) does not appear. Moreover, the term $k'\ .\ 4a/R$ is, for high polymer systems, small compared with $\tfrac{1}{2}$. Equation (4) thus reduces to the simple equation:

$$\frac{t_{sp}}{c} - \frac{\ln t_r}{c} = \frac{4a}{R} \cdot [\eta] + \frac{1}{2}\,[\eta]^2 c \qquad (5)$$

and hence a value of $[\eta]$ that is unaffected by 'absorption' can be evaluated from the slope of the plot of:

$$\frac{t_{sp}}{c} - \frac{\ln t_r}{c} \text{ vs. } c$$

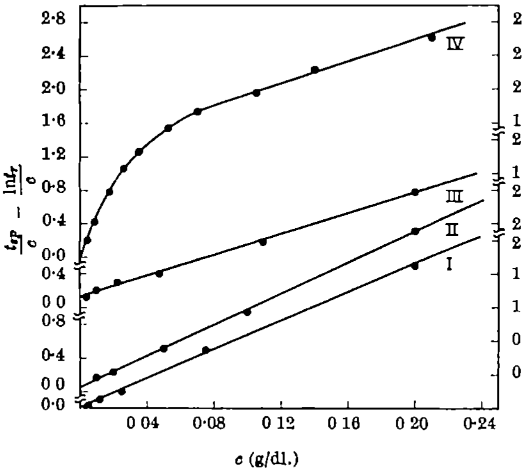

Fig. 1 Plot of $\frac{t_{sp}}{c} - \frac{\ln t_r}{c}$ versus c for data from refs. 6, 7, 8 and 9

Scale for I ordinate × 10^{-1}, abscissa × 10
II ,, × 10 , ,, × 10
III ,, × 10 , ,, × 1 in volume per cent

(*b*) The method we proposed for detecting absorption or wall effects and eliminating their influence on the intrinsic viscosity is based on equation (5) of the corrected paragraph in (*a*) above. Its soundness depends, therefore, on the validity of the assumption that the term $k' . 4a/R$ in equation (4) may be neglected. Some typical examples illustrate that this term is indeed negligible. For polymers (excluding polyelectrolytes) k' falls in the range 0·25–0·6, and a, the thickness of the absorbed layer, will be in the range of a typical particle diameter, 1000–6000 Å. In viscometers the smallest practicable capillary radius, R, is about 0·025 cm. With the typical values $k' = 0{\cdot}3$, $a = 1250$ Å, and $R = 0{\cdot}025$ cm, $k' . 4a/R$ equals $0{\cdot}6 \times 10^{-3}$. This term is therefore very small in comparison with $\frac{1}{3}$; its neglect introduces an error of only 0·1 per cent. Even with extreme values ($k' = 0{\cdot}6$, $a = 6000$ Å) the error would be only 1 per cent, and the error in the determination of $[\eta]$ using equation (5) would be well within the usual experimental error of determining $[\eta]$. Use of the more complete equation (4) would be necessary only if k' or a (or both) were exceptionally large; we have been unable to find data for such systems among polymer-solvent systems in the literature. (With polyelectrolytes in solvents of low ionic strength, however, the magnitude of k' and hence of $k'.4a/R$ may be such as to preclude the use of our method. Such cases will be considered elsewhere.)

It should be emphasized that use of equation (5) makes it possible to determine whether measured viscosities (or related functions) are affected by absorption or wall effects. Upward curvature, as c decreases, in a plot of t_{sp}/c vs. c has been attributed to absorption[2], to a wall effect[3], to expansion of the polymer coil[4], to electroviscous effects (with polyelectrolytes)[5], etc. If in the region where a plot of t_{sp}/c vs. c is curved, a plot of $(t_{sp}/c - \ln t_r/c)$ vs. c is a straight line passing almost through the origin, one may conclude that absorption and/or wall effects are significant and responsible for the curvature. If, on the other hand, the $(t_{sp}/c - \ln t_r/c)$ vs. c plot also is curved, some other explanation must be sought.

To illustrate the validity and value of these procedures we have applied them to four different systems for which data are available in the literature. (For each of these systems, plots of η_{sp}/c vs. c have been published displaying marked upward curvature that might be ascribed to absorption.)

For the systems polyvinyl acetate in butanone[6] and polyisobutylene in decalin[7], data plotted as $(t_{sp}/c - \ln t_r/c)$ vs. c yield the lines I and II of Fig. 1. Each of these is a straight line—a result consistent with the explanation that absorption effects are responsible for the upward curvature in the η_{sp}/c vs. c plots. Moreover, with each system the intrinsic viscosity calculated from the slope of the straight line (using equation (5)) is in good agreement with the value obtained by extrapolation using η_{sp}/c values obtained at higher concentrations.

With silver iodide sols (ref. 8, 'solutions' *IB*), and with aqueous solution of sodium pectinates[9], the data yield lines III and IV of Fig. 1. For the silver iodide sols a straight line is obtained. This provides support for the suggestion made[8] that the upcurve may be due to an absorption effect—a "slight flocculation on the wall of the capillary". (Again, the intrinsic viscosity calculated from the slope is in good agreement with the intrinsic viscosity obtained, with higher concentration data, in the usual way.) On the other hand, the sodium pectinate data do not yield a straight line, but a line with the downward curvature that would be expected with a polyelectrolyte effect.

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Gas Chromatographic Analysis of C_1–C_4 Hydrocarbons with open Tubular Columns

SINCE their invention by Golay[1], the use of open tubular columns with liquid phase coating on the inside wall became one of the most important techniques of gas chromatography. The use of open tubular columns permits the analysis of complex mixtures with high efficiency. It was, however, felt that the use of such columns for low-boiling hydrocarbons (below C_4) is possible only at 'very low' temperatures[2]. According to our best knowledge, the separation of methane, ethane and ethylene with open tubular columns was not yet reported.

During a recent work, we checked the gas hold-up (the 'retention time of the air peak') of some open tubular columns coated with silicone oils and greases which were used in connexion with a flame ionization detector, by using methane or more correctly mixtures of C_1–C_3 hydrocarbons. The use of methane for such measurement is generally accepted since the flame ionization detector has no response for air. To our surprise, we did find some separation for these components.

Using a 300-ft. long column with 0·010 in. inside diameter and coated on the inside wall with '*DC*–550' phenyl silicone oil, we found that when operating it at

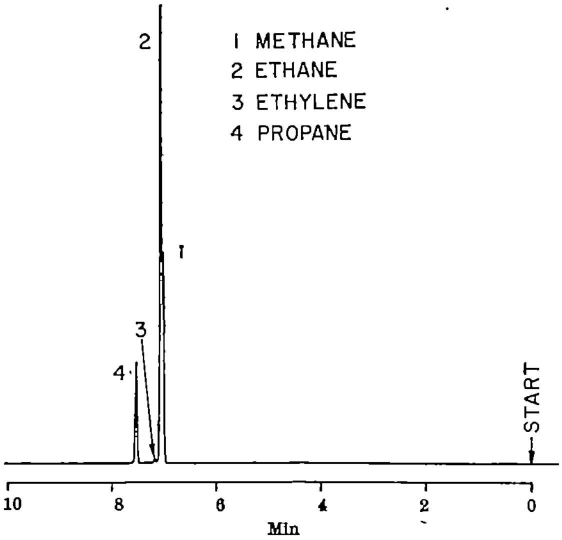

Fig. 1. 300-ft., 0·010-in. internal diameter open tubular column with '*DC-550*' phenyl silicone oil as liquid phase. Column temperature, 22° C. Sample volume, 1 ml. gas mixture, approx. 1/100 split. Carrier gas (helium) inlet pressure: 30 lb./sq. in.

room temperature, a partial separation between methane and ethane and a complete separation from ethylene and propane could be observed (Fig. 1). For this chromatogram, a chart speed of 1 in./min and a potentiometer recorder with 1 sec full-scale pen speed (Leeds and Northrup 'Speedomax *G*', 5-mV recorder) was used. We are quite sure that with faster pen and chart paper, the separation between the first two peaks would be even better.

By cooling the column, the separation could be improved. However, 'very low' column temperatures are not needed. The chromatogram on Fig. 2, for example, was made keeping the column at 5° C.

Another very interesting result was obtained when a second gas mixture was injected into a 150-ft.-long open tubular column with 0·010-in. internal diameter, prepared by using Dow Corning silicone high-vacuum grease as liquid phase. As shown in Fig. 3, excellent separation between methane and ethane could be obtained at room temperature.

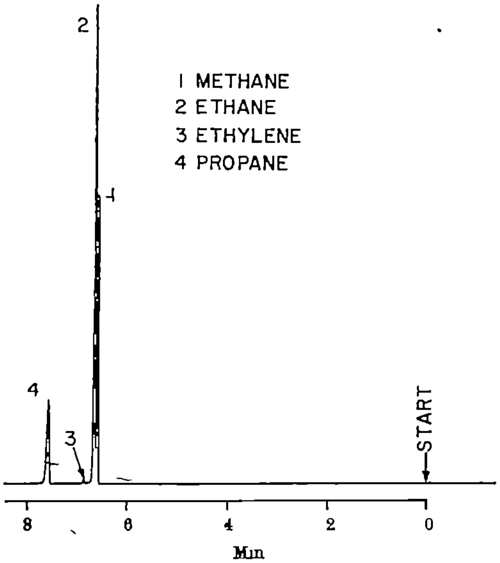

Fig. 2. Column and sample volume as on Fig. 1. Column temperature, 5° C. Carrier gas (helium) inlet pressure: 35 lb./sq in.

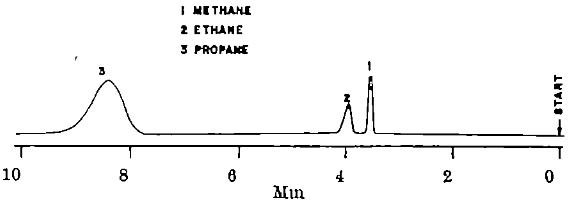

Fig. 3. 150-ft., 0·010-in. internal diameter open tubular column with Dow Corning silicone high vacuum grease as liquid phase. Column temperature, 22° C. Sample volume, 1 ml. gas mixture, about 1/100 split. Carrier gas (helium) inlet pressure. 20 lb./sq. in.

It should be mentioned that the silicone high-vacuum grease used for the preparation of this column also contains some colloidal silica. Thus, in our opinion, the chromatogram on Fig. 3 can actually be attributed partly to gas adsorption chromatography rather than to gas-liquid partition chromatography alone. However, adsorption columns in which the active adsorbent is distributed in a liquid phase coated on the inside wall of a capillary tube were not yet reported.

Naturally, such columns were used many times by different authors at higher temperatures. In such circumstances, however, the liquid phase—and not the adsorbent—is the active components.

We thank Dr. P. F. Varadi, of Machlett Laboratories, Springdale, Conn., for directing our attention to this problem.

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Quantitative Determination of Griseofulvin

For the determination of griseofulvin or 7-chloro-4,6-dimethoxy cumaran-3-one-2-spiro-1′-(2′-methoxy-6′-methyl*cyclo*hex-2′-ene-4′-one)[1], spectrophotometric[2–4], and spectrophotofluorometric[5] methods have been described. Colour reactions[6] are known which have been used for the identification of this antibiotic.

A method based on a colour reaction has been evolved in this laboratory for the quantitative determination of griseofulvin. In the course of experiments the feasibility of coupling an azo-component to the unsubstituted C_5-atom of the aromatic ring of the molecule was investigated.

Theoretical considerations and experimental evidence have shown that this coupling cannot be carried out on the intact molecule because the carbon atom in question does not possess the electron density necessary for an electrophilic substitution reaction.

A product amenable to coupling with a diazo-component is obtained through hydrolysis when griseofulvin is subjected to the action of a strong acid, or of a strong base, in suitable circumstances. The concentration of griseofulvin is indicated by the intensity of the colour of the azo-derivative of this hydrolysis product.

The optimum conditions for hydrolysis in solutions of hydrochloric acid or potassium hydroxide of different concentrations, and as functions of time, have been determined experimentally. The method consists of mixing 4 ml. of a solution of griseo-

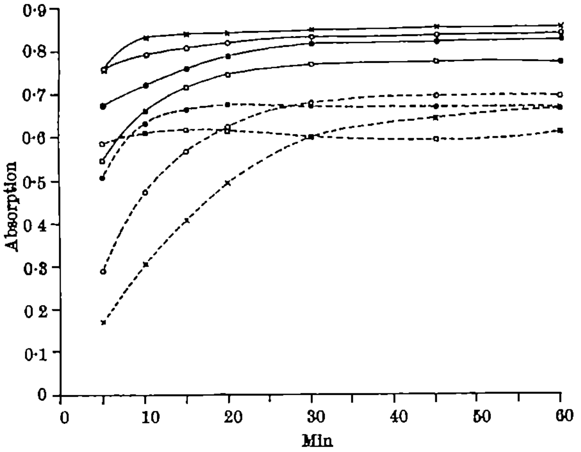

Fig. 1. Colour intensity of the azo-dye as a function of the circumstances of hydrolysis of griseofulvin. Hydrolysing agent, N HCl (— — × — —); 2 N HCl (— — ○ — —); 4 N HCl (— — ● — —); 8 N HCl (— — □ — —); N KOH (——×——); 2 N KOH (——○——); 4 N KOH (——●——); 8 N KOH (——□——)

fulvin (25 μg/ml. in 85 per cent v/v ethanol) with 2 ml. of hydrochloric acid or potassium hydroxide solutions of varied concentrations and keeping this mixture in a thermostat at 80° C for various times. After this, neutralization, respectively with a solution of pyridine or acetic acid, follows. Then 2 ml. of a 0·5 per cent aqueous solution of the stabilized diazonium salt of 4′-amino-2′,5′-dimethoxybenzanilide ('Echtblau *RR*') is added, and this mixture (final volume 10 ml.) is placed in a thermostat at 80° C for 8 min to achieve full development of colour. After cooling to room temperature, extinction is measured at $\lambda = 495$ mμ against a blank solution. The results are shown in Fig. 1.

It can be seen that for hydrolysis treatment with potassium hydroxide is more effective than treatment with hydrochloric acid. The optimum stage is reached with N potassium hydroxide after treatment for 30 min. Using other diazo-components for coupling, products with other colours are obtainable; Fig. 2 shows the adsorption curves of some of these.

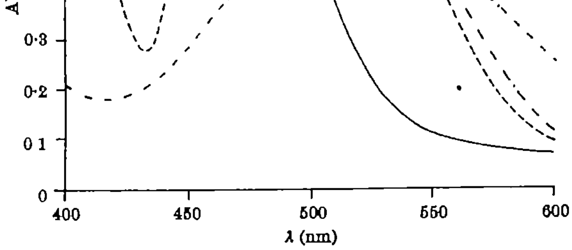

Fig. 2. Absorption spectra of different azo-dyes formed with different azo-compounds. Bases corresponding to the diazo-components: *o*-dianisidine (————); 4′-amino-2′,5′-dimethoxybenzanilide (— — —); *N*-phenyl-*p*-phenylenediamine (—·—·—); 2,6-dichloro-2′,5′-dimethoxy-4-nitro-4′-aminoazobenzene (—··—)

When hydrolysis is carried out with an N potassium hydroxide solution for 30 min., there is a linear relationship between the extinction values measured at maximum absorption, and the concentrations of griseofulvin in the coloured solutions prepared with the different diazo-compounds. The calibration chart shows a straight line meeting the origin of the co-ordinates. Average deviation from the straight line within the concentration limits of 5 μg/ml. and 50 μg/ml. is ± 0·5 per cent. The colour of the solutions to be measured remains stable for two hours.

Measurements of absorption were carried out on a Unicam *SP* 500 spectrophotometer, using a 10-mm cell.

Crystalline griseofulvin was used. Based on this colour reaction, experiments are being carried out to establish a method for the determination of griseofulvin in various preparations, and fermentation broths. It is to be hoped that disturbing effects of impurities can be eliminated to allow adequate spectrophotometric determinations to be carried out.

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A Comparison of Polysaccharides of *Hypnea musciformis* and *Chondrus crispus*

A REVIEW of the literature gives some evidence which indicates that the polysaccharides of *Hypnea* species and *Chondrus crispus* (carrageenin) are identical. Clingman's study of *H. specifera*[1] showed these polysaccharides to contain D-galactose and 3,6-anhydro-D-galactose, the same basic components found on analysis of *Chondrus* extracts[2]. *Hypnea* extractives have a potassium-sensitive fraction (termed the κ-fraction[3]), as does *Chondrus*[3], which are degraded by a cell-free bacterial preparation from *Pseudomonas carrageenovora*. This preparation is reported to be specific not just for carrageenin but for its κ-fraction[4].

Evidence suggesting that polysaccharides from the algae are not identical may also be found in recent literature. Pronounced differences in gel strength between extractives of *H. musciformis* and *C. crispus* have been reported[5]. Furthermore, the cell-free bacterial preparation described by Yaphe[4] has been found to degrade another polysaccharide, fucoidin[6].

It was felt that an examination of the oligosaccharides produced by mild hydrolysis of the algal extracts would show any differences in arrangement of the D-galactose and 3,6-anhydro-D-galactose units, thus helping to explain the dissimilarities which have been noted.

Samples of *H. musciformis* and *C. crispus*, picked clean by hand, were washed well with sea-water, rinsed with a minimum of distilled water and lyophilized. The algae were ground in a Wiley mill to pass an 80-mesh screen. Weighed amounts were suspended in borate buffer (*p*H 9·0, i.e. 0·05) and disrupted in a Lourdes mixer for 20 min at 15,000 r.p.m. with cooling. The samples were then made to 1·0 per cent (w/v) in the buffer and stirred at 80° C for

1 h. The resultant mixture was clarified by centrifugation at 2,000*g* and the cell debris discarded. The supernatant was exhaustively dialysed first against the buffer and then against water adjusted to *p*H 9·0 with sodium hydroxide. A portion of each algal extract was treated with three volumes of 95 per cent ethanol and the precipitate collected and dried to constant weight *in vacuo* at 35° C over sodium hydroxide. The remainder of the extracts were fractionated by the method of Smith[3]. A portion of each of the fractions thus isolated was subjected to mild hydrolysis using the procedure of Painter[7], with one modification, that is, a small amount of 'Dowex 50' (H+ form) was mixed with the samples in the dialysis bag in an effort to force the hydrolysis to completion. The dialysates so obtained were passed through a cation exchange column ('Amberlite 200', H+ form, 16–20 mesh) to convert the barium salts of the sulphated sugars to their free acids and to remove the excess barium remaining in solution. The effluents were concentrated by evaporation under reduced pressure at 60°–80° C.

The original, untreated fractions as well as the dialysates and dialysants of the treated fractions were compared chromatographically using the following solvents and papers: (*a*) 1-butanol/acetic acid/water (50 : 12 : 25), Whatman No. 3*MM*, descending; (*b*) 1-butanol/ethanol/water (3 : 1 : 1) made to 3 per cent with cetylpyridinium chloride ascending: (*c*) water saturated methyl ethyl ketone made to 3 per cent with cetylpyridinium chloride, Whatman No. 54 and No. 3*MM*, ascending, both after Rees[8]. Electrophoretic separation was carried out on a horizontal cell in borate buffer (*p*H 8·3, i.c. 0·05) using Whatman papers No. 54, No. 4, No. 3*MM* and *S* and *S* 507 and cellulose acetate strips (Gerlman Instrument Co., 'Microphore').

By all chromatographic methods, a total of five distinct spots could be easily observed in the dialysates of the whole, previously unfractionated *C. crispus* and *H. musciformis* polysaccharides. In all methods the R_F and R_{gal} of the spots from the two polysaccharides appeared identical. The electrophoretic evidence supported this conclusion. The precipitates prepared by potassium chloride fractionation of the above polysaccharides, termed the ϰ-fraction by Smith[3], showed the same five components after hydrolysis. The hydrolysates of the λ-fractions of the two polysaccharides, which do not precipitate on treatment with potassium chloride, again appeared identical to each other. They only showed, however, two components in solvent system (*a*) (8-h and 24-h irrigations) and four in systems (*b*) and (*c*). Chromatography of unhydrolysed and unfractionated polysaccharides, as well as their unhydrolysed ϰ- and λ-fractions, yielded only one spot, at the origin. Similar results were obtained with the dialysants, making it apparent that the hydrolysis had not gone to completion, even with the addition of extra resin.

From the results presented, together with that of others[1,3,4], we consider it reasonable to assume that both the basic structural units and the mode of their linkage in the polysaccharides derived from *C. crispus* and *H. musciformis* are identical. Observed differences between the two may, therefore, be due to such other factors as cation concentration, degrees of hydrogen bonding, or polysaccharide concentration (particularly in commercial preparations). More specifically the concentration of the ϰ-fraction, which possesses great gel strength, may vary seasonally[6] and is present normally in greater proportion in *H. musciformis* than in *C. crispus*.

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BIOPHYSICS

Concentration Dependence of Sedimentation Coefficient of Deoxyribonucleic Acid with added Sodium Chloride

The addition of sodium chloride has a marked effect in reducing the electrostatic interaction between deoxyribonucleic acid (DNA) molecules in solution, as is shown by the decrease in the slope of the concentration dependence of reduced viscosity ($\eta_{sp}/c.$) with the increase of added salt[1,2]. This work shows that the same information can also be obtained from the variation of the concentration dependence of sedimentation coefficient of DNA with the addition of sodium chloride. Fig. 1 is a plot of the reciprocal of the sedimentation coefficient, $1/S_{20,w}$ versus DNA concentration, at sodium chloride concentrations of 0·001 M, 0·01 M, 0·1 M, 0·15 M and 0·2 M. Changes in the slope of the curves as well as a constant extrapolated value of sedimentation coefficient at zero concentration, $S^0_{20,w} = 22{\cdot}7 \times 10^{-13}$ sec, at different concentrations of salt, reveal a close similarity between the concentration dependence of reduced viscosity and that of the reciprocal of sedimentation coefficient, in the case of DNA in solution.

It is also evident from Fig. 1 that with increase of concentration of sodium chloride, the sedimentation-rate increases and the region of rapid variation of sedimentation coefficient is shifted to higher concentrations of DNA. The charge on the DNA macro-ion increases with the addition of sodium chloride to DNA solution along with a shift of the region of rapid variation of the charge to higher concentrations of DNA[3].

This shows a close resemblance between the variations of the charge and the sedimentation-rate with concentration of DNA. Thus the two explanations of hypersharp boundary observed at high concentrations of DNA (one, on the basis of rapid variation of sedimentation with concentration[4], and the other, on the rapid variation of charge[3]), perhaps, do not contradict each other.

In this investigation, it has also been observed that the sedimentation boundary of calf thymus DNA remains more hypersharp in 0·001 M sodium chloride up to 0·0045 per cent compared with about 0·009 per cent in higher concentrations of sodium chloride. The schlieren refractive boundary, recorded with the help of a Spinco *E* ultracentrifuge using standard 12-mm aluminium centre-piece cell, is shown in Fig. 2.

In view of the close qualitative similarity between the modes of variation of charge, reduced viscosity and sedimentation with DNA concentration as the

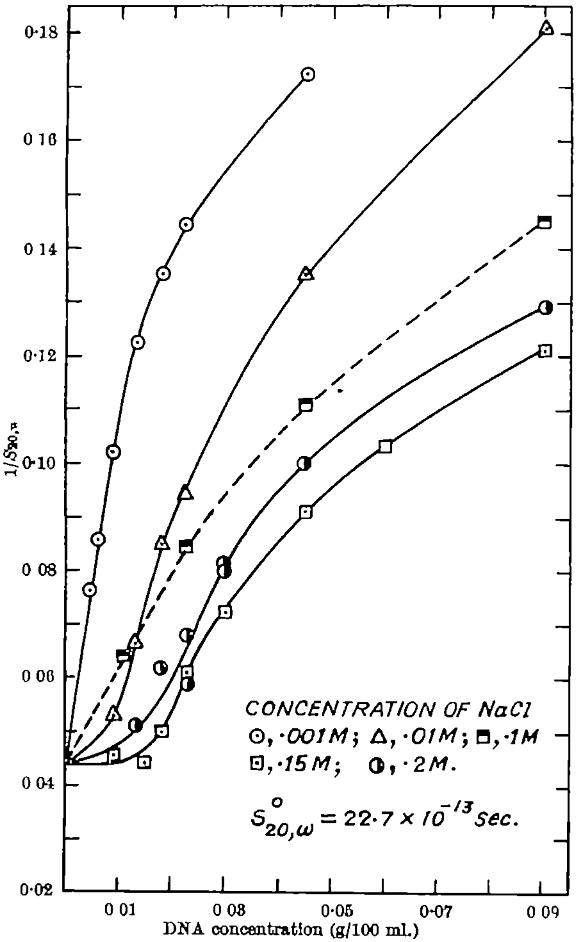

Fig. 1. Variation of the reciprocal of the sedimentation coefficient ($1/S_{20,w}$) with concentration of calf thymus DNA in sodium chloride solvents 0·001, 0·01, 0·1, 0·15 and 0·2 M

result of addition of sodium chloride, a quantitative correlation might provide more information about the DNA molecules in solution.

It is well known that physico-chemical measurements are affected by the quality of DNA preparation and the method of handling the DNA solutions. The sample of calf thymus DNA used in this work (Lot No. 3354, Mann Research Laboratory, New York; phosphorus 8·82 per cent and nitrogen 16·4 per cent) showed the following characteristics. The ultra-violet spectra measured in a *PMQ* II spectrophotometer (Carl Zeiss) showed $E_{259}/E_{231} = 2·5$, $E_{259}/E_{280} = 1·85$, and maximal rise in extinction at 260 mμ 49·3 per cent and $E_{259}/E_{280} = 1·42$ at *p*H 3 (ref. 5).

Fig. 2. Hypersharp schlieren boundary of calf thymus DNA in 0·001 M sodium chloride sedimenting from right to left at 8, 16 and 24 min from the time of reaching 2/3 of the 39,460 rev./min. Bar angles are 18·5°, 18° and 17·5°, respectively. DNA concentration 0·0045 per cent (O.D.$_{259 m\mu}$ = 0·865)

The critical salt concentration for denaturation was between 10^{-3} and 10^{-4} M sodium chloride and the extinction was practically constant between 10^{-3} and 1 M sodium chloride[6]. $E^{1\%}_{1\text{cm}} = 193$ at 0·15 M sodium chloride. DNA solution was prepared according to a recent method[1]. 100 mg of fibre was initially dispersed in 10^{-3} M sodium chloride for 75 h in cold without agitation (0·2 per cent DNA), and 0·09 per cent concentration of DNA solutions in 0·001, 0·01, 0·1, 0·15, 0·2 M sodium chloride were prepared. These were spun after 45 h in a Servall rotor in the cold for 1 h, which resulted in no gel or precipitate. Freshly prepared de-ionized double-distilled water was used and the filling of the ultracentrifuge cell was done with the help of a No. 22 needle. The rotor speed selected was 39,460 rev./min.

Apart from the general conclusions made here from Fig. 1, there are other aspects of the mode of concentration dependence of sedimentation-rate. The inflexion in the curves in 0·01, 0·15 and 0·2 M sodium chloride near 0·01 per cent concentration of DNA is the result of presence of undispersed aggregates, which seems to be the consequence of the mode of dilution[7], namely, each dilution was made from the highest concentration of the stock solution (0·09 per cent). This is also evident from the fact that in the 0·1 M sodium chloride series (stock solution was stored for 4½ months), where dilution was made successively from the next higher concentration, the shape of the curve is different. The former method of dilution did not affect the 0·001 M sodium chloride series, where evidently dispersion of DNA in solution was rapid. In all the cases the minimum time allowed for dispersion in cold was 16 h without agitation.

It is evident that the method of handling the solution of DNA affects the mode of concentration dependence in the region of rapid variation of sedimentation with concentration. But this did not affect the extrapolated value of $S^{0}_{20,w} = 22·7 \times 10^{-13}$ sec. The solution stored for 4½ months in 0·1 M sodium chloride was also not degraded.

It is also observed that the sedimentation-rate is more sensitive to small variation of electrolyte in the lower ionic strength. Hence, any previous salt concentration in the DNA sample will affect the shape of the curve at lower ionic strength. The sedimentation is, however, less sensitive to change of moderately high concentration of salt. As seen in Fig. 1, at extremely low concentration region below 0·01 per cent, under identical methods of handling, the sedimentation-rates may be practically independent of moderately high salt concentrations. But at higher concentrations of DNA the constancy of sedimentation-rate at salt concentrations > 0·1 M sodium chloride, as reported by others[8], has not been found in this work.

The values of $S_{20,w}$ in 0·2 M sodium chloride agree with the recent values of others[3] in the concentration range 0·04–0·09 per cent. The values in 0·15 M sodium chloride series are consistently higher than those in 0·2 M sodium chloride series. The interpretation of this result may be tentatively made as follows. If the rod-like shape for DNA is assumed, the sedimentation-rate will measure the *M*/*L* (mass/length)[4]. Then higher $S_{20,w}$ value at 0·15 M sodium chloride will mean greater *M*/*L*. Similar results on the basis of light scattering measurement showing greater *M*/*L* at 0·15 M sodium chloride compared with that at 0·2 M sodium chloride can be seen in the work of others[2].

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Coefficient of Thermal Conduction: Homogenized Tissues: Thermal Conduction and the Rate of Blood Flow

THE coefficient of thermal conductivity has already been measured for slices of various dog tissues[1], and found to vary from 0·6 × 10^{-3} for fresh lung to 5·5 × 10^{-3} for fresh muscle. The same apparatus can be used for measuring K for homogenized tissues; in general, these have values of K which vary less from each other than do those for tissue slices.

The technique used is the same as that already described, except that chamber II of the apparatus is filled with homogenized tissue instead of fresh tissue slices. The homogenization is carried out for 5 min in a Waring blender; the homogenized tissues are then transferred to plastic centrifuge tubes and spun for 15 min at 10^3g to free them from air. The measurement of K is made after three relaxation times for the tissue have elapsed; chamber I is kept at 28° C, the temperature in chamber II is initially 20° C, and the quantity of heat passing through it to chamber III (initially at about 6° C) during the fourth and fifth relaxation times is found. The steady-state equations are applied to this to find K.

The average values of $K \times 10^3$ (6 determinations for each tissue) for the homogenates are: muscle 3·6, liver 2·5, brain 1·6, and lung 2·0. These can be compared with the values of $K \times 10^3$ for tissue slices: muscle 5·5, liver 3·3, brain 1·7, and lung 0·6. Homogenization thus reduces the value of K for muscle and liver to about 70 per cent, affects the value of K for brain only a little, and raises the value of K for lung by about three-fold. The value of K for water is 1·5 × 10^{-3}, which agrees well with the accepted value. In all cases the variation from the average is not more than ± 10 per cent; this applies to both tissue slices and tissue homogenates.

The conclusion is that the thermal conduction in tissues such as muscle and liver is reduced by homogenization, heat being able to move better in the presence of structural pathways than in their absence; in the case of brain, homogenization makes little difference, and the effect of homogenization in the case of lung is clearly due to the removal of air.

With reference to the hypothermic state in which blood at low temperatures is pumped into the circulation, it is known that all parts of the body are not cooled equally. This may be the result of the flow in some regions or organs being greater than that in others, the result of some tissues conducting heat to a greater extent than do others, or the result of both factors. Suppose that the rate of cooling of an organ, $\Delta\theta/\Delta t$, is entirely dependent on the extent to which it is a thermal conductor. If it is supplied with a constant flow of cold blood, $\Delta\theta/\Delta t$ should be highly and positively correlated with the coefficient of thermal conductivity K; if the correlation is small, $\Delta\theta/\Delta t$ cannot be largely dependent on K, and the conclusion must be that the flow of the blood through different organs varies.

The rate of cooling of a tissue is measured by perfusing cold blood into the femoral artery of the dog and collecting the venous return from the superior jugular and femoral veins. This return is measured in ml./min, and passes to a disk oxygenator, then through a DeBakey rotary pump, then to a Brown–Harrison heat exchanger, and then back to the animal. Calibrated needle thermistors are placed in the tissues in which the rate of cooling is to be measured; these are usually the brain, lung, liver, muscle, and kidney, and thermistors are also placed in the arterial and venous streams. The flow of blood is 10–50 ml./min/kg of the animal's body-weight, and is constant for the time Δt, which is usually an interval of 10–20 min from the beginning of the perfusion of cold blood. The temperature of the tissues falls during this interval and tends to approach that of the arterial blood, the form of the falling temperature curve being that of Newton's law of cooling, that is, log θ being linear with time.

The coefficient of thermal conductivity K is measured for each tissue as already described[1]; $\Delta\theta/\Delta t$ is then correlated with K. The coefficient of correlation r is 0·364 ± 0·181, that is, it is scarcely significant, and so the conclusion is that the rate of cooling of the tissues is determined by the different rates of flow of cold blood through them, and not to a significant extent by their thermal conductivity, variable though this is (a ten-fold variation).

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BIOCHEMISTRY

Physical Significance of Michaelis Constants

Briggs and Haldane[1] showed that for the reaction sequence $E + S \rightleftharpoons ES \rightarrow E + P$, the same form of initial rate equation is obtained by the steady-state assumption as by the more restrictive equilibrium assumption of Michaelis and Menten[2]. It was evident, therefore, that the Michaelis constant, K_m, defined[3] as the substrate concentration with which half the maximum rate is attained, is not necessarily equal to the substrate constant, K_s, the dissociation constant of the enzyme–substrate complex. Nevertheless, it is still sometimes assumed[4,5] that the Michaelis constant is a measure of the affinity of the substrate for the enzyme. In a recent text-book[6], it is stated that the Michaelis constant must be either equal to or greater than the substrate constant. It seems worth while to point out that this is not so, and to indicate relationships between K_m and K_s values derived from reaction schemes for reversible reactions involving one and two substrates.

Single Substrate Reactions. For the simple reaction sequence postulated by Michaelis and Menten[1]:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_{+2}} E + P$$

steady-state treatment[2] gives $e/v_0 = 1/V + K_m/V[S]$. where $K_m = (k_{-1} + k_{+2})/k_{+1}$. The necessary and sufficient condition for $K_m = k_{-1}/k_{+1} = K_s$ is that $k_{+2} \ll k_{-1}$, whereby the breakdown of ES to give product is too slow to disturb the equilibrium with enzyme and substrate. (The conclusion[7,8] that this requires $k_{+2} \ll k_{+1}$, deduced from $K_m = K_s + k_{+2}/k_{+1}$, is incorrect, since K_s may also be much less than unity.)

Obviously K_m cannot be smaller than K_s if this simple postulate is valid. The situation is otherwise if two or more enzyme complexes are present in comparable concentrations in the steady state:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E + P$$

Steady-state treatment gives the same form of initial rate equation as before, but the Michaelis constant for S is a complex function of rate constants[9] which may be written:

$$K_m = K_s\left[\frac{k_{-2} + k_{+3} + k_{+2}\,(k_{+3}/k_{-1})}{k_{-2} + k_{+3} + k_{+2}}\right]$$

If $k_{+3} \gg k_{+2} + k_{-2}$, then $K_m = (k_{+2} + k_{-1})/k_{+1}$, as before; the restrictions merely ensure that $[EP]$ is negligible. In general, however, K_m may be greater or smaller than, or equal to, K_s, according to the relative values of k_{+3} and k_{-1}. These are the specific rates of the last steps of the forward and reverse reactions respectively. Therefore, by symmetry, for a reversible reaction, either $K_m = K_s$ in both directions, or if $K_m > K_s$ in one direction, the reverse must be true in the opposite direction.

If three intermediate complexes are postulated, the relations are more complicated[9], but it is still true that if the specific rate of the last dissociation step, which gives the product, is less than the specific rate of dissociation of the ES complex, the dissociation constant for the latter, K_s, will be greater than the Michaelis constant for the substrate.

Two-Substrate Reactions. The Michaelis constant for each substrate in a two-substrate (or coenzyme-substrate) reaction varies in general with the concentration of the other[10]. It seems best[11] to adopt the convention of infinitely large concentration for the other substrate, and to define K_m for S_1, for example, as the concentration which gives a partial maximum rate ($[S_2] \rightarrow \infty$) equal to half the true maximum rate, $V([S_1],[S_2] \rightarrow \infty)$. The initial rate equations derived for three main types of mechanism postulated for such reactions are then most closely analogous to that for a single substrate reaction. The equation:

$$\frac{e}{v_0} = \frac{1}{V} + \frac{K_{m_1}}{V[S_1]} + \frac{K_{m_2}}{V[S_2]} + \frac{K_{m_2}K_{s_1}}{V[S_1]\,[S_2]} \qquad (1)$$

has been derived for two types of mechanism, discussed in detail elsewhere[11,12]. Briefly, they are: (1) an alternative pathway mechanism in which binary complexes of each substrate with the enzyme, and a ternary complex, are assumed to be in equilibrium with the free substrates; (2) compulsory order mechanisms in which only one substrate (or coenzyme) forms a binary complex.

For mechanism (1), the Michaelis constants defined as here are equal to the dissociation constants of the *ternary* complexes, for example, K_{m_1} is the dissociation constant for $ES_1S_2 \rightleftharpoons ES_2 + S_1$, and may be greater or smaller than K_{s_1}, the dissociation constant for $ES_1 \rightleftharpoons E + S_1$. Moreover, the ratios K_{m_1}/K_{s_1} and K_{m_2}/K_{s_2} must be equal, because of the thermodynamic relation between these four equilibrium constants. (The case in which the ratio is unity, that is the combination of the enzyme with one substrate does not affect its affinity for the other, can be distinguished experimentally by a relationship between the initial rate parameters[12].)

For mechanisms of type (2), the Michaelis constant is a function of rate constants for reactions which do not involve either the free or bound substrate, and does not have any simple physical significance in general. The simplest is the Theorell–Chance mechanism[10], in which ternary complexes are either not formed or are not rate-limiting[12].

$$E + S_1 \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES_1$$

$$ES_1 + S_2 \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} ES_1' + S_2'$$

$$ES_1' \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E + S_1'$$

For S_1, the leading substrate, or coenzyme, in the forward reaction, $K_m = k_{+3}/k_{+1}$ and therefore $K_m/K_s = k_{+3}/k_{-1}$. The corresponding quantities for S_1', in the reverse reaction, are the reciprocal ratios, k_{+1}/k_{+3} and k_{-1}/k_{+3}. Thus, the Michaelis constants and substrate constants will be equal only if the rates of dissociation of the reactant and product enzyme complexes are equal; and if $K_m > K_s$ in one direction, the reverse must be true in the reverse reaction. This is illustrated by experimental data for liver alcohol dehydrogenase: at *p*H 6·0, the Michaelis constants for nicotinamide-adenine dinucleotide (NAD) and NADH are 4·1 μM and 14 μM respectively[13], and the dissociation constants for the compounds of the coenzymes with the enzyme are 266 μM and 0·22 μM respectively[14].

If one or more rate-limiting ternary complexes are included in the foregoing reaction scheme, it can be shown[12] that for S_1, $K_m < k_{+3}/k_{+1}$. In general, with this type of mechanism, if the specific rate of dissociation of the product complex is smaller than that of the leading substrate complex, the dissociation constant for the latter will be greater than the Michaelis constant, as in the case of a single substrate reaction.

The third type of mechanism for two-substrate reactions is that in which the enzyme reacts with each substrate in turn, via a binary complex, to form a product, and itself undergoes intermediate chemical change by oxidation or reduction or group transfer[11,12]. The initial rate equation is:

$$\frac{e}{v_0} = \frac{1}{V} + \frac{K_{m_1}}{V[S_1]} + \frac{K_{m_2}}{V[S_2]} \qquad (2)$$

Again it can be shown that the relative values of K_m and K_s for each substrate depend on the relative rates of dissociation of the two binary enzyme–

substrate complexes. If $K_m > K_s$ for the substrate which reacts with one form of the enzyme, the reverse must be true in the reverse reaction for the substrate which reacts with the same form of the enzyme.

It has been shown[15,16] that competitive inhibitors present as impurities in coenzymes have quite different effects on initial rate parameters according to the relative values of K_m and K_s for the coenzyme. It may also be worth pointing out that, in a two-substrate reaction, a concentration of one substrate much greater than its Michaelis constant is not necessarily sufficient to ensure that the system becomes kinetically equivalent to a single substrate reaction. For example, in equation (1), if $[S_1] \gg K_{m_1}$ the second term will be negligible, but the last term may not be if $K_{s_1} > K_{m_1}$. This is of importance in kinetic studies of inhibition in such systems.

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Production of Staphylococcal Coagulase in Stirred and Aerated Culture

AN interest in possible production methods for staphylococcal coagulase made us consider whether a submerged culture technique could be developed for this purpose since experience in these laboratories with diphtheria toxin production[1] had shown submerged culture to be a convenient and rapid method with many advantages over the older techniques of static culture. Submerged culture of coagulase had been attempted by Duthie and Haughton[2], who used the vortex system of aeration[3], but they had found only small yields of coagulase. On the other hand, Davies[4] had found that aeration of his cultures improved the coagulase yield, and Fahlberg and Marston[5] found an increase in coagulase production in shaken flasks as compared with unshaken flasks. It appeared therefore that, by a suitable choice of conditions, good yields of coagulase might be obtained in stirred aerated culture and small-scale experiments were started.

We used as a fermenter a 7-lb. Kilner jar, fitted with a metal top, which carried a rotating seal and a guide for the stirrer shaft. The top had an opening for inoculating and sampling and an air inlet and outlet. The impeller was a conventional turbo-mixer, 1/3 of the diameter of the tank, and was driven at 1,425 r.p.m. 1·75 l. medium were added to the jar which was standing in a constant-temperature water-bath at 35° C. Air was passed over the liquid at 200 ml./min and 25 per cent 'Antifoam *A*' (Midland Silicones, Ltd.) in butyl acetate was added as required. The organism used was *Staphylococcus aureus 5R* (*CN* 4575 in the Wellcome Bacterial Collection); this strain was known to produce high titres of coagulase when grown in the rough form[6]. It was also known to produce γ-lysin[7]. The inoculum was prepared by picking rough colonies from a chocolate agar plate and growing these in a baffled shake flask containing 100 ml. medium. The flask was shaken for 24 h on a rotary shaker (120 r.p.m. 3/4 in. throw) in an incubator at 35° C. 0·1 ml. of the resultant culture was used as an inoculum for the Kilner jar. Coagulase was measured by a modification of the method of Rammelkamp *et al.*[8] and titres are expressed as reciprocals of the highest dilutions causing clots. γ-lysin titres are reciprocals of the highest dilutions showing lysis of rabbit red blood cells.

Two media were used in this work; the first was nutrient broth which was sterilized by autoclaving. Using this medium growth and coagulase production were complete 28 h after inoculation, a coagulase titre of 3,200 was found and this was not altered after a further 18 h. The organisms did not appear to back mutate to the smooth type, all colonies arising from samples plated on chocolate agar being rough. The disadvantage of using this medium was the production of γ-lysin, titres of up to 64 occurring; it was therefore decided to try a different medium to see if this could be avoided. The medium selected was based on a papain digest of beef[9]. It was sterilized by filtration and had a final *p*H of 8·4. The first experiments using this medium were not encouraging. Growth was good, but 24 h after inoculation only low titres of coagulase were present. Further investigation, however, showed that high values could be obtained provided that growth was stopped after about 8 h and that if this was not done there was rapid destruction of the already formed enzyme (Table 1).

The formation of γ-lysin was, however, negligible in the 8 h sample; thus it seemed worthwhile to attempt to produce coagulase using this method and typical results are given in Table 2.

Table 1

Exp. No.	Coagulase titre after: 6 h	8 h	10 h	12 h	14 h
CT 5	160	1,600	320	80	80

Table 2

Exp. No.		Coagulase titre after. 3 h	4 h	5 h	6 h	7 h	8 h	9 h	10 h
CT	7	64	256	256	1,024	1,024	3,200	ND	ND
	16	ND	4	8	16	320	640	1,280	320
	17	80	320	640	2,560	2,560	1,280	ND	ND
	22	40	160	160	1,280	1,280	ND	ND	ND
	28	16	64	320	640	2,560	ND	ND	ND
	29	4	8	32	320	640	2,560	ND	ND
	31	4	16	64	640	1,280	640	ND	ND
	39	8	16	128	640	1,280	2,560	ND	ND

Coagulase was produced rapidly in these cultures and over-incubation by as little as an hour led to considerable destruction. The γ-lysin titre never exceeded 4. Since coagulase assay required 3 h it would be necessary to use a more rapid assay (see, for example, ref. 2) to determine the correct harvesting time. Exploratory work using aluminium tanks containing 11 l. medium has indicated that the scaling up of this process should not present any difficulty.

The reason for the rapid destruction of coagulase in these cultures has not been determined. It did not appear to be mechanical destruction since coagulase stirred in the absence of organisms remained stable, and addition of gelatin to cultures as a protective colloid did not prevent destruction. When 0·01 per cent 'Thiomersalate' was added to the organisms destruction ceased immediately and culture filtrates of organisms were not active in destroying the enzyme. It seems, therefore, that it was the organisms which were destroying the enzyme and it is of interest to note that this phenomenon may explain the fact that Duthie and Haughton found only small amounts of coagulase when they attempted production by submerged culture.

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Malignolipin and Malignant Tumours

Kosaki *et al.*[1–3] have recently reported the isolation of a new specific phospholipid, malignolipin, from malignant human and experimental tumours. They have described its role as a promoter of carcinogenesis[4] and have claimed to have evolved a histological test for the identification of malignolipin[5] and a diagnostic test for cancer[6,7] based on the detection of this phospholipid in blood. Anti-malignolipin sera have been reported[8] to reduce the viability of Yoshida sarcoma cells *in vitro*. These results await confirmation from other laboratories. The following therefore represents an attempt at isolating malignolipin from malignant tumours.

The material used in the work reported here comprised two types of human tumours (human tumours were kindly supplied by Dr. M. V. Sirsat, Pathology Department, Tata Memorial Hospital, Bombay)—breast and œsophagus carcinomas; three types of experimental tumours—a transplantable mouse fibrosarcoma originally induced by Waravdekar and Randive[9] and maintained in Swiss mice, spontaneous and transplantable breast tumours carried in *C3H* (*Jax*) mice and a spontaneous reticulum cell sarcoma in golden hamsters; and normal mouse liver.

Each of the foregoing types of material was worked up in at least two separate experiments. The method of Kosaki *et al.*[2] was carefully followed in all its details for the isolation of malignolipin picrate.

All the solvents used for the extraction of malignolipin and the preparation of its picrate were previously purified by standard procedures. In each experiment, at least 12–15 g of the material were ground with 9 volumes of absolute ethanol. The alcohol extract was precipitated with acetone. The precipitate was filtered through a sintered glass funnel, dried and eluted with petroleum ether. This eluate was further precipitated with acetone, filtered through a sintered glass funnel, dried and finally eluted with benzene. The benzene extract was mixed with an equal volume of saturated solution of picric acid in benzene. The picrate formed was filtered through the sintered glass funnel, washed free of excess of picric acid with benzene and crystallized three times from dioxan/benzene (1 : 7). It was finally crystallized from acetone.

A lemon-yellow picrate melting at 258°–65° C was obtained from all the malignant tumours as well as from normal mouse liver. The mixed melting points of the picrate derived from these materials remained undepressed. The melting point of the picrate, however, was different from that quoted[3] for malignolipin picrate (123° C). Unlike the bluish-white fluorescence in the ultra-violet reported[3] for the crystals of malignolipin picrate, this compound (m.p. 258°–65° C) does not exhibit any fluorescence in the ultra-violet either in the solid state or in solution. It is readily soluble in water, ethanol, acetone and dioxan but sparingly soluble in benzene, petroleum ether and chloroform. It did not show the presence of sulphur, halogens and phosphorus by Lassaigne's test[10]. Phosphorus could not be detected in any of the picrates even by Lowry's micromethod[11]. Analysis of the picrate shows C, 28·7 per cent, and H, 2·5 per cent by combustion method and N, 22·5 per cent by Johnson's method[12].

Unidimensional ascending paper chromatography of the picrates in butanol/pyridine/water (8 : 2 : 1) at 28° C yielded two spots with R_F values 0·6 (I) and 0·26 (II). Spot (I) was identified as picric acid from its R_F value and absorption spectrum. It appears that the complex decomposed during chromatography. Similar decomposition of the picrates during chromatography has also been reported by other workers[13,14]. The spot (II) with R_F value 0·26, when eluted with water, has no absorption in the visible range but has a λ_{max} at 256 mμ. The picrate has no absorption in the ultra-violet, but in the visible region it has an absorption maximum at 360 mμ due to the picric acid part of the molecule.

In this investigation so far, I have been unable to isolate malignolipin picrate (m.p. 123° C) from as many as five different types of pathologically confirmed malignant tumours listed above. Instead, a non-specific picrate has been obtained; its properties are much different from those reported for malignolipin picrate.

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Differences between Urinary Albumin and Serum Albumin

In the work reported here, the molecular size and the peptide patterns of the albumins found in normal urine were compared with those of serum albumin.

Urine obtained from normal subjects was dialysed against distilled water and concentrated by freeze drying. The dry powder was dissolved in 0·0175 M phosphate buffer at *p*H 6·3 and applied to columns of diethylaminoethyl cellulose (DEAE). After separation of the first fraction eluted with the starting buffer at *p*H 6·3[1], a linear concentration and *p*H gradient was applied to the cellulose column (0·0175→0·4 M phosphate; *p*H 6·3→5·2). The fractions eluted by the buffer between 0·3 and 0·4 M phosphate were pooled, dialysed and concentrated. The proteins were further fractionated on a block of 'Pevikon *C*–870' using a borate buffer of *p*H 8·6, μ 0·064. The extinction coefficient of fraction *A* (corresponding to area *A*, 10 and 11 cm from the origin, Fig. 1,*A*) $E^{1\%}_{1\text{cm}}$ was 11·0, a value considerably higher than that obtained for serum albumin, which was 5·32. This high value was presumably due to the presence of bound urinary components rather than to an increased concentration of aromatic amino-acid residues; dialysis of serum albumin against normal urine, followed by extensive dialysis against 0·15 M sodium chloride, gave a solution of serum albumin with an $E^{1\%}_{1\text{cm}}$ = 49.

Fig. 1,*C* shows the immunoelectrophoretic pattern obtained when fraction *A* and normal human serum were reacted with an antiserum prepared by the injection of a horse with normal human serum (Inst. Pasteur, Lot No. 13411). Fraction *A* gave a single precipitin line corresponding to a protein that was slightly more electronegative than serum albumin at *p*H 8·6. It also gave a reaction of complete identity with crystalline serum albumin when reacted with an antiserum albumin in two-dimensional agar gel diffusion plates (Fig. 1,*F*). It did not react with an antiserum prepared in a goat against human orosomucoid (Hyland Labs. Lot No. *GABS* 88).

Increasing dilutions of a known weight of fraction *A* and of a standard solution of crystalline serum albumin were titrated by gel diffusion (in an Ouchterlony plate) against a standard solution of the antialbumin antiserum. Using as the end-point the greatest dilution of antigen (serum albumin or fraction *A*) which gave a visible precipitin line with antiserum, such lines were not discernible with less than 9·3 μg of fraction *A* or 6·3 μg of serum albumin.

Ultracentrifugal analyses of 5 samples of fraction *A* from urines of different individuals each gave a single peak with an average sedimentation constant, $S_{w,20}$ of 2·6 ± 0·2 (uncorrected for protein concentration) as compared to $S_{w,20}$ 4·2 for crystalline serum albumin of comparable concentration.

Samples of urinary albumin and crystallized serum albumin were denatured at *p*H 8·0 by heating at 100° C for 45 min, then hydrolysed at *p*H 8·0 with chymotrypsin in the *p*H-stat[2] in an enzyme to substrate ratio of 1 : 8 and the peptides separated in two dimensions on No. 1 Whatman filter paper, first by high-voltage electrophoresis in one dimension followed by ascending chromatography in the second dimension as previously described[3]. The resulting peptide patterns are shown in Fig. 1,*D* and *E*. Although the 'albumins' obtained from urines of different individuals had similar mobilities in zone electrophoresis, some of them were found to be more electronegative than serum albumin at *p*H 8·6 when analysed by immunoelectrophoresis. Fig. 1,*D* shows a representative peptide pattern obtained with the more electronegative urinary albumin. A poor differentiation of spots was obtained on the peptide patterns when the more electropositive albumins were analysed. The urinary albumins that were more negatively charged at *p*H 8·6 gave patterns which had little or no resemblance to those obtained after digestion of serum albumin. Whether this difference is due either to a unique denaturation of urinary albumin following renal filtration or to a splitting of the peptide bonds *in vivo*

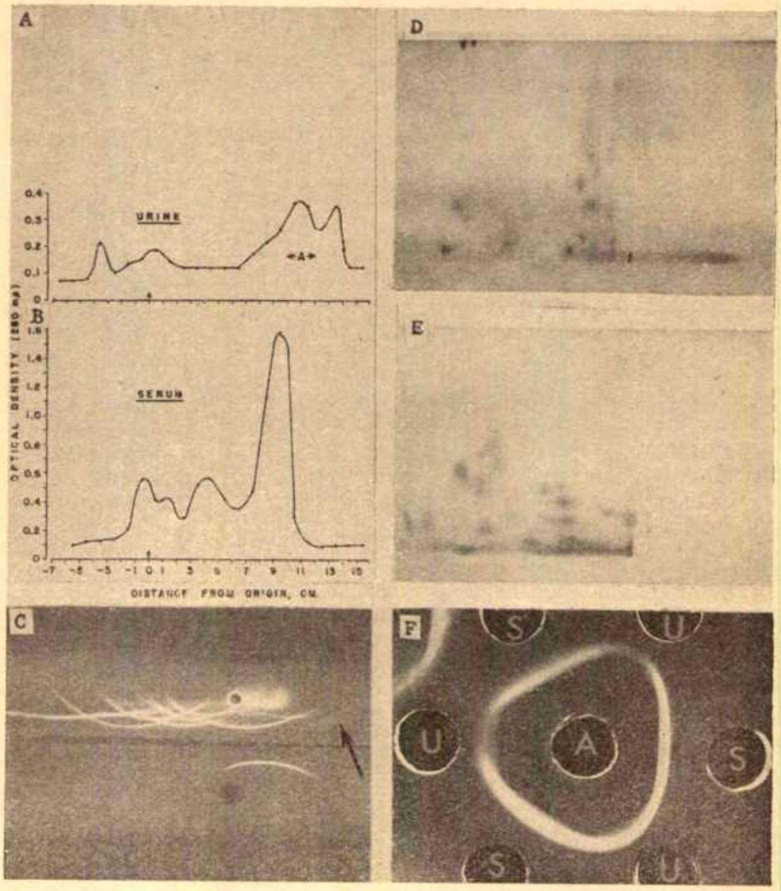

Fig. 1. Starch-block electrophoresis: pattern of urine (*A*) and serum (*B*). Immunoelectrophoresis (*C*) of the more electronegative (at *p*H 8·6) urinary albumin (lower well) and normal human serum (upper well). Centre trough contains horse anti-human serum. The precipitin line corresponding to serum albumin is marked by arrow. Representative peptide pattern of the urine albumin (*D*) and serum albumin (*E*). Ouchterlony plate (*F*) *U*, urine albumin; *S*, crystalline human serum albumin; *A*, anti-human serum albumin (horse)

with formation of smaller fragments held together by disulphide bonds is not known.

If serum albumin is re-absorbed by the tubules following glomerular filtration[4] it is interesting that at least some of these smaller molecules of albumin appear in the urine and presumably escape re-absorption.

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Quantitative Analysis of Sugars in Royal Jelly

THE amount of carbohydrate in royal jelly, determined in terms of total reducing substances, has been investigated. The results are seen in tabular form in a review by Johansson[1] and show concentrations of carbohydrate in fresh royal jelly ranging from 8·3 to 29·1 per cent by weight. During one of these investigations[2] an additional test was conducted, which was based on selective fermentation procedures, whereby a sample of native royal jelly, having a total reducing-sugar concentration of 29·1 per cent (calculated in terms of glucose), was found to have an apparent glucose concentration of 12·2 per cent, a fructose concentration of 15·2 per cent and a concentration of 1·7 per cent for non-fermentable carbohydrates.

It has been determined that D-glucose, D-fructose, and D-sucrose are the principal sugars in royal jelly[3]; the results having been based on the isolation and identification of crystalline sugars and their derivatives. Working with a sample of royal jelly from the same source[4] as that used in the work just cited, the investigation now described is a quantitative analysis of the principal sugars in royal jelly, conducted using the methods of paper partition chromatography and quantitative colorimetric assay, following an established procedure[5]. This method of analysis, involving the fractionation of sugars prior to colorimetric quantitative analysis of each sugar, is believed to be more reliable than methods based on the fermentation or reducing characteristics of one sugar in the presence of others.

Standard curves for the sugars to be analysed were prepared in the following way. Standard solutions of D-glucose, D-fructose and D-sucrose were prepared and aliquots of each, analysed in triplicate, ranging in concentration from 5 to 30 μg of sugar per ml. of solution, were transferred to matched colorimeter tubes. Aqueous phenol (0·100 ml. of 80 per cent w/w) and sulphuric acid (5·00 ml. of 36 normal) were added and the contents of the tubes carefully mixed, stoppered lightly, and then allowed to cool for 0·5 h. Absorbances of the yellow-orange solutions were determined with a 'Spectronic 20' spectrophotometer at a wave-length setting of 490 mμ. The absorption data for the three sugars, after compensation with blank samples, are shown in Table 1. Standard curves for the sugars were then constructed as shown in Fig. 1.

Table 1. ABSORPTION DATA FOR D-GLUCOSE, D-FRUCTOSE AND D-SUCROSE (STANDARD SOLUTIONS)

Compound	Concentration (μg/ml.)	Absorbance Per sample	Absorbance Average
D-Glucose	7·8	0·102	0·101
		0·102	
		0·098	
	13·6	0·187	0·185
		0·182	
		0·186	
	26·2	0·337	0·329
		0·321	
		0·329	
D-Fructose	6·6	0·130	0·131
		0·132	
		0·130	
	13·5	0·256	0·258
		0·260	
		0·257	
	28·0	0·520	0·515
		0·512	
		0·513	
D-Sucrose	7·7	0·112	0·116
		0·116	
		0·119	
	15·6	0·224	0·225
		0·225	
		0·226	
	22·3	0·318	0·317
		0·315	
		0·320	

In order to separate and analyse the sugars in royal jelly, an aliquot portion (20–30 mg) of royal jelly, weighed by difference using a micropipette, was placed on the 'starting line' of Whatman No. 1 chromatography paper (18¼ in. × 22½ in.). The sugars were subjected to partition in the usual way[3,5,6], using *n*-butanol/ethanol/water (4 : 1 : 5, upper layer) as the irrigating solvent, during a 24-h period. By cutting small strips from the paper and spraying these strips with Tollens reagent according to a method described in a previous paper[3], the positions of the sugars on the chromatography paper were determined. Using this solvent the sugars were differentiated from each other by relating their R_F values to those of D-glucose (R_F, 0·16), D-fructose (R_F, 0·19), and D-sucrose (R_F, 0·03).

After eluting the sugars from the paper with water (20·00 ml., 15 min) the solutions were filtered through glass wool and aliquot portions (1·00 ml.) transferred (in triplicate) to matched colorimeter tubes. Blank samples were prepared by cutting from the chromatogram segments of paper which held no sugars, but which were equal in area to those segments from which sugars were eluted. Thus, each sugar fraction separated from the paper had a corresponding blank fraction. These blank segments were washed in water (20·00 ml., 15 min) and the extracts filtered through glass wool.

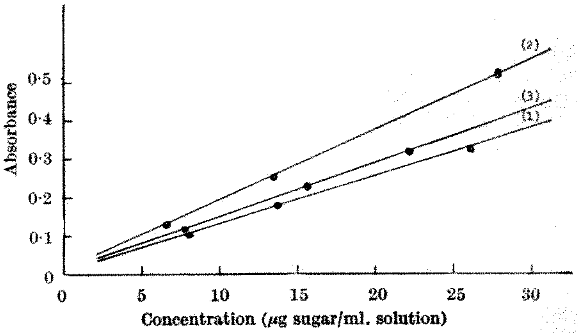

Fig. 1. Standard curves. 1, D-Glucose; 2, D-fructose; 3, D-sucrose

Aliquot portions (1·00 ml.) were transferred (in triplicate) to matched colorimeter tubes. Aqueous phenol (0·100 ml. of 80 per cent w/w) and sulphuric acid (5·00 ml. of 36 normal) were added to all sugar and blank samples. The contents of the tubes were carefully mixed and were then stoppered lightly and allowed to cool. After 30 min the absorbances of the solutions were determined using a 'Spectronic 20' spectrophotometer set at 490 mμ. Average values were determined and referred to the standard curve for each particular sugar, after compensating for the corresponding blank values. The concentration of each sugar in royal jelly was thereby determined; the results of three independent analyses are shown in Table 2. Based on an average of the three analyses, as shown in Table 2, the royal jelly sample which was analysed contained (per cent by weight) 9·76 D-glucose, 11·32 D-fructose and 0·94 D-sucrose.

Table 2. ANALYSIS OF SUGARS IN ROYAL JELLY
Concentration (% w/w) of sugars in royal jelly

	Trial No. 1	2	3	Average value
D-Glucose	9·82	9·77	9·66	9·76
D-Fructose	11·29	11·31	11·35	11·32
D-Sucrose	0·91	0·92	0·99	0·94

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Effect of Urea on the Synthesis of Polynucleotides with Crude Bacterial Extracts

A CRUDE bacterial extract is normally not capable of catalysing a net synthesis of poly-A from ADP[1]. If, however, the myokinase-like enzymes are blocked by the addition of ATP one observes a considerable net synthesis of polynucleotides with crude extracts of *Azotobacter vinelandii*, as we have found recently[2,3]. In this way the degradation of ADP, which leads to nucleosides and nucleobases via AMP, is inhibited. A complete inhibition of the adenylate kinase occurs if the ratio of ATP/ADP is 1 : 1 or higher; at lower ratios only an incomplete inhibition of the adenylate kinase and correspondingly a lower net synthesis of polynucleotide is observed. The amount of poly-A decreases after several hours because of phosphorolysis (Fig. 1).

We have now found that this phosphorolytic splitting of the polymer can be inhibited by adding 0·4 M urea. The course of the reactions is illustrated in Fig. 1.

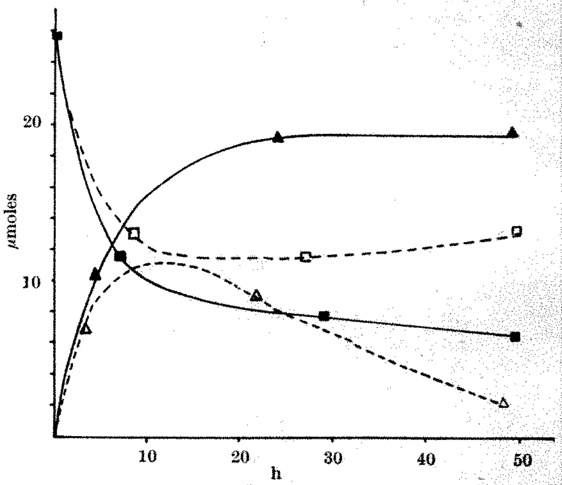

Fig. 1. Course of poly-A synthesis from ADP with a crude extract of *Azotobacter vinelandii*: 25 μmoles of ADP, 6 μmoles of ATP, 10 μmoles of magnesium chloride, 200 μmoles of *tris* buffer (*p*H 8·1), crude extract from *Azotobacter* containing 1·0 mg protein. Final volume 1·0 ml, temperature 30°. △, poly-A; □, sum of ADP + ATP without addition of urea; ▲, poly-A; ■, sum of ADP + ATP in the presence of 0·4 M urea

A. vinelandii was grown according to Hyndman *et al.*[4]. 23-g portions of wet bacteria were homogenized in 46 ml. of distilled water by means of a 'Zellhomogenisator' (Braun, Melsungen, Germany) with glass balls (0·11–0·12 mm) for 120 sec under cooling with carbon dioxide. The resulting suspension was sucked through a cool glass filter and centrifuged at 20,000*g* at 0° for 1 h. The supernatant liquid was frozen and kept at – 18°; it was directly used for the experiments. Protein was determined by the biuret method. Phosphate exchange was assayed according to Grunberg-Manago *et al.*[5]. The crude extracts had average specific activities of 0·5 unit/mg protein.

The course of the reactions was examined by means of paper chromatography. 1 per cent ammonium sulphate / isopropanol (1 : 2)[6] was used as the solvent. For quantitative analysis the spots, which had been located by means of a short-wave ultra-violet lamp, were cut out separately and eluted with 4·0 ml. of 0·05 M *tris* buffer (*p*H 7·0) for 15–20 h. Extinction coefficients used:

$$\varepsilon_1(\text{for ADP and ATP}) = 15{\cdot}78 \times 10^3;\ \lambda_{\max} = 259\ \text{m}\mu^7.$$

$$\varepsilon_2\ (\text{for poly-A}) = 9{\cdot}8 \times 10^3;\lambda_{\max} = 257\ \text{m}\mu^8.$$

A corresponding paper blank was subtracted.

The identity of the poly-A was proved by means of alkaline degradation, which resulted in a mixture of 2′- and 3′-AMP, and also through the examination of the ultra-violet spectrum[8]. The reaction product could not be dialysed. In paper chromatography and in cellulose layer chromatography[9] it remained at the origin of the chromatograms.

In the presence of urea, part of ATP is also used up indirectly for poly-A synthesis through the myokinase equilibrium. We do not, however, believe that in this case ATP is a direct substrate of the enzymatic polycondensation. This opinion is also supported by experiments in which ATP alone was incubated with the crude extract. Here a net synthesis of poly-A only started after ADP had been added. We therefore presume that ADP is the substrate of the reaction and that the enzyme in question is polynucleotide phosphorylase[5].

The effect of urea can also be used in the preparative synthesis of polynucleotides. 2·86 m.moles of ADP, 0·89 m.mole of ATP, 1·0 m.mole of magnesium chloride, 20 m.moles of *tris* buffer (*p*H 8·1) and crude extract of *A. vinelandii* (containing 100 mg protein) were made up with water to 100 ml. Urea was added to give a final concentration of 0·4 M. The solution was kept at 30° for 32 h, and the poly-A isolated according to Olmsted *et al.*[10]. The yield was 1·46 m.moles of poly-A (51 per cent). [*Note added in proof.* The molecular weight of the product was measured ($S^0_{20} = 2{\cdot}55$; corresponding molecular weight $= 3{\cdot}1 \times 10^4$) by H. Seidel and R. Iyanger.]

We cannot yet give a full explanation of the effect of urea. Urea might act as a competitive inhibitor[11]. The other possibility is that through the presence of urea the secondary structure of the poly-A chain might be changed in such a way that it can no more be attacked by the enzyme.

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Treatment of Sub-cellular Particles prior to Assay of the Enzymatic Marker, Succinic Dehydrogenase

IT is very useful to be able to pin-point the activity of a tissue enzyme in a morphologically distinct sub-cellular unit, and this is frequently attempted by measuring the activity of the enzyme in fractions or sub-fractions obtained by centrifuging tissue homogenates. Succinic dehydrogenase is generally accepted and frequently used as an enzymatic marker for mitochondria in fractionation studies. It appears, however, that it is difficult to recover 100 per cent of the activity of succinic dehydrogenase of a homogenate after fractionation, particularly after centrifugation of particles in gradients of dense sucrose[1–8], and the hopeful assumption has been made that the distribution of the recovered activity (ranging from 52 to 136 per cent) accurately reflects the true distribution (that is, that the errors or losses of activity are uniformly spread among the fractions). Experience has shown that this assumption can be far from the truth.

100 per cent recovery of activity is necessary in meaningful distribution studies and is usually, though not necessarily, achieved most easily when each fraction sample is made to show the maximum activity of which it is capable with the chosen assay system. For fractions obtained from brain homogenates by centrifuging in 0·3 M sucrose, suspension of the sedimented particles in water or very hypotonic buffer for an hour before assay was found to be a sufficient pre-treatment to produce maximum activity with a consequent high recovery of homogenate activity in the fractions[3]. It was presumed that the pre-treatment effectively reduced a limiting permeability barrier by osmotic swelling. However, this pre-treatment was not so successful with sub-cellular particles which had been centrifuged through high concentrations of sucrose or of Ficoll polymer in 0·3 M sucrose[2,4], nor do experimental conditions always permit the particles to be sedimented and resuspended in a medium of low tonicity prior to assay. This note describes an improvement in the treatment of sub-cellular fractions before succinic dehydrogenase assay such that good recoveries and nearly maximum activities are measured even after density gradient separation and even if the tonicity of the pretreatment medium cannot be reduced very far. By this procedure, recovery of succinic dehydrogenase activity among the *A*, *B* and *C* sub-fractions of a brain P_2 ('mitochondrial') fraction prepared by a modification of the method of Gray and Whittaker[9,10] was 96 ± 3 per cent (4 experiments).

The improved pretreatment is as follows: The suspended particles are diluted to a suitable volume with 3–10 mM phosphate buffer (ammonium or potassium), preferably containing 4 mM sodium succinate, and stored at 0° for 1–2 h. The suspension is frozen to − 20° or lower in a stoppered 'Pyrex' tube. It may be stored overnight in this state or else thawed after 20 min. The thawed suspension is kept at 0° for at least a further hour before assay. For the comparisons in Table 1, brain mitochondria were either suspended in mildly hypotonic sucrose (0·1 M) containing 10 mM ammonium phosphate buffer *p*H 7·4 (sample *A*), or were suspended in 5 mM potassium phosphate buffer *p*H 7·4 after they had been sedimented by centrifuging for 90 min at 75,000*g* in 1·1 M sucrose (sample *B*). Addition of 1–4 mM ethylenediamine tetraacetic acid to the suspension medium had little effect, but inclusion of magnesium chloride was deleterious. Inclusion of succinate seemed to increase the activity little, but sample activity was generally preserved for a longer period in its presence. Assay of activity of succinic dehydrogenase was by the manometric method of Aldridge and Johnson[3] using a hypotonic medium with ferricyanide as electron acceptor. This acceptor is suitable for assay of the particulate succinic dehydrogenase complex[11,12] and is preferred here over phenazine methosulphate both for its sensitivity and for the fact that a linear rate of carbon dioxide evolution is maintained throughout a 45-min assay enabling more accurate determinations to be made than with the latter acceptor which permits very brief assays only. The activities are recorded in Table 1 as percentages of that measured after the improved standard pre-treatment described here.

The results in Table 1 show that a freeze/thaw cycle applied to swollen particles was the best pre-treatment. A freeze/thaw cycle applied to particles suspended in 0·3 M sucrose raised the apparent activity from 35 to 84 per cent so the freezing is not

Table 1. COMPARISON OF PRE-TREATMENTS OF BRAIN MITOCHONDRIAL SUSPENSIONS BEFORE SUCCINIC DEHYDROGENASE ASSAY

	Pre-treatment	Sample *A*	Sample *B*
(*a*)	Stand 0°/15 min		
(*b*)	Stand 0°/1 h (unbuffered)	48 (2)	
(*c*)	Stand 0°/2½–4½ h (unbuffered)	86 (4)	
(*d*)	Stand 0°/2 h	70 (4)	63 (3)
(*e*)	Nil; add 'Triton *X*-100' to assay medium in concentration listed (%)		
	(i) 0·04	38	
	(ii) 01	67	
	(iii) 0·2	45	
	(iv) 0·5	18	
(*f*)	Standard	100 (6)	100 (6)
(*g*)	Standard (unbuffered)	90 (3)	
(*h*)	As (*f*) but ultrasonics* instead of freeze/thaw	85 (4)	
(*i*)	As (*f*) but also add 'Triton *X*-100' (0·125%) to assay medium	89	

* 90 sec (optimal) ultrasonic treatment at 20 kc/s

Succinate was not included in the pre-treatment media of these experiments. Numbers of determinations are noted in parentheses.

completely effective without some prior swelling of the particles. It should be noted that particles left in buffer suspension at 0° for periods of 5 h or more have been found to suffer loss of activity. The failure of ultrasonic treatment to produce full activity did not appear to be due to inactivation of the enzyme since a further 30-sec treatment did not lower the activity. The effect of increasing amounts of 'Triton *X*-100' is not surprising since the ferricyanide acceptor is not suitable when the particulate succinic dehydrogenase complex is disintegrated by various solubilizing treatments[12].

For some tissue preparations, a simple pre-treatment with calcium ions (0·75 mM) without freezing and thawing seems sufficient to obtain full activity using phenazine methosulphate as electron acceptor, but this was inadequate when applied to brain mitochondria[5]. The method described here has not been tested on a wide variety of tissues, but gives satisfactory results with liver and muscle fractions besides those from brain.

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Iso-Butyric Acid in Silage

The presence of iso-butyric acid in silage has not been reported since Klinc[1], using a qualitative chemical method of detection, claimed to have found it in the material in the surface layers of a silo. Barnett[2] during comprehensive investigations into the formation of the volatile fatty acids in silage found no trace of it, but the results of Lessard, Briggs and Scaletti[3] suggest its presence in samples of alfalfa silage made in the laboratory. No quantitative determinations of the acid have been reported.

In the course of the determination of the volatile fatty acids present in 400 samples of silage, in this Department, iso-butyric acid has been found in measurable amounts in 38 of them. The silages were made from grass, or grass and clover mixtures, and were taken from farms in the south-east of England in the winter of 1961–62. The levels of the acid found were low with an average value of 0·35 mg/ml. in the silage juice, or 0·13 per cent when calculated to a dry matter basis. The iso-butyric acid occurred in the same range of silage *p*H as the *n*-butyric acid, but it made up only a very small proportion of the total butyric acid present. Table 1 shows the *p*H-range in which the iso-acid occurred, and also the substantial proportion of the total number of samples in the range which contained it.

These results suggest that iso-butyric acid occurs widely in small amounts probably as the result of secondary fermentation processes in the silage. Its distribution in samples, therefore, might be expected to be even more widespread in winters following poor silage-making seasons.

Table 1

	*p*H			
	4·0–4·5	4·6–5·0	5·1–5·5	5·5–6·0
No. of samples containing iso-butyric acid	6	6	23	3
Percentage of total samples containing iso-butyric acid	4·9	13·0	57·5	42·6

The method used for the separation and determination of the volatile fatty acids in this work was based on a gas-liquid chromatography technique. The acids were steam distilled from a sample of the silage juice, extracted by means of ether and injected directly on to the column. The column was 8½ ft. long with a stationary phase of silicone 550 containing 12 per cent behenic acid coated on to 100–120 mesh 'Celite 545'. Nitrogen was used as the carrier gas and the temperature of the column was 135° C. The acids were detected by automatic titration with N/100 sodium hydroxide.

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Production of Humic Substances in Decomposing Peat and Compost Samples

During an investigation into the physical properties of humic acids, humic products were extracted from mixtures of papyrus and various additives such as different types of clays, which had been decomposing in natural tropical swamps for periods of up to 20 months, and from core samples collected from deep

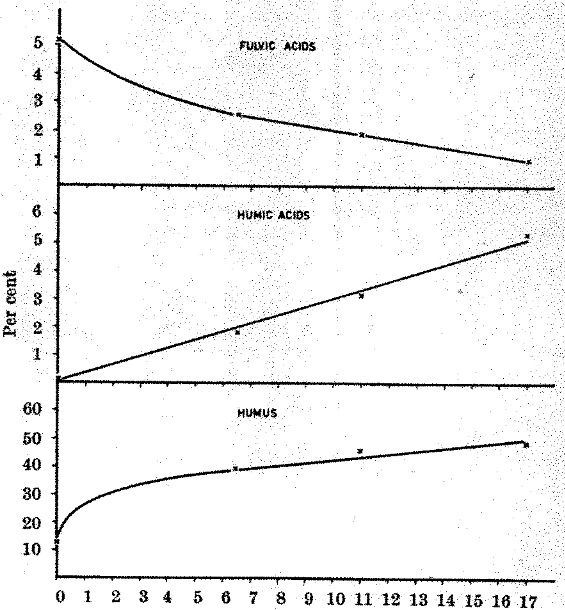

Fig. 1. Changes in the concentrations of the humic products in decomposing papyrus samples

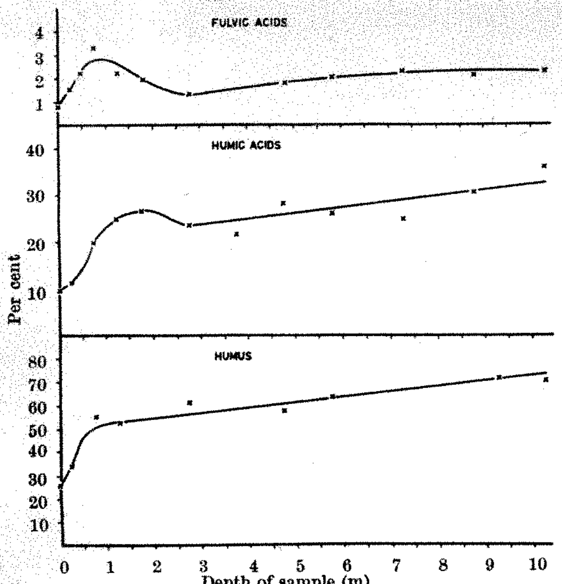

Fig. 2. Changes with depth in the concentration of the humic products in sphagnum peat

papyrus and sphagnum peat deposits (down to 20 m), by which the time-scale for the period of decomposition could be extended to durations exceeding 15,000 years.

It appeared (Figs. 1 and 2) that with progressing decomposition (and hence with older peat and compost samples) there were proportional increases in the contents of humic acids (extracted with 0·5 per cent sodium hydroxide and purified by reprecipitation in sodium hydroxide/hydrochloric acid) and humus (as the organic residue which was insoluble in acetyl-bromide).

It was found that the percentage of the coloured components of the fulvic acid fraction (estimated as the insoluble barium salts at *p*H 7·0 of the organic material soluble in the range *p*H 2·0–10·0)[1] could be used as an indicator of the speed of the decomposition process: if this had been fast, then the production of these acids greatly exceeded their polymerization, condensation or breakdown. Values were reached of up to 13 per cent for very rapidly decomposing samples, while for samples decomposing at a normal rate at the time of sampling, the fulvic acid content was consistently lower than 2 per cent.

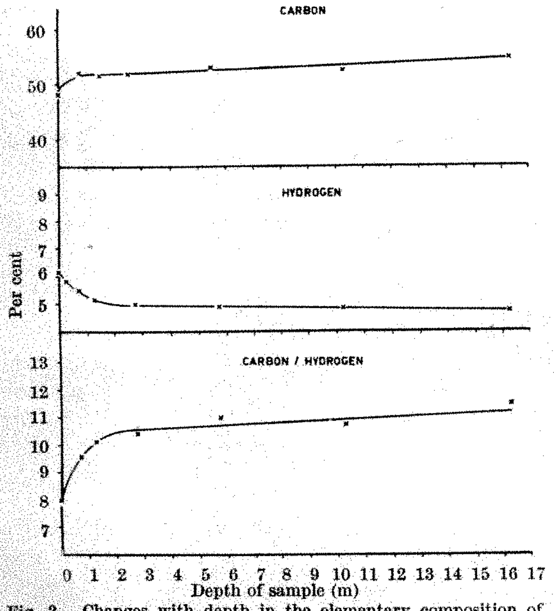

Fig. 3. Changes with depth in the elementary composition of the humic acids from a sphagnum peat

For the determination of the rate and course of the decomposition process previous to and at the time of sampling, several ratios of the humic products also proved to be of importance. The ratio fulvic acids/humic acids, for example, proved to be particularly high (1–40) in fresh and still actively decomposing material, while the ratio fulvic acids/humus was very low (< 0·05) in material which had actively decomposed in the past, but where the rate of decomposition had since slowed down.

For the core samples changes in these ratios with depth could also be well correlated with changes in the vegetation (as shown by pollen analysis) which contributed to the formation of the deposit layer.

The humic acids, with regard to elementary analyses, had a very different composition from what was previously recorded in the literature[2]. The carbon contents (34–55 per cent) were lower than the values which had hitherto been accepted as valid for these acids (55–60 per cent), and most of the percentages of hydrogen (5–11 per cent) and oxygen (40–55 per cent), on the other hand, were higher than had been expected (respectively 3–5 and 30–40 per cent).

As the carbon contents and the carbon/hydrogen ratios increased with progressing humification, and the percentages of hydrogen decreased (Fig. 3), it therefore seems likely that the humic products investigated, even those extracted from the deepest core samples (more than 16 m in depth and more than 15,000 years in age), are less humified than the more recently formed humic acids obtained by other investigators.

Table 1. AMOUNTS OF HUMIC PRODUCTS FORMED AFTER DECOMPOSITION OF PAPYRUS FOR 20 MONTHS UNDER VARIOUS CONDITIONS AND THE ELEMENTARY ANALYSIS OF THE HUMIC ACIDS

	Aerobic decomposition	Anaerobic decomposition	Anaerobic decomposition with kaolinite	Anaerobic decomposition with montmorillonite
Fulvic acids (%)	0·52	0·60	1·53	2·60
Humic acids (%)	0·13	0·27	2·07	1·27
Humus (%)	12·5	27·2	55·5	12·00
Elementary analysis of humic acids				
Carbon (%)	38·96	40·33	37·73	47·82
Hydrogen (%)	5·62	4·79	7·36	4·86
Oxygen (%)	53·65	53·21	52·50	41·40
Nitrogen (%)	1·77	1·67	2·41	5·92

It was also found (Table 1) that, in contrast with montmorillonitic clays, the presence of kaolinite results in a more rapid breakdown but in a less-advanced humification; therefore, although fewer humic acids are produced in the presence of montmorillonite, they are more humified.

It followed from most analyses that anaerobic or semi-aerobic conditions are more favourable for decomposition and humification than are aerobic ones. Indications were obtained that alternating anaerobic and aerobic conditions would be even more favourable.

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Effect of Pyridoxal 5-Phosphate in the Presence of Vanadyl Ions on the De-iodination of Thyroxine

In the course of a recent investigation of the effects of metal ions on the de-iodination of thyroxine, it was suggested that sulphydryl groups are involved in the regulatory mechanism of de-iodination on the cellular level[1,2].

Pyridoxal 5-phosphate has been shown to induce *in vitro* in the presence of certain cations, for example, vanadyl or aluminium, the catabolism of sulphydryl-carrying amino-acids[3-5]. It was of interest to check whether the pyridoxal phosphate–vanadium complex can affect the rate of deiodination *in vivo*.

Groups of 10 mice of local strain were used. 0·1 ml. of the investigated material in saline solution was injected intraperitoneally. One hour after injection, the mice were injected with thyroxine labelled with iodine-131. Two hours later, the mice were killed and the amounts of labelled protein-bound iodine and of iodide in serum were determined as follows: the serum proteins were precipitated by trichloroacetic acid (TCA), in the presence of iodide hold-back carrier, and centrifuged. Then the supernatant and washings were collected and the iodide was precipitated with 1 M silver nitrate. The labelled protein-bound iodine and the labelled silver iodide were counted, and the ratio of $^{131}I/^{131}PBI + ^{131}I$ was calculated. This ratio increases when de-iodination is enhanced.

Oxygen consumption-rate was also determined and the procedure adapted was as follows: the measuring system consisted of an Erlenmeyer flask of 1,200 ml. which was attached to a differential manometer filled with a silicon fluid (density 1·10). A known volume (100 ml.) of soda-lime inside a meshed container was introduced into the flask. Air-flow through the flask could be controlled by a stopcock. The measurements were carried out in a thermostat at 22 ± 0·5° C using three mice at a time, the weight of which was determined. Before each measurement, air was passed through the flask for a few minutes, the stopcock was then closed, and the change in pressure was followed by the differential manometer.

The results given in Table 1 show that whereas pyridoxal phosphate alone has no effect on the rate of de-iodination, vanadyl ions have a certain effect of enhancement. However, when vanadyl ions are administered simultaneously with pyridoxal 5-phosphate, the catalytic effect of vanadyl ions is doubled. It can be further seen from Table 1 that the oxygen consumption-rate diminishes as the rate of de-iodination increases.

The effect of thyroxine on pyridoxal phosphate-level in liver[6,7] and the effect of the latter compound on the deamination of thyroxine[8] have been reported. Our observation is, however, of a different phenomenon, namely, an effect on the rate of thyroxine de-iodination. The fact that pyridoxal phosphate alone has no effect on thyroxine metabolism shows that, within the short time of our experiments, there is no interference with the different modes of action of pyridoxal phosphate. The activation of pyridoxal by vanadyl ions is rather specific to alpha-beta elimination[3,9] and strongly suggests that it decreases the level of sulphydryl groups in the organism.

The results corroborate our former observations[2] that sulphydryl groups are involved in the process of de-iodination and that this process is not parallel to but reciprocal with the oxygen consumption-rate.

Table 1. EFFECT OF PYRIDOXAL 5-PHOSPHATE IN THE PRESENCE OF VANADIUM ON THE RATE OF DE-IODINATION OF THYROXINE AND ON RATE OF OXYGEN CONSUMPTION

Compound	Dose per mouse (mg)	$^{131}I/^{131}PBI + ^{131}I$ (× 100)	Difference (%)	*S.D.*	Oxygen consumption rate (ml. O_2/min/g)	Difference (%)
Controls	—	3·0 ± 0·28	—	—	0·065	—
Pyridoxal phosphate	0·4	2·6 ± 0·28	−13·3	< 70	0·065	—
VO⁺⁺	0·2	3·7 ± 0·28	+23·3	> 99	0·039	−40
Pyridoxal phosphate + VO⁺⁺	0·4 0·2	4·5 ± 0·19	+50·0	> 99	0·028	−57

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PHYSIOLOGY

Effect of Cortisone on the Urinary Excretion of Histamine in the Hyperthyroid Rat

Recent work has emphasized that histamine metabolism in the rat may be influenced by adreno-cortical[1,2] and thyroid[3,4] hormones. Various observations also indicate that these two groups of hormones may be functionally related in certain respects. It was therefore of interest to examine the action of cortisone on the urinary excretion of histamine in the hyperthyroid rat. It has been shown[3] that the rat treated with thyroxine has a high urinary histamine-level, and there is evidence that the high urinary histamine is the result of an increased rate of histamine synthesis[4].

The urine of female, non-pregnant Sprague–Dawley rats (initial body-weight about 150 g) was collected in 24-h portions under hydrochloric acid. The rats were given 10 g of a semi-synthetic, histamine-free food. The free urinary histamine (μg histamine base per 24 h) was determined by bioassay on the ileum of guinea pigs[2]. All rats were given aminoguanidine to prevent inactivation of histamine by histaminase[4] (diamine oxidase).

Liothyronine was given subcutaneously in a dose of 0·05 mg/rat/day. Cortisone was given intramuscu-

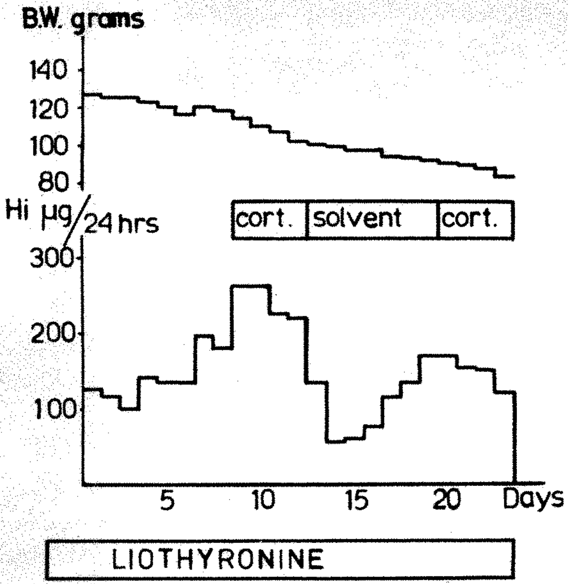

Fig. 1. Changes in urinary histamine μg base per 24 h (lower diagram) and body-weight in g (upper diagram) in response to continuous treatment with liothyronine (0·05 mg daily) and intermittent administration of cortisone (5 mg daily). Liothyronine was given 2 days before starting urine collection. The rat received aminoguanidine 20 mg/kg subcutaneously once daily

larly in a dose of 5 mg/rat/day. As controls the respective solvents were injected.

The previous observation[4] of an increase in the urinary histamine-level in response to the prolonged administration of liothyronine was confirmed. The increase started after 4–5 days of treatment. It may be seen from Fig. 1 that the administration of cortisone for 4 days brought the urinary histamine of the hyperthyroid rat down to a normal level, from which it rose again, when only the cortisone solvent was injected. On renewal of cortisone treatment the urinary excretion of histamine was again reduced.

It was also found that liothyronine, when given to a cortisone-treated rat, did not raise the urinary histamine as it did in control animals given the cortisone solvent.

Apparently, cortisone can both prevent and abolish the increase in urinary histamine brought about by liothyronine in the rat. A direct antagonism between cortisone and liothyronine as regards histamine metabolism is thus likely. The results may help to explain the finding of Spencer and West[6] that cortisone normalizes the increased sensitivity to histamine of the hyperthyroid mouse. The enhanced sensitivity to histamine of hyperthyroid animals may well be caused by an accelerated rate of endogenous synthesis of the amine. A similar mechanism may explain the high sensitivity to histamine of the adrenalectomized rat and of animals treated with haemophilus pertussis vaccine[7].

It is still too early to say whether hyperthyroidism in man and the beneficial effects of adrenocortical steroids in hyperthyroid crises[5] may involve changes in the metabolism of histamine.

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Immuno-electrophoretic Demonstration of Differences in Serum Proteins of New-born Infants of Normal and Diabetic Mothers

In a previous communication we reported that newborn infants of diabetic mothers have raised serum glycoprotein-levels when compared with infants of normal mothers of similar gestational age[1]. In continuation of this investigation we have subjected the same sera to immuno-electrophoresis in agar gel, using a modification of Scheidegger's micro-method[2]. Commercially available anti-sera from the Pasteur Institute were used as a mixture from horses No. 223 and No. 224.

Sixteen diabetic infants and fourteen controls were examined. In the diabetic babies we found a pattern of serum proteins which was consistently and distinctly different from patterns of normal controls. Representative patterns observed in an infant of a normal and a diabetic mother are reproduced in Fig. 1. It can be seen that the infant of the diabetic mother, in comparison to its control, has a larger number of distinct arcs, of which that in continuation of the orosomucoid and that in the siderophilin region are most striking.

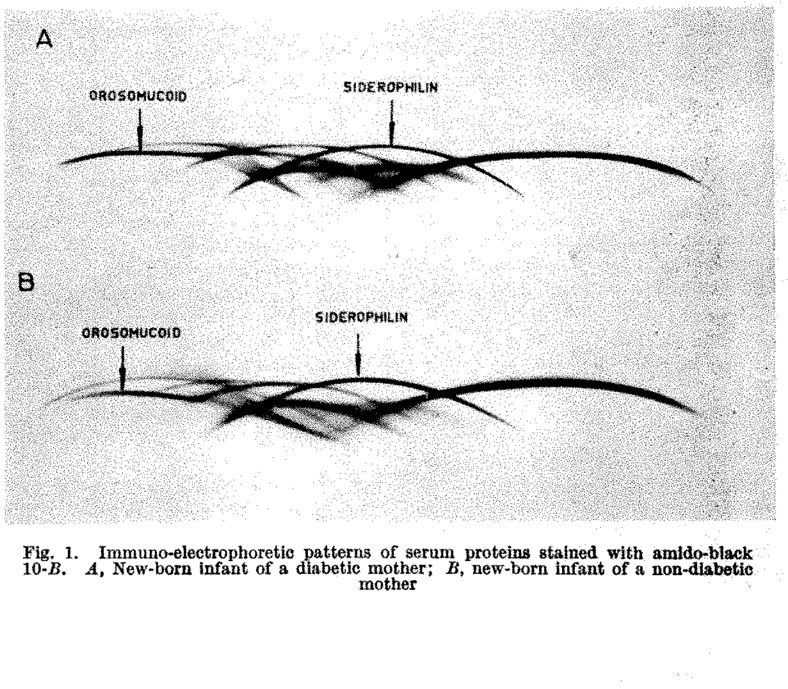

Fig. 1. Immuno-electrophoretic patterns of serum proteins stained with amido-black 10-*B*. *A*, New-born infant of a diabetic mother; *B*, new-born infant of a non-diabetic mother

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ANATOMY

Extracellular Single Fibre Recording Technique for Peripheral Nerves without Section of Nerve or Incision of Perineurium

Two main techniques are at present in use to obtain electrophysiological data from single afferent fibres in mammalian and reptilian nerves. (1) The nerve trunk is severed and successively fractionated until the stage is reached at which a fine strand (or strands) gives a unit response. This method suffers from the disadvantage that the nerve trunk is severely damaged and only a few fibres can be investigated in each experiment—an inevitably non-representative sample of the nerve fibre (or receptor) spectrum. (2) A micro-electrode is inserted into the dorsal root or its ganglion, from which extra- or intra-cellular single unit recordings are made. In this case a representative sample of all dorsal root fibres can be taken, but it is impossible to obtain a true sample of the fibres supplying a particular area of skin. Several isolated reports also describe extracellular[1-5] and intracellular[6] records made with micro-electrodes inserted blindly into nerve trunks, but no generally applicable technique has yet emerged.

The technique described here allows the sampling of most of the fibres supplying any specific area of the skin. 1 cm of nerve trunk (in our experiments the great auricular nerve of the rabbit) is exposed near to its origin and a pair of platinum stimulating electrodes, 5 mm apart, are hooked under it in a pool of mineral oil. A few mm of the same nerve are exposed 3 cm distal to the stimulating electrodes and hooked up on a single platinum recording electrode. Square wave pulses are applied to the stimulating electrodes and the threshold for activity of all fibres is determined. The recording electrode is then removed and a 'Perspex' ('Lucite') support placed under the nerve. Supra-threshold pulses at about 5/sec are applied to the stimulating electrodes and a glass micropipette filled with 3 M sodium chloride is driven radially into the nerve bundle until a characteristic single-fibre action potential shows on the oscilloscope.

The criteria we used for single-fibre activity were: the sudden onset and cut-off of the action potential as stimulus intensity was raised or lowered respectively, and the constancy of the latency and waveform of the action potential when the stimulus was increased up to ten times the threshold value. The amplitudes varied between 0·2 and 10 mV. Conduction velocities of 105 fibres from six rabbits are shown in the histogram (Fig. 1). The range, 1·5–66 m/sec, shows that even some 'unmyelinated' fibres were being sampled and preliminary histological controls indicate that there was little bias in the selection of fibres within this group. The method gives a highly localized lead and very rarely was more than one unit recorded at once.

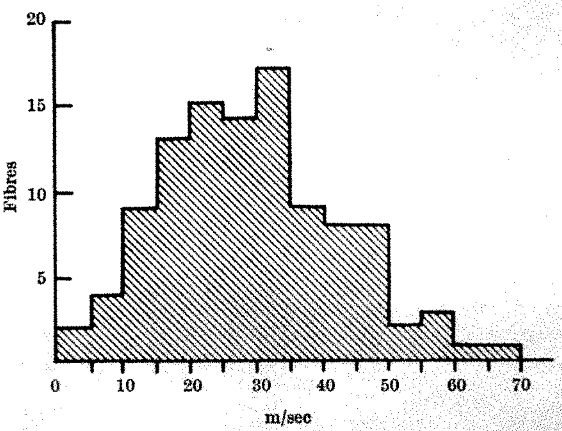

Fig. 1. Histogram of the conduction velocities of 105 single nerve fibres measured at 36°–37° C

Various types of microelectrode, used for single unit recording in the central nervous system, have been tried. Stainless steel[7] or tungsten[8] wire, etched to 0·5μ and dipped in lacquer, buckled too easily against the tough connective tissue of the peripheral nerve bundle. Silver[9], stainless steel or tungsten wire, similarly etched and threaded into glass sleeves drawn down to 1μ internal diameter gave the required structural rigidity, but presented too large a surface area, with the result that during electrical stimulation the compound action potential tended to mask the AP of the single fibre. Glass micropipettes, however, of fairly abrupt taper less than 0·5μ tip, filled with 3 M sodium chloride, of resistance 35–90 Mohms, give consistent results.

When a single fibre has been located by electrical stimulation, the stimulator is switched off and the skin explored distally for the receptive field. With the head of the animal firmly clamped and the micromanipulators rigidly mounted, the electrode tip remains close to the fibre located and we have regularly made recordings for 30 min without deterioration. In some experiments up to 25 per cent of the myelinated fibres have been sampled from bundles containing 50–100 fibres and their receptive fields plotted.

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Radial Bands in the Optic Nerve Myelin Sheath

WHILE examining the histochemistry of the optic nerve, we observed beaded transverse band-like areas, which were more dense than the other areas in the myelin sheath in osmium-tetroxide-fixed tissues

(Fig. 1 *A*, arrows). These beads of dark areas in the myelin sheath were fairly constantly observed in cross as well as in longitudinal sections. The bands became very clear when the osmium-tetroxide-treated, paraffin-embedded sections were cleared in xylol, immersed in turpentine or dioxane for 1½ h, and then cleared again in xylol and mounted in dibutylphthalate polysterene xylene mounting medium. With this procedure, banded arrangements of beads were clearly seen (Fig. 1 *B*, arrows), and the lipid material which is in between these beads had been extracted, whereas the beads themselves were left unextracted. This suggests that these dark beaded areas in the myelin sheaths of the optic nerve, which are seen in osmium-tetroxide-treated material, are resistant to fat solvents. It is probable that these banded areas are made up of some kind of proteolipid or polysaccharide material which is resistant to fat solvents.

The bands extend from the axon throughout the myelin sheath; the number is not constant. We have seen the same type of banded appearance in the trigeminal and facial nerves[1], and Tewari and Bourne[2] have recorded it in the sciatic nerve.

While we were preparing our material on the optic and cranial nerves for publication, a paper by Peters appeared on electron microscopic studies of optic nerve of rats, mice and toads in which he describes a radial component in optic nerves fixed in osmium tetroxide, embedded in araldite, and stained with potassium permanganate or lead acetate. In low-power electron micrographs of transverse sections of central sheaths, these dense lines can be observed, arranged radially (Fig. 1 *C*, 1) across the thickness of some of the sheaths at right angles to the lamellæ. These lines tend to occur in groups, and most of the lines extend throughout the thickness of the sheaths.

With higher resolution, Peters[3] has shown that the lines are not continuous, but are composed of a series of thickenings of intraperiod lines (Fig. 1 *C*, 2). A somewhat similar appearance has been observed in longitudinal sections of sheaths.

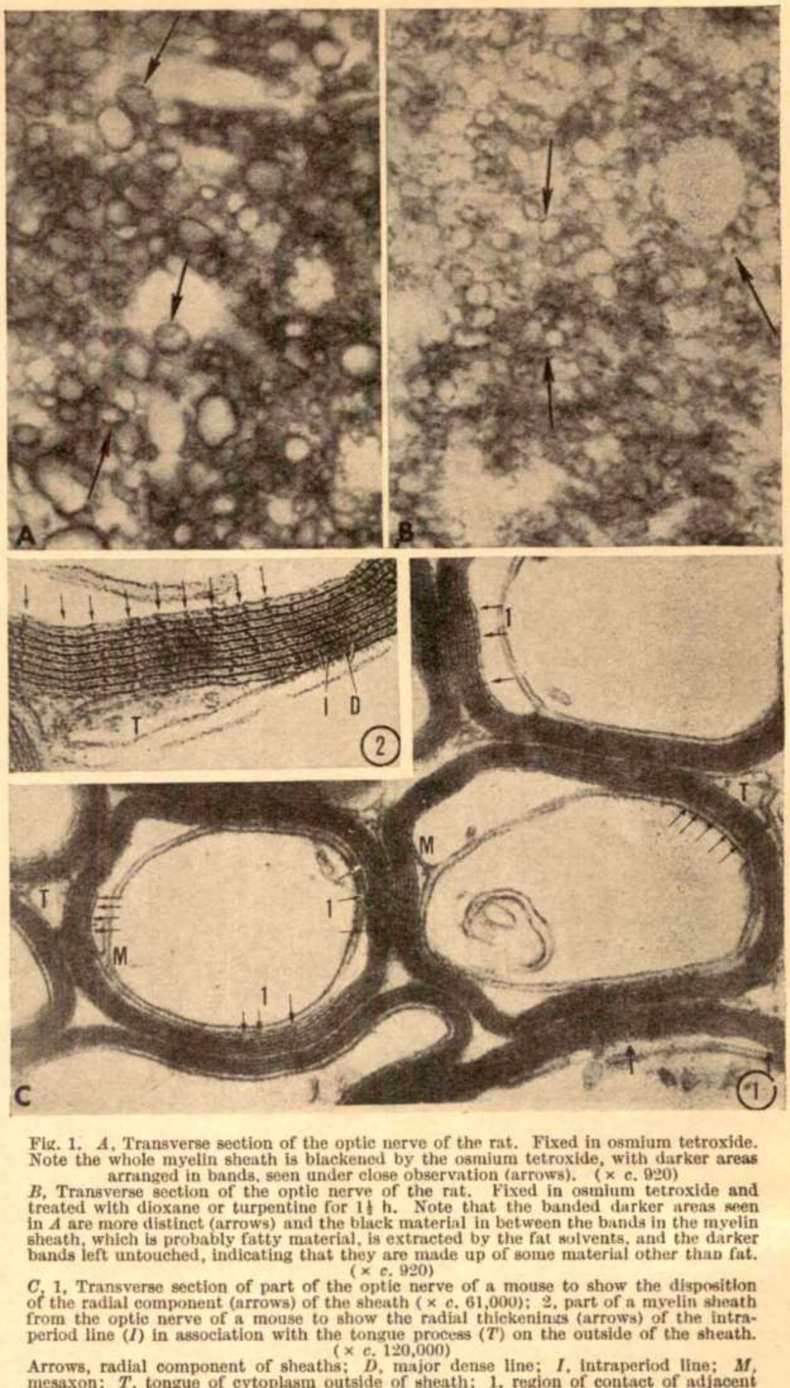

Fig. 1. *A*, Transverse section of the optic nerve of the rat. Fixed in osmium tetroxide. Note the whole myelin sheath is blackened by the osmium tetroxide, with darker areas arranged in bands, seen under close observation (arrows). (× *c*. 920)
B, Transverse section of the optic nerve of the rat. Fixed in osmium tetroxide and treated with dioxane or turpentine for 1½ h. Note that the banded darker areas seen in *A* are more distinct (arrows) and the black material in between the bands in the myelin sheath, which is probably fatty material, is extracted by the fat solvents, and the darker bands left untouched, indicating that they are made up of some material other than fat. (× *c*. 920)
C, 1, Transverse section of part of the optic nerve of a mouse to show the disposition of the radial component (arrows) of the sheath (× *c*. 61,000); 2, part of a myelin sheath from the optic nerve of a mouse to show the radial thickenings (arrows) of the intraperiod line (*I*) in association with the tongue process (*T*) on the outside of the sheath. (× *c*. 120,000)
Arrows, radial component of sheaths; *D*, major dense line; *I*, intraperiod line; *M*, mesaxon; *T*, tongue of cytoplasm outside of sheath; 1, region of contact of adjacent sheaths.
(*C* is taken from Peters, A., *J. Biochem. Biophys. Cytol.*, **11**, 3, 735 (1961) by kind permission of Dr. Peters and the Editors of the *Journal*)

Gray (cited from Peters[3]) has observed similar radial component in sheaths of cerebral cortex fibres also.

Until recently very little evidence has been brought forward that the peripheral nerves also contain such a banding which can be seen under the electron microscope. The only report hitherto available is that of Robertson[4], whose figures show fine radial filaments passing through the peripheral nerve myelin, indicating the presence of such a banding.

The radial banded beads in the myelin sheaths of the optic and cranial nerves, which become very clear in our preparations after turpentine or dioxane extraction, appear to represent a whole group of the radial bands seen under the electron microscope. Due to the low resolution of the light microscope and due to an optic illusion, they appear as broad bands in the myelin sheath.

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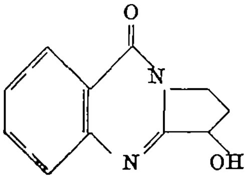
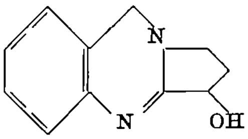
PHARMACOLOGY

Bronchodilating Action of Vasicinone and Related Compounds

Amin and Mehta have claimed[1] that vasicinone (I), which they obtained by aerial oxidation in sunlight of crude extracts of *Adhatoda vasica* or of pure vasicine (II), the shrub's main alkaloid, has a bronchodilating action. They suggested that this property is attributable to the quinazol-4-one ring system for they found it to be common to the parent substance (III) and various simple derivatives[2]. Vasicine, on the other hand, proved to have a bronchoconstricting action, and the bronchodilator effects variously attributed[3] to preparations containing it they ascribed to contamination by small amounts of vasicinone.



I



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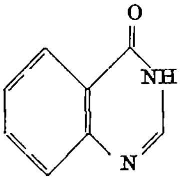

III

To make some quantitative assessment of these claims we have examined quinazol-4-one (III), *l*-vasicine and *dl*-vasicinone. As we were unable to repeat the photochemical oxidation of vasicine mentioned by the Indian authors we prepared racemic vasicinone in moderate (30 per cent) but reproducible yield by acetylating *l*-vasicine with acetic anhydride, oxidizing the product with chromic oxide in acetic acid and finally removing the protecting acetyl group by alkaline hydrolysis.

In vitro tests on guinea pig tracheal rings showed that vasicinone and quinazol-4-one produced relaxation at 100 μg/ml., thus having about 1/2,000 the activity of adrenaline, whereas vasicine caused slight relaxation at 10 μg/ml. but contraction at higher concentrations.

Against histamine-induced contraction of the isolated tracheal chain vasicinone was about 1/5 as active as 4-quinazolone and about 1/3,800 as active as adrenaline.

In vivo studies on guinea pigs under urethane anæsthesia were made using the Konzett–Rössler[4] technique. Vasicine in doses above 400 μg/kg intravenous produced an increase in resistance of the lungs to inflation, taken as an indication of bronchoconstriction. No evidence of bronchodilation was seen even in the presence of atropine 1 mg/kg (cf. Chopra[3]). This is in agreement with the finding of Southwick and Cremer[5] in the dog and Amin and Mehta[1] in the guinea pig. This bronchoconstrictor action may be related to the reported acetylcholine potentiating properties of vasicine[6].

When given intravenously 4–6 min before the administration of bronchoconstrictor doses of histamine, the alkaloid showed no antagonistic action, but when given 30 sec before, the histamine antagonism was obtained with recovery of the histamine response within 5 min. From dose-response lines it was established that 38 μg of vasicine were required to reduce the response produced by 2 μg of histamine to that elicited originally by 1 μg of histamine. The dose required to produce a similar effect against 5-hydroxytryptamine was 160 μg and the response to acetylcholine could not be blocked with a dose below that which produced bronchoconstriction. The antihistamine effect, although specific, is very weak and of short duration.

With vasicinone and quinazol-4-one the same degree of inhibition of the histamine response could not be achieved as was possible with vasicine. Doses up to 100 μg produced some reduction in response to 2 μg of histamine, but on increasing the dose of either substance a decrease in antagonism was obtained. These doses were well below those required to produce bronchoconstriction.

In summary, therefore, we may say that on isolated tracheal muscle quinazol-4-one and vasicinone are about 1/2,000 as active as adrenaline in producing relaxation and 1/700 and 1/3,800 (respectively) as active against histamine-induced contraction. In the intact guinea pig the compounds showed no bronchodilator activity and were bronchoconstrictor at high doses. Vasicine was the most active compound against histamine-induced bronchospasm; it was fairly specific and had a very short duration of action. Vasicinone and quinazol-4-one showed only slight activity against histamine and antagonism decreased on increasing the dose.

The differences *in vivo* and *in vitro* suggest that we are measuring very different effects and the short duration of action *in vivo* indicates rapid removal or metabolism of these compounds. Their bronchodilator activity is in no way comparable with known bronchodilator drugs.

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HÆMATOLOGY

Levels and Uptake of Ascorbic Acid from Plasma by White Cells, Platelets and Leukæmic Cells

It has previously been shown that white blood cells are capable of absorbing ascorbic acid against a concentration gradient from plasma, or from plasma diluted in a solution containing saline, ethylenediamine tetraacetic acid and dextran[1]. This investigation has been continued and extended to include an assessment of uptake by granulocytes, lymphocytes and platelets.

Where estimation of absorption of ascorbic acid by white cells from plasma was carried out for varying periods of time, controls (consisting of separated white cells and plasma) were kept at the same temperature. At the end of the experiment, trichloroacetic acid (which acts as a protein precipitant and stabilizer of ascorbic acid) was added, and the ascorbic acid was estimated by the 2,4-dinitrophenylhydrazine method[1].

Table 1. PLASMA AND CORRESPONDING WHITE BLOOD CELL ASCORBIC ACID LEVELS IN RANDOM BLOOD SPECIMENS

Plasma (μg/ml.)	White blood cells (μg/10^8)	Plasma (μg/ml.)	White blood cells (μg/10^8)
1·1	15·6	8·2	30·7
1·1	25·5	8·9	64·3
2·9	16·7	10·8	32·8
3·2	46·9	12·0	36·8
5·2	33·6	16·5	28·5
7·1	26·7	17 0	45·9

Table 2

	Plasma ascorbic acid (μg/ml.)	White blood cell ascorbic acid (μg/10^8 cells)	White blood cell ascorbic acid after cells have been 4 h in contact with plasma in diluent at 4° C (μg/10^8 cells)
Fasting	5·2	22	33
3 h after 700 mg of ascorbic acid	17·0	27	46
Fasting	7·1	26	30
3 h after 700 mg of ascorbic acid	20·0	27	52

Plasma and white cell ascorbic acid levels in the fasting state and 3 h following an oral dose of 700 mg of ascorbic acid.

Table 1 shows no correspondence between white blood cell and plasma ascorbic acid levels when blood was taken at random times from patients who were not fasting. Because of this poor correlation the levels of plasma and white blood cell ascorbic acid and the uptake by the white cells were determined before and 3 h after an oral dose of 700 mg of ascorbic acid. Two cases giving typical results are shown in Table 2. It can be seen that there is only a small rise in white blood cell levels, coinciding with a much greater rise in plasma levels. However, the uptake of ascorbic acid by the white cells is greater when they are left *in vitro* in contact with the plasma having the higher level. It can thus be appreciated why a high plasma level is not always associated with a high white blood cell ascorbic acid level, and it is conceivable that when body stores of ascorbic acid are depleted, a low plasma fasting level may be obtained in the presence of a high white blood cell ascorbic acid level. When white blood cells from patients with low fasting levels were suspended for four hours in plasma obtained from a normal person 3 h after an oral loading dose of 700 mg of ascorbic acid, rises from 9·7 to 30·7 and 20·9 to 64·3 μg of ascorbic acid per 10^8 white blood cells were obtained.

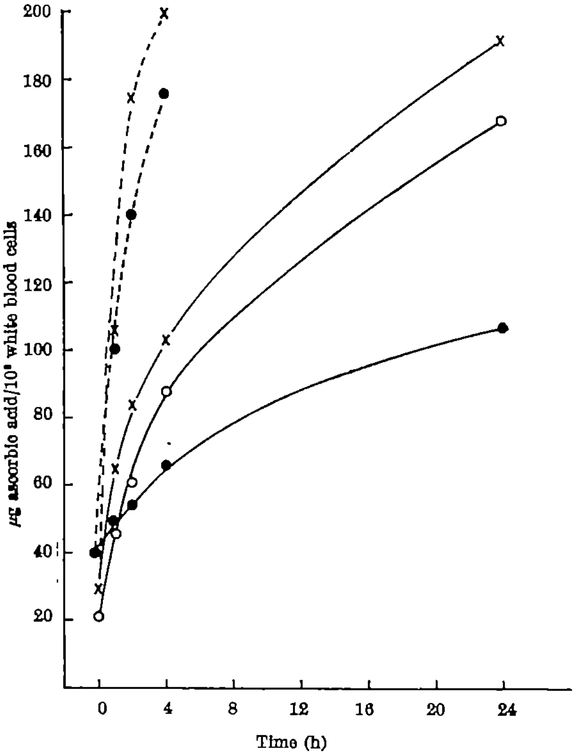

Fig. 1. Uptake of ascorbic acid by normal white blood cells suspended in plasma and diluent with ascorbic acid added at a concentration of 2·5 mg/100 ml. ——, 4° C; – – –, 37° C. The results shown are representative of 10 cases

In the present and all previous estimates of white blood cell ascorbic acid levels and white blood cell uptake, no attempt has been made to separate the platelets, and all assessments of cell content and uptake of ascorbic acid have included any contribution by platelets as a contaminating fraction. To achieve a separation, blood was collected in siliconed apparatus, and platelet rich, leucocyte free plasma obtained by differential centrifugation. The ascorbic acid content of the platelets and their uptake from plasma were determined as previously described for leucocytes. The results of one such experiment are shown in Table 3, in which it can be seen that uptake of ascorbic acid by the platelets is accompanied by a loss of similar amount from the plasma. The results for the ascorbic acid content of platelets shown in Fig. 2*a* are expressed as μg/5 × 10^9 platelets in comparison with values for white blood cells expressed as μg/10^8 white blood cells. Because of the small number of cases examined and the errors involved in platelet counting and fragmentation during preparation, it is difficult to compare the results with those for white cells.

The differential separation of neutrophils, lymphocytes and platelets was next carried out by the albumin gredient method of Spriggs and Alexander[2].

Table 3. UPTAKE OF ASCORBIC ACID BY PLATELETS FROM 1 ML. OF PLASMA AND THE CORRESPONDING DECREASE OF ASCORBIC ACID IN THE PLASMA

μg ascorbic acid in platelets separated immediately	μg ascorbic acid in platelets separated after 4 h	Difference (μg)	μg ascorbic acid in plasma before absorption	μg ascorbic acid in plasma after 4 h in contact with platelets	Difference (μg)
3·1	7·8	4·7	15·2	10·8	4·4

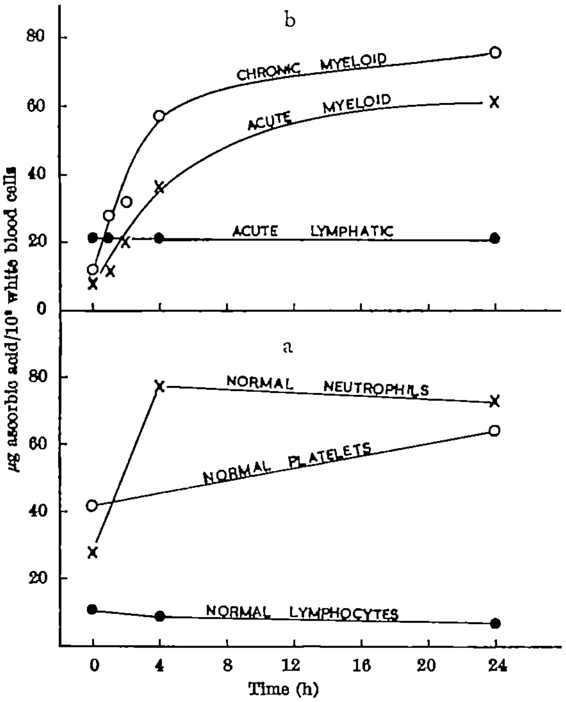

Fig. 2. *a*, Uptake of ascorbic acid as in Fig. 1 by neutrophils, lymphocytes and platelets. The levels for platelets are shown as μg/5 × 10^7 platelets. *b*, Uptake of ascorbic acid as in Fig. 1 by leukæmic cells

The fractions were suspended in diluent plus ascorbic acid at a final concentration of 2·5 mg/100 ml. and the uptake at 4° C after 4 and 24 h determined. The results are shown in Fig. 2*a*. The increases in ascorbic acid content of the neutrophils after exposure to diluent plus ascorbic acid are less than would be anticipated in comparison with the normals of Fig. 1. This may be due to cell damage occurring during the process of separation. The lymphocytes, which appeared morphologically intact after the separation, showed no uptake of ascorbic acid.

The white blood cell ascorbic acid levels and uptake of leukæmic cells at 4° C are shown in Fig. 2*b*. In each case, the amount of blood added to diluent was adjusted so that the ratio of cells to diluent was comparable with that in the normals. The results support the findings of Waldo and Zipf[3], who obtained low levels of plasma ascorbic acid in leukæmia and other conditions involving blood-forming organs. Fig. 2*b* also shows that immature granulocytes take up ascorbic acid, but the data are too few for relative assessment. It is conceivable that this is an apparent result, and that a higher uptake is masked by a greater utilization of ascorbic acid by immature cells. Abnormal cells of the lymphocyte series in common with the normal lymphocytes of Fig. 2*a* showed no uptake of ascorbic acid.

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Free Amino-Compounds in Blood Platelets

PLATELETS contain 0·31–0·39 mg nitrogen per 10^9 platelets. Of this, 83–92 per cent is protein nitrogen[1]. Although platelets are the main source of histamine and serotonin (5-hydroxytryptamine) in blood[2], nothing is known of the free amino-acids therein. It was thus of interest to determine the total free amino-acid content of platelets and to identify individual components.

Total α-amino nitrogen: The concentrations in platelets from normal individuals and certain patients, some with platelet anomalies, are given in Table 1.

Table 1. TOTAL FREE AMINO-NITROGEN IN PLATELETS

Condition	No. of cases	Platelet count/mm³ (10³)	Total amino-nitrogen (μg/10^9 platelets)
Thrombocytopenia			
No operation*	2	90–170	9·1–16·0
Post-splenectomy	6	275–620	4·9–8·2
Allergic purpura	4	350–525	5·1–5·7
Thrombocythæmia	1	1,200	10
Polycythæmia	2	276–672	6·4–9·0
Haemochromatosis	2	210–240	5·4–9·0
Normal individuals	5	220–280	5·8–9·2

* Treated with steroids.

Identification of amino-acids: Using two-dimension paper chromatography, four amino-acids, namely, aspartic and glutamic acids, serine and glycine, were identified as well as a peptide with R_F 0·55–0·60 in phenol and 0·10–0·12 in butanol–acetic acid. After acid hydrolysis, alanine, tyrosine and leucine(s) appeared and the spots produced by glycine and serine showed increased density. This distribution would appear to be specific, being different from that in plasma[3] and in normal and leukæmic white blood-cells[4].

The possible identification by paper chromatography of serotonin and histamine in appropriate extracts of platelets was also investigated. Table 2 shows the R_F values of these substances in 3 solvents. To detect these substances, however, aliquots containing at least 10 µg nitrogen were required. Since 10^9 platelets contain less than 0·1 µg histamine and 0·2–0·4 µg serotonin[2] no corresponding spots could be identified in platelet extracts with a total amino-nitrogen of 45 µg.

Table 2. R_F VALUES OF HISTAMINE AND SEROTONIN

Compound	Solvent		
	Phenol	Butanol–acetic acid	77% ethanol
Histamine	1*	0·16	0·7
Serotonin	0·96	0·58	0·8

* Moving with solvent.

Effect of glass contact: All the above investigations were carried out in siliconed glass. Further experiments demonstrated that using untreated glass for washing platelets before extraction resulted in an appreciable loss of amino-acids, the peptide being the only compound detected on a chromatogram.

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Serum Protein Synthesis in Fœtus Haptoglobins and Group-Specific Components

Methods of starch-gel and immuno-electrophoresis allow a division of the haptoglobins (Hp), the group-specific components (Gc) and the transferrins into several genetically determined types[1-3].

The existence of haptoglobin, group-specific component and transferrin proteins in cord sera, which differ from the corresponding maternal protein types, seem to show that new-born children in some cases synthesize their own haptoglobin, group-specific component and transferrin proteins[4-6].

We have recently investigated a twin-couple of still-born fœtuses which were of different sexes and also belonged to different ABO-, MN- and Rh-groups. Both twins had well-developed haptoglobin and group-specific component patterns qualitatively and quantitatively indistinguishable from the adult normal patterns, as determined by starch-gel and immuno-electrophoresis. The blood and serum types of the mother and her two children are given in Table 1 and in Table 2 (Nos. 13–15). The differences in haptoglobin and group-specific component-types between the twins and the mother strongly imply that both twins were able to synthesize their own haptoglobin and group-specific component proteins. As regards the Gm types, the twins and the mother lacked the Gm^a antigen and are thus not informative on the development of the Gm^a character.

Table 1. Blood- and Serum-Groups of Mother and Her Two Still-born Fœtuses

	Blood group	Serum group Gm-type	Hp-type	Gc-type
Mother	A MN Rh$_1$rh	*a*–	2–1	2–2
Twin 1	O N Rh$_1$rh	*a*–	2–2	2–1
Twin 2	A M Rh$_1$Rh$_1$	*a*–	1–1	2–1

In addition, determinations of haptoglobin were carried out on three, and determinations of group-specific components on six, further fœtal sera ranging in age from 17 to 30 weeks. In two sera (Nos. 4 and 8) haptoglobins were detected with the conventional starch-gel electrophoretic technique with borate buffer and benzidine-staining, and in one of these sera (No. 4) the child belonged to a haptoglobin type different from that of the mother (Table 2). In no case could the group-specific component precipitate be demonstrated (Table 2). Two of the fœtal sera (Nos. 4 and 10) were used in absorption of an anti-immune serum containing antibodies both against the haptoglobins and the group-specific components. Both sera had the capacity of absorbing the antibodies against the group-specific component as revealed by immuno-electrophoretic analysis of the absorbed immune serum against normal sera containing the haptoglobin and group-specific component protein. Serum No. 4 also absorbed the antibodies against the haptoglobins, whereas serum No. 10 did not. The absorption was carried out in the gel according to a technique described previously[7].

Table 2. Clinical and Laboratory Data on Seven Mother–Fœtal Samples

No.		Length (cm)	Age (weeks)	Hp	Gc
1	Mother	—	—	n.d.	2–1
2	Fœtus	18	17	n,d.	absent
3	Mother	—	—	1–1	2–1
4	Fœtus	29	21	2–1	absent
5	Mother	—	—	n.d.	1–1
6	Fœtus	22	20	n.d.	absent
7	Mother	—	—	1–1	1–1
8	Fœtus	17	17	1–1	absent
9	Mother	—	—	2–1	1–1
10	Fœtus	38	30	absent	absent
11	Mother	—	—	n.d.	2–1
12	Fœtus	20	17	n.d.	absent
13	Mother	—	—	2–1	2–2
14	Fœtus 1	30	25	2–2	2–1
15	Fœtus 2	30	25	1–1	2–1

Investigation of cord blood from 71 new-born children in all instances gave well-developed group-specific component patterns; where in 29 cases the group-specific component pattern of the child belonged to another type than that of the corresponding mother (Table 3). As regards the haptoglobins, only 10 per cent of 120 investigated cord sera could be haptoglobin-typed[8]. In a recent work by Bergstrand *et al.*[9], no haptoglobin could be demonstrated in six sera from fœtuses between 16 and 26 cm, nor in 29 cord blood sera.

Table 3. Gc-Types of 71 Mother-Cord Samples (Data from Ref. 5 are included)

Gc type	Mother Obs.	Exp.		Child Gc 1–1	Gc 2–1	Gc 2–2
1–1	42	39·5	obs.	33	9	0
			exp.	31·4	10·7	0
2–1	27	26·9	obs.	12	9	6
			exp.	10·1	13·5	3·5
2–2	2	4·6	obs.	0	2	0
			exp.	0	1·5	0·5

Expected frequencies calculated from Gc-frequencies of 2,259 unrelated Swedes. $Gc^1 = 0·7459$, $Gc^2 = 0·2541$ (ref. 10).

In conclusion, it seems as if the synthesis of haptoglobin and group-specific component proteins in the fœtus is subjected to great individual variations, which might indicate differences in 'biochemical maturity'. Further work is in progress of using the haptoglobin and group-specific component protein polymorphisms as a 'physiological tagging' in the evaluation of protein synthesis in the fœtus as well as in the evaluation of turnover of passively transferred serum proteins in new-born children and adults.

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PATHOLOGY

Induction of Fibrosarcomas in Mice given a Minute Quantity of 3-Methylcholanthrene or Dibenz(*a,h*)anthracene as New-borns

The susceptibility of new-born mice to chemical carcinogenesis has been reported from several laboratories within the past two years[1-4]. New-born mice (Swiss, *C3H/P*, *C57BL*) given a single low dose of either 3-methylcholanthrene (3-MC) or dibenz(*a,h*)-anthracene (DBA) developed a higher incidence of pulmonary tumours and more tumour nodules per lung in a shorter time than did adult mice[5].

The experiment reported here was designed to determine the minimum carcinogenic dose of 3-MC and

Table 1. INCIDENCE OF SUBCUTANEOUS FIBROSARCOMAS IN NON-INBRED ALBINO MICE GIVEN A SINGLE SUBCUTANEOUS INJECTION OF 3-METHYLCHOLANTHRENE OR DIBENZ(a,h)ANTHRACENE AS NEW-BORNS

Dose (μg)*	Total No. of mice†	No. of mice with tumours Weeks following treatment 13–25	26–35	36–45	46–55	Total	(per cent)	Median latent period of tumours (weeks)
3-Methylcholanthrene								
11·1	42	10	4	2	1	17	40·5	24
3·7	45	9	6	3	1	19	42·2	27
1·2	31	9	3	0	1	13	41·9	19
0·41	46	1	1	1	0	3	6·5	34
0·14	48	0	0	0	0	0	0	—
0·046	48	0	0	0	0	0	0	—
0·015	40	0	0	0	0	0	0	—
0·0051	37	0	0	0	0	0	0	—
Dibenz(a,h)anthracene								
6·7	49	26	3	1	0	30	62·2	21
2·2	41	11	0	2	0	13	31·7	19
0·74	47	5	5	1	0	11	23·4	30
0·25	48	4	1	0	0	5	10·4	23
0·083	40	0	2	1	1	4	10·0	37
0·028	54	0	0	0	0	0	0	—
0·0092	46	0	0	0	1	1	2·2	48
0·0031	61	0	1	0	0	1	1·6	33
Olive oil controls 0·0	85	0	0	0	0	0	0	—

* In 0·05 ml. olive oil. † Mice living to 13 weeks of age or older.

DBA in new-born non-inbred albino mice (NIH General Purpose mice). Eight logarithmically spaced dose-levels of each carcinogen were used. For 3-MC, the doses varied from 11·1 μg to 0·0051 μg; for DBA the range was 6·7 μg–0·0031 μg. The carcinogens were dissolved in olive oil in such concentrations that the desired dose was contained in 0·05 ml. of vehicle. Control mice received 0·05 ml. of olive oil. 130 litters were randomly distributed among treatment and control groups, 6–8 litters in each of the 16 treatment groups and 13 in the control group. Each mouse was given a single subcutaneous injection of carcinogen (or vehicle) within 12 h after birth. The animals were weaned at 4 weeks of age, separated by sex and housed in groups of 5–8 animals per cage. The mice were observed for more than a year for the development of tumours; animals were killed only if they developed tumours or became moribund. Because of persistent fighting among the males, all surviving male mice were killed and autopsied at 55 weeks of age. Surviving females, however, are still under observation. As of age 55 weeks there was no apparent difference in the incidence of subcutaneous tumours or in latent period between males and females, and the results for both sexes have been combined in Table 1. The subcutaneous tumours measured 0·3–3·4 cm in diameter, and all were diagnosed histologically as fibrosarcomas. Only subcutaneous fibrosarcomas appearing within 55 weeks after administration of carcinogen are reported here. The incidence of other tumours and any later subcutaneous fibrosarcomas which develop will be reported when the experiment is terminated and the final results are tabulated.

The results with 3-MC show no change in the incidence of fibrosarcoma at doses between 11·1 μg and 1·2 μg. There is a sharp drop in the incidence at the 0·41-μg level, and at the 4 lowest doses of 3-MC (0·14–0·0051 μg) no subcutaneous tumours were induced in 173 mice within 55 weeks. With DBA, however, there is a more gradual decrease in tumour incidence with decreasing dose. Tumours appeared within the 55-week period in 2 of 161 mice administered the 3 lowest doses (0·028–0·0031 μg). Both the time of appearance of the earliest tumours (13–16 weeks) and the median latent period to tumour appearance were about the same for the two carcinogens used. Although the latent period of tumour formation was not closely related to the dose, it tended to be somewhat longer at the lower dose-levels.

The doses of carcinogen at which fibrosarcomas were induced in this experiment (0·41 μg of 3-MC; 0·0031–0·083 μg DBA) are, to the best of our knowledge, the lowest amount of chemical carcinogen ever reported to induce tumours. It may be that use of the new-born mouse provides a more sensitive system than the adult for assaying the carcinogenic activity of polycyclic hydrocarbons, and perhaps also of other chemical agents.

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Effect of Starvation and of Adrenalectomy on Compensatory Hyperplasia of the Kidney

COMPENSATORY hyperplasia of the kidney has frequently been used as a model for the investigation of regeneration. However, the effect of the intake of food on the mitotic response has not to my knowledge been commented on, and the effect of adrenalectomy is disputed[1,2]. Some observations made during a study of the control of compensatory hyperplasia seem interesting enough to justify a short report.

The animals used were male albino rats weighing 118–180 g from a colony of Wistar origin. Groups of animals were bilaterally adrenalectomized and maintained on a normal diet (M.R.C. diet 14*B*) with 0·9 per cent sodium chloride solution as their water supply. In addition, one kidney was removed from some animals at the time of adrenalectomy: others had a unilateral nephrectomy without adrenalectomy, and others a sham operation: the last three groups were either starved or allowed free access to food post-operatively, and groups were killed at 8-h intervals

Table 1

	8 h		16 h		24 h		32 h		40 h		48 h	
A	(40)	4·2 ± 0·4	(40)	3·6 ± 0·4	(40)	7·6 ± 0·75	(40)	4·2 ± 0·4	(40)	3·6 ± 0·4	(40)	7·6 ± 0·75
B	(4)	4·7 ± 1·7	(4)	2·2 ± 0·75	(15)	10·0 ± 1·8	(8)	12·5 ± 1·6	(15)	26·1 ± 2·9	(12)	19·5 ± 2·9
C		—		—		—	(11)	3·8 ± 0·8	(9)	7·7 ± 0·9	(11)	6·9 ± 1·0
D	(3)	1·7 ± 1·7	(3)	2·0 ± 2·0	(6)	9·7 ± 3·1	(6)	13·0 ± 4·0	(5)	19·2 ± 4·0	(7)	20·6 ± 3·8
E	(3)	1·3 ± 0·9	(3)	3·3 ± 1·3	(6)	6·2 ± 1·1	(5)	5·8 ± 0·8	(6)	7·7 ± 1·8	(5)	9·4 ± 1·6
F	(3)	3·7 ± 1·8	(3)	4·2 ± 1·8	(5)	8·2 ± 2·0	(6)	2·7 ± 2·2	(13)	6·6 ± 1·4	(5)	6·0 ± 1·6
G		—		—		—		—	(9)	1·4 ± 0·8		

Total mitotic counts: the number of animals in each group is given in parentheses and the mean count is given with the standard deviation of the mean.

A, Unoperated normally fed animals killed at that time of day; *B*, unilaterally nephrectomized animals allowed free access to food; *C*, bilaterally adrenalectomized animals allowed free access to food; *D*, bilaterally adrenalectomized unilaterally nephrectomized animals allowed free access to food; *E*, bilaterally adrenalectomized unilaterally nephrectomized animals starved post-operatively; *F*, unilaterally nephrectomized animals starved post-operatively; *G*, sham-operated animals starved post-operatively.

over the 48 h after operation. All operations were performed at 2–4 p.m. The remaining kidneys were removed and fixed immediately after death, and 5μ paraffin sections prepared and stained with hæmatoxylin and eosin. Mitoses were counted over a standard area (1·1 mm^2) in each of the four zones of the kidney as in previous work[3]. The total number of cells of all types examined per kidney was approximately 32,000, of which some 17,000 belonged to the renal tubules: as in previous investigations, mitoses were found to be almost confined to the tubules. Only one kidney of the animals which had been adrenalectomized without unilateral nephrectomy was used to avoid the risk of under-estimating the variance due to agreement between the counts of the two kidneys. The kidneys of animals showing pyelonephritis or hydronephrosis were discarded. The total number of mitoses seen in all four zones was calculated and the results are given in Table 1.

Series *A* and *B* are based partially on previously published material[3] and show: *A*, the normal diurnal rhythm; *B*, the early mitotic response in the remaining kidney of fed animals, with a sharp peak at 40 h. It can be seen from series *F* that in animals which have been starved post-operatively this peak is greatly diminished, and compared with the mitotic counts in unoperated controls is almost abolished: however, the sham-operated controls (series *G*) starved after operation have a very much lower mitotic rate than the normally fed animals of series *A* ($P < 0{\cdot}02$), and compared with series *G* the unilaterally nephrectomized starved animals show a very considerable mitotic increase ($P < 0{\cdot}01$). Adrenalectomy alone (series *C*) considerably distorts the normal mitotic rhythm; unilaterally nephrectomized adrenalectomized rats (series *D*) show a definite increase in the mitotic rates over the adrenalectomized controls of series *C* at 32–48 h ($P < 0{\cdot}01$ in each case). Unilaterally nephrectomized rats from which both adrenals have been removed and which had been starved post-operatively had counts not significantly above those of starved uninephrectomized animals with intact adrenals (series *F*), except at 24 h when the chance probability of the difference is 0·05–0·02: the significance of this single reading in the series is dubious. All probabilities were calculated by the *t* test: it has been shown[3] that the distribution of mitotic rates between animals is often somewhat skew, though this was not obvious in the small groups studied. However, as the *t* test is not very sensitive to small deviations from normality, the calculated probabilities are probably valid.

The conclusions would seem to be: (1) that starvation depresses the normal mitotic rate; (2) that the mitotic response of compensatory hyperplasia is considerably diminished by starvation compared with that in fed animals, but only slightly, if at all, relative to starved controls, and that this diminution is not affected by adrenalectomy; (3) that adrenalectomy alters the normal diurnal rhythm and the pattern of the mitotic response of compensatory hyperplasia but does not abolish this.

Bullough and Laurence[4] have found that starvation produces a depression in the mitotic rate of mouse epidermis, and that this effect is abolished by adrenalectomy; however, adrenalectomy does not seem to affect the reduced renal hyperplasia of starved rats. Goss and Rankin[2] stated that adrenalectomy inhibited compensatory renal hyperplasia on the basis of mitotic counts 48 h after operation: however, their controls were not adrenalectomized, and it would seem that hyperplasia will in fact occur, though the peak is less sharp than normally. The effect of starvation is obviously important in planning experiments on compensatory hyperplasia and may produce misleading results if ignored[5].

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Catechol-O-Methyltransferase Activity in Neuroblastoma Tumour

Von Studnitz[1], Greenberg and Gardner[2], and Voorhess and Gardner[3] have reported marked elevations in the urinary excretion of 3-*O*-methylated metabolites in the urine of patients with neural tumours. It is possible that these urinary products arise mainly from *O*-methylation of their precursors in the liver. However, the fact that some of these patients have a normal blood pressure in the presence of a large endogenous production of norepinephrine suggested to us that metabolism of the norepinephrine may occur within the tumour.

We have used the assay method described by Axelrod *et al.*[4] with minor modifications to examine the tumour for catechol-*O*-methyltransferase activity. This method is very sensitive because of the use of DL-epinephrine-7-^{3}H.

An ovoid tumour (6 × 5 × 4 cm) was excised from the region of the right suprarenal gland of a 13-month old, white, male patient. The tumour was chilled on ice and a 100·3-mg portion was homogenized in 0·6 ml of ice-cold 1·15 per cent potassium chloride solution. The homogenate was centrifuged at 14,500*g* for 20 min. The supernatant solution was examined for catechol-*O*-methyltransferase activity at the same time as a non-particulate fraction of tumour obtained at autopsy from two other patients with neuroblastoma and of rat liver prepared in the same manner.

Three tubes were prepared for each sample; 10 μM of potassium phosphate buffer (0·5 M, *p*H 7·6), 0·5 μM of magnesium chloride (1 M), and 0·6 mμM (2·8 μc.)

DL-epinephrine-7-^{3}H-hydrochloric acid, were added to all the test-tubes. To the first and third tubes, 10 mμM of *S*-adenosylmethionine was also added. The co-factor, *S*-adenosylmethionine, was omitted from the second tube of each set. 50 μl. of the supernatant fraction was added to the first and second tubes of each set; but before it was added to the third tube, the supernatant fraction was heated for 3 min in a 100° water-bath. Therefore, the first tube of each set contained the complete reaction mixture; in the second tube the co-factor was omitted, and in the third tube the enzyme was denatured. The total volume of the reaction mixtures was 100 μl. The reaction mixtures were incubated in 10 × 98 mm test-tubes in a water-bath at 37° for 90 min. After incubation, 10 μl. from each tube were transferred to 0·5 ml. of *p*H 10 borate buffer (0·13 M) and the mixture was extracted with 5 ml. of toluene/isoamyl alcohol (3 : 2). After centrifuging, duplicate 1-ml. portions of the upper phase were added to a fluor solution consisting of 10 ml. of toluene containing 0·4 per cent 2,5-diphenyloxazole and 0·01 per cent 1,4-*bis*-2′(5′-phenyl-oxazolyl)-benzene and 4 ml. of ethanol. The solutions were assayed for radioactivity in a liquid scintillation spectrometer (Tri-Carb scintillation spectrometer, Packard Instrument Co., La Grange, Illinois).

Table 1. CATECHOL-*O*-METHYLTRANSFERASE ACTIVITY IN NEUROBLASTOMA

	C.p.m.* in toluene/isoamyl alcohol extract		
	With *S*-AMe	Without *S*-AMe	Boiled extract
P. H. W.†	1,435	655	955
J. K. S.†	8,015	880	885
D. H. W.‡	1,685	950	1,105
Rat liver	23,385	970	1,135

* Corrected for background See text for details of the reaction mixtures and extraction procedure.

† Tumour tissue was obtained at autopsy from these patients.

‡ In the case of this patient, tumour tissue was obtained at laparotomy.

The results of the assay are presented in Table 1; it can be seen that significantly more radioactivity was extracted by the toluene–isoamyl alcohol from the complete reaction mixtures. If the co-factor was omitted, or if the enzyme was denatured, less radioactivity was extracted. Under conditions of the assay, only about 2 per cent of the radioactivity of the ^{3}H-epinephrine is extracted into the toluene–isoamyl alcohol, but 50 per cent of the ^{3}H-metanephrine is extracted. Portions of the toluene–isoamyl alcohol extracts were evaporated to dryness under a stream of nitrogen, and the residues were transferred to a sheet of Whatman No. 1 filter paper with ethanol. The chromatogram was developed in isopropanol/7 N ammonia solution (4 : 1), dried, and scanned for radioactivity. One large peak of radioactive material was present, and this corresponded to added metanephrine. In addition, 10-μl. portions of each of the incubation mixtures were chromatographed in the *n*-butanol/glacial acetic acid/water system (4 : 1 : 1). Two radioactive areas were detected on the chromatograms of the complete reaction mixture from each tumour; these areas corresponded to epinephrine and metanephrine. The chromatograms of the incubation mixtures from which *S*-adenosylmethionine was omitted and the mixtures to which boiled enzyme was added showed only one radioactive area which corresponded to epinephrine.

This evidence indicates that catechol-*O*-methyltransferase is present in extracts of the neuroblastoma tumours; under the conditions used ^{3}H-epinephrine was converted to ^{3}H-metanephrine.

The only other report of an examination of catechol-*O*-methyl-transferase activity in tumour of neural crest origin, of which we are aware, is that of Greenberg and Gardner[2]. These authors found no catechol-*O*-methyltransferase activity in tumour tissue from a patient with ganglioneuroma. In the work described here, the presence of this enzymatic activity in extracts of neuroblastoma tumours provides evidence that norepinephrine may be at least partially metabolized within the tumour, and in this way elevated levels of norepinephrine metabolites may be formed in the absence of a corresponding elevation in blood norepinephrine.

The difference between the findings of Greenberg and Gardner and ours may represent a difference in the tumours or may result from the much greater sensitivity of the assay method using tritium-labelled epinephrine.

In addition to the findings reported here, a similar result has been obtained using ^{3}H-norepinephrine in our laboratory.

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RADIOBIOLOGY

Influence of Ionizing Radiation on Glutaminase Activity in Mouse Brain and Liver

IN 1940, Dale[1] demonstrated that carboxypeptidase activity is inhibited if submitted to small doses of X-rays. Bekkum[2], Potter[3] and Nadson[4] showed that under ionizing radiations primary changes occur in the mitochondria. We have investigated the variations of the *in vivo* behaviour of glutaminase activity in liver and brain of mice submitted to an overall 58-min irradiation with 500 r.

Table 1 shows that after 2 h following irradiation there is still a significant inhibition of glutaminase activity which increases progressively 24 and 48 h after irradiation. Table 2 shows that 2 h after irradiation brain glutaminase does not exhibit any significant change in its activity. After 24 h, and especially after 48 h following irradiation, the activity of this enzyme is inhibited significantly.

Table 1. CHANGES OCCURRING IN GLUTAMINASE ACTIVITY AFTER A TOTAL IRRADIATION WITH 500 R., FOR 58 MIN IN MOUSE LIVER (TOTAL HOMOGENATE)

Activity (refs. 6 and 7) determined in phosphate buffer solution at *p*H 7·4 in the presence of pyruvate

	M mol. NH_3/g liver/h
Normal	0·401
2 h after irradiation	0·276
24 h after irradiation	0·183
48 h after irradiation	0·120

Table 2. CHANGES IN GLUTAMINASE ACTIVITY IN MOUSE BRAIN IRRADIATED *in vivo* WITH 500 R. FOR 58 MIN

Determination performed in phosphate buffer solution at *p*H 8·5 (refs. 7 and 8)

	M mol. NH_3/g brain/h
Normal	1 620
2 h after irradiation	1·564
24 h after irradiation	0·892
48 h after irradiation	0·440

The decrease of the activity of these enzymes situated in the mitochondria could be explained as a consequence of changes in the synthesis of new proteins, and therefore also of enzymal proteins, effects known to be induced by ionizing radiations[4]. Furthermore, both enzymes having active —SH groupings, they may be inhibited by the direct action of X-rays on these groupings as described by Barron[5].

Inhibition of these two enzymes once more supports the fact that ionizing radiations act in the first place on the —SH groupings, oxidizing them, and that they cause primary changes in the mitochondrial structure.

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BIOLOGY

Plankton from the East African Area of the Indian Ocean

AN earlier communication[1] has described how a series of plankton samples was taken from the coastal strips of Kenya and Tanganyika, extending outwards for some 50 miles. An analysis of some of the dry-weight data shows the following results.

For Figs. 1*a* and *b* all night samples, shallow water samples (that is, where the total depth was less than 200 fathoms), samples taken within 1 h after sunrise and within 1 h before sunset have been ignored. The remaining samples represent a scattering throughout an area of many thousand square miles.

In association with all these plankton samples Secchi disk readings were taken, using a 50-cm disk, to give a measure of the water transparency.

In Figs. 1*a* and *b* the Secchi disk readings, that is, the water transparency, has been plotted against the dry weight of plankton per m³, this latter being on a logarithmic scale. The regression coefficient has been calculated for each graph and the calculated regression lines indicated. The independent equations are:

$$\text{Fig. 1}a \quad \log y_1 = 1{\cdot}6433 - 0{\cdot}01717x_1$$
$$\text{Fig. 1}b \quad \log y_2 = 1{\cdot}9441 - 0{\cdot}02585x_2$$

In both the regression is significant, $P = < 0{\cdot}001$; the difference between the regression lines is not significant.

Thus the data are consistent with equal rate of fall-off in the plankton dry weight/m³ in the topmost 200 m with increase in transparency.

It might be argued that the relationship between the plankton and the water transparency is direct, the transparency depending on the plankton concentration. There is always a certain amount of suspended inorganic matter in the sea, plus varying concentrations of the microplankton; these might be the direct cause of variations in transparency. It is considered unlikely that the type and quantity of plankton taken in a 74 m.p.i. net, with apertures of 0·205 mm, will influence the transparency to a significant degree.

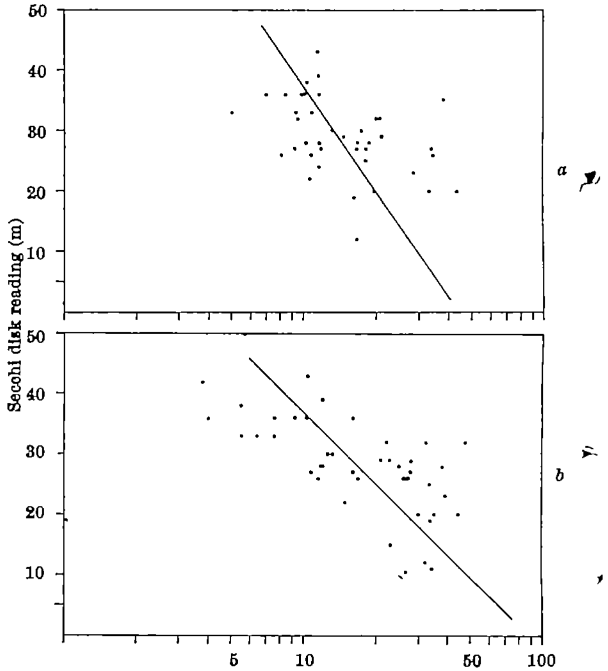

Fig. 1. Diagrams showing the relation of water transparency and plankton dry weight per m³ (on logarithmic scale) from vertical samples taken from 200 m to the surface (*a*) and 50 m to the surface (*b*); the calculated regressions are indicated

A further argument is that as the transparency increases so there is an increased rate of loss of plankton due to net avoidance. There was no evidence to support this.

My conclusions from the data available, applying therefore to the area within the limits of the survey, are as follows.

A small part of the plankton is a normal inhabitant of the topmost layers of the sea; therefore, theoretically, there would always be plankton at the surface even with water of infinite transparency.

The bulk of the plankton reacts according to the degree to which the transparency of the water permits penetration of light; this reaction modifies vertical movements due to negative geotropism, inherent vertical migration patterns, etc. Thus, throughout the area of the survey where the total depth exceeds 200 fathoms, there is, during daylight, no significant variation in the plankton dry weight per unit volume in the topmost 200 m. (The fact that all the points of the graphs do not lie on, or much closer to, the regression lines is no doubt due to the discrepancies inherent with net sampling.) Variations of this plankton indicated previously[1] are not indicative of richer or poorer areas, but of the relative water transparency at the time of sampling.

Clearly this does not preclude the possibility that an assessment of the plankton of the complete water column, extending to beyond 200 m, would show wide variations in relative richness; but it does indicate that plankton estimations based on daylight samples from the topmost 200 m only can be misleading unless the samples are considered in relation to the water transparency.

The variations shown in the shallow water coastal areas do not necessarily conform to the deeper water plankton pattern and are true variations of plankton abundance indicating relatively rich or poor areas.

In Fig. 2 all shallow-water samples have been ignored; the remainder include samples taken at various times throughout the 24-h period. Time and Secchi disk readings are shown as 3-hourly means.

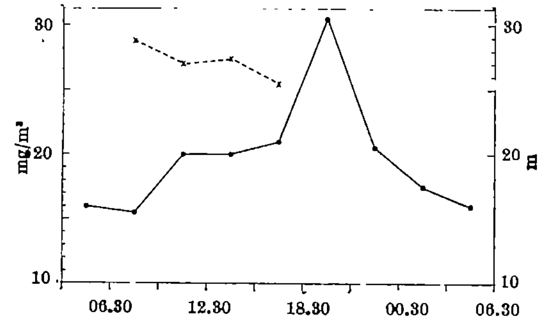

Fig. 2. Diagram showing the variation of the plankton dry weight (mg/m³), taken in vertical samples from both 200 m and 50 m to the surface, throughout 24 h; the former is shown as consecutive 3-hourly means of the latter. Secchi disk readings (m) are also indicated as four consecutive 3-hourly means of the 12-h daylight period

A vertical migration pattern is shown with the single peak occurring between 18.30 and 21.30 h (the annual range of sunset times here is between 18.15 and 18.45 h). Some indication is also given here of the relationship between water transparency and the plankton. As is to be expected the theoretical cross-over point values, about 23 mg/m³–23 m, are very close to the regression line in Fig. 1*b*, less so in Fig. 1*a*.

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Effect of Additions of Volatile Fatty Acids on the Viability and Fertility of Diluted Bovine Semen

Although the lower fatty acids will prolong the respiratory activity of washed mammalian spermatozoa for a considerable time[1], we can find no reference to the effects of added fatty acids on the viability and fertility of diluted semen.

Trials have been conducted in this laboratory to compare the viability of semen in diluents with, and without, added volatile fatty acids. The control diluent was 14*G*, which has been shown to give significantly higher conception-rates than 2·9 per cent sodium citrate[2]. (Composition of 14*G* was: 2·0 per cent sodium citrate; 0·3 per cent glucose; 1·25 per cent glycerol; 1·0 per cent glycine; 1,250 units of penicillin and 1,250 μg of streptomycin per ml. The diluent, when finally prepared, consists of 80 per cent buffer and 20 per cent egg yolk.) This diluent was supplemented with acetic, propionic, butyric, valeric, caproic and octoic acids, both separately and in combination with each other. The addition of these fatty acids improved viability at both 5° C and 37° C. The greatest increase in viability was obtained, however, with caproic acid at a final concentration of 0·025 per cent v/v in the diluent.

Consequent on good results in the laboratory, a field trial was conducted in the 1961 spring mating season. The experimental diluent selected for the trial was 14*G* supplemented with 0·025 per cent caproic acid.

This trial was conducted for twelve weeks by the Hamilton A.B. Centre and for six weeks by the Palmerston North Centre. On one day of each of the weeks the trial was operated all semen issued for use in the field was diluted with the experimental diluent. During the period of the trial all bulls were routinely collected every third day. Thus after three weeks semen from all bulls was diluted once with the experimental diluent. All semen used in the trial was used on the day of, or the day after, collection. Semen was diluted to contain 12½ or 18¾ million sperm per ml. depending on the fertility of the bull. While semen from all bulls was used on the same day of collection, only semen from those bulls exhibiting good storage characteristics was used on the day after collection[3].

Conception-rates were measured as the percentage of cows reported as in calf to first inseminations 49 days after service.

The difference of 0·4 percentage units in favour of the control diluent, 14*G*, with semen used on the day of collection, was not significant ($\chi^2 = 3{\cdot}3$ $P > 0{\cdot}05$). With semen used on the day after collection, however, there was a highly significant difference of 2·6 percentage units in favour of the experimental diluent ($\chi^2 = 39{\cdot}9$ $P < 0{\cdot}001$).

Table 1. Conception-Rates of Control and Caproic Acid Supplemented Diluents Percentage of Cows reported in Calf 49 Days after First Service

	Diluent			
	14*G*		14*G* + 0·025% caproic acid	
Age of semen (h)	No. of inseminations	% successful	No. of inseminations	% successful
6–18	224,275	64·1	41,997	63·7
24–36	95,587	64·3	16,831	66·9

The function of volatile fatty acids in prolonging viability and improving fertility in stored semen is not known. It is possible that it is involved as an energy-providing metabolite under the aerobic conditions existing in stored semen. As its utilization is oxygen dependent a comparison was made for viability between samples stored normally and samples stored under 'anaerobic' conditions.

Split ejaculates from 12 bulls were diluted in 14 *G*, 14 *G* saturated with nitrogen (14 *GN*), 14 *G* plus caproic acid (14 *GC*) and 14 g plus caproic acid saturated with nitrogen (14 *GCN*). Comparisons between treatments were made by estimating the viability of samples incubated at 37° C after one to seven days storage at 5° C.

Viability at 37° C for the various treatments was 14 *G*, 24·9 h, 14 *GC* 33·0 h, 14 *GN* 38·7 h, and 14 *GCN* 47·2 h. Each estimate of viability is the mean for 96 samples. The increase in viability obtained by the addition of caproic acid was as great in the 'anaerobic' (8·5 h $P < 0{\cdot}01$) as in aerobic samples (8·1 h $P < 0{\cdot}01$). While some aeration of samples may have occurred in opening the test-tubes during the incubation period, the difference in viability between the aerobic and 'anaerobic' samples is so great (14·1 h $P < 0{\cdot}01$) as to indicate a very real difference in the oxygen status of the two treatments.

While the possibility that caproic acid is utilized as an energy-providing substrate in aerobic samples cannot be excluded, it is apparent from the viability of 'anaerobic' samples that depletion of the energy-providing substrate is not the reason for cessation of motility in these samples, and that the observed increase in viability is probably due to some other function.

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In vitro and *in vivo* Survival of Morphologically Atypical Embryos in Rabbits

IN a series of investigations of the storage of rabbit embryos, different types of morphologically atypical embryos were encountered[1,2]. These abnormalities were: presence of excessive red blood cells or air bubbles in mucin coat, persistence of corona radiata cells until 48 h, ruptured zona pellucida, embryos with angularly shaped blastomeres, irregular mucin coat and abundance of hypha-like projections, flattened and lentil-shaped morulæ.

The structural abnormalities of the embryos may be a result of cytological, physiological, genetical, environmental or pathological disturbances. Some of the observed abnormalities are likely to be a result of several causes such as incomplete maturation of ova, faulty fertilization or ageing of gametes. Although not shown by actual investigations, it seems highly probable that aberrations occur in oögenesis similar to those found by Knüdsen[3] in spermatogenesis. There is evidence that the frequency of morphological abnormalities of the embryo is influenced by hereditary factors[4] and the season of the year[5].

The purpose of this experiment was to test the suitability of morphologically atypical embryos (2 days *post coitum*) for storage and subsequent transfer.

Donor (pubertal) and recipient (adult) does of the New Zealand large breed were used. During the main investigation, which is not described here, some 150 donors were superovulated using pregnant mare serum (P.M.S.) (commercial preparation 'Equinex' from Ayerst Laboratories) and human chorionic gonadotrophin (H.C.G.) (from Upjohn). Only 15 donors had typical and atypical embryos in the same animal; these were used in the present study. A total of 96 morphologically atypical and 83 morphologically typical (control) embryos were obtained from the superovulated donors which were bred to fertile bucks and autopsied approximately 40 h *post coitum*. The Fallopian tubes were removed and the embryos flushed into watch glasses and examined with a stereomicroscope (× 60). The atypical embryos were placed in storage tubes in a serum/saline, 1 : 1 media and stored for two days at 10° C. Morphologically typical embryos from the same donor were stored in a second tube of the same media to serve as control.

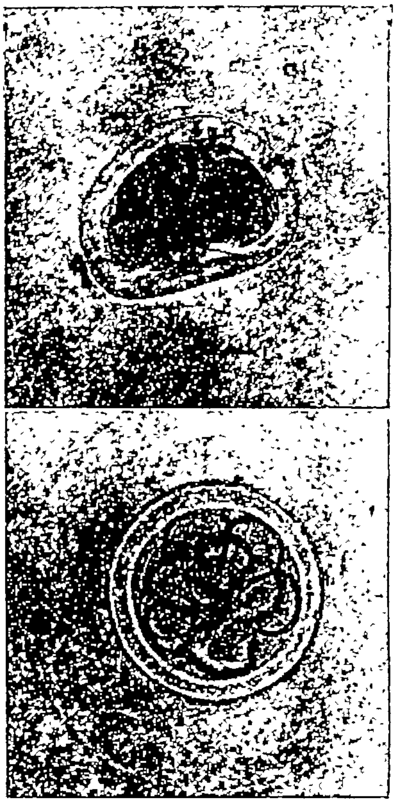

Fig. 1. Atypical (top) and typical (bottom) rabbit embryos recovered from superovulated donors 40 h *post coitum* (× 185). Note the flattened zona pellucida in the atypical embryo and the even size and distribution of blastomeres in the typical embryo

Eighteen recipients were ovulated with an intravenous injection of 15 I.U. of H.C.G. (Upjohn) two days after the donors were bred. Two days post-injection, the embryos were transferred to the Fallopian tube through the fimbriæ via a flank laparotomy. Approximately six embryos were transferred to each Fallopian tube; one side morphologically atypical embryos and the other side control embryos from the same donor. The recipients were laparotomized at 8 days *post coitum* and implantation sites were counted. The pregnant recipients were autopsied 15 days *post coitum* and survival of embryos determined by their heart beat.

Four morphologically atypical groups of embryos were found: embryos with cellular elements between the zona pellucida and the mucin coat (perhaps as a result of delayed denudation of the corona radiata); oval-shaped zona pellucida; flattened zona pellucida; and embryos with indistinct blastomeres (Fig. 1). These four types survived during storage for 48 h at 10° C. The percentage of implantation at 8 days *post coitum* of atypical embryos were statistically similar to that of morphologically typical embryos. However, the percentage of fœtal survival at 15 days

Table 1. IMPLANTATION AND FŒTAL SURVIVAL OF MORPHOLOGICALLY ATYPICAL EMBRYOS AFTER STORAGE FOR 48 H AT 10° C.

	Atypical embryos						Control (typical embryos)				
Morphological characteristics of atypical embryos	No. of recipients	No. of embryos transferred	Implantation‡ Total	Implantation‡ Per cent	Fœtal survival§ Total	Fœtal survival§ Per cent	No. of embryos transferred	Implantation‡ Total	Implantation‡ Per cent	Fœtal survival§ Total	Fœtal survival§ Per cent
Delayed denudation of corona radiata	4	21	7	33	4	57	19	8	42	7	88
Ovoid zona pellucida	4	22	15	68	6*	86	18	12	67	6*	100
Flattened zona pellucida	2	12	9	75	5	56	12	10	83	8	80
Indistinct blastomeres before storage†	7	41	30	73	20	67	34	20	59	10	50

* Two recipients dripped off before slaughter. † All embryos had distinct blastomeres after storage. ‡ At 8 days *post coitum*. § At 15 days *post coitum*.

post coitum was lower in three types of atypical embryos than in control. This phenomenon may be due to a 'carry-over effect' in the atypical embryos. It is emphasized that embryos should be carefully selected for subsequent transfer especially in quantitative studies on implantation and embryonic survival. The embryos which showed indistinct blastomeres prior to storage had distinct blastomeres after storage. It is possible that the embryos with the indistinct blastomere were in an incomplete stage of cleavage when taken from the donor. It is also possible that such incomplete cleavage, once started, is completed even when stored at 10° C.

Kvasnitskii[6] had reported that lentil-shaped ova do not normally develop although ovoid-shaped ova develop normally. Hertig *et al.*[7] described different types of morphological abnormalities in human embryos: absence of chorionic cavity, shallow implantation, maloriented germ disk, and different degrees of trophoblastic hypoplasia. Such abnormalities might be subject to subsequent degeneration. It has been suggested that a lack of adequate direct or indirect stimulation by a defective trophoblast may cause the failure of the corpora lutea to undergo secondary hyperplasia, as in normal pregnancy[7]. Further physiological, biochemical, genetic and immuno-embryological studies are needed to understand the relationships between implantation and fœtal survival in a wide spectrum of mammalian species.

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Resolution of Endopolygalacturonase and a Macerating Factor in a Fungal Culture Filtrate

THE brown rot fungi, *Sclerotinia fructigena* Aderh. and Ruhl., and *S. laxa* Aderh. and Ruhl., are known to penetrate infected fruits by dissolution of the middle lamella[1], and the macerating effect of culture filtrates of these fungi on disks of plant tissue has been ascribed to pectolytic enzymes[2]. The separate identity of 'protopectinases' (using the nomenclature of Demain and Phaff[3]) has been questioned in recent reviews[3,4]; but the presence of distinct fungal macerating enzymes, without characterized substrates, has been reported in a commercial pectic enzyme[5] and in culture filtrates of two plant-pathogenic fungi[5,6].

In the course of studies on the pectolytic enzymes of *S. fructigena*, dextran gel filtration on 'Sephadex *G*.75'[7] has proved useful for the partial purification of an extracellular endopolygalacturonase. We have examined the macerating activity of the fractions (10 ml.) using Brown's method[8], based on the loss of coherence of 4 disks of potato (1 cm diameter, 0·35 mm thick), immersed in the test solution (2 ml.). Fig. 1 shows the results obtained, together with those of an assay of endopolygalacturonase by the agar plate method[9].

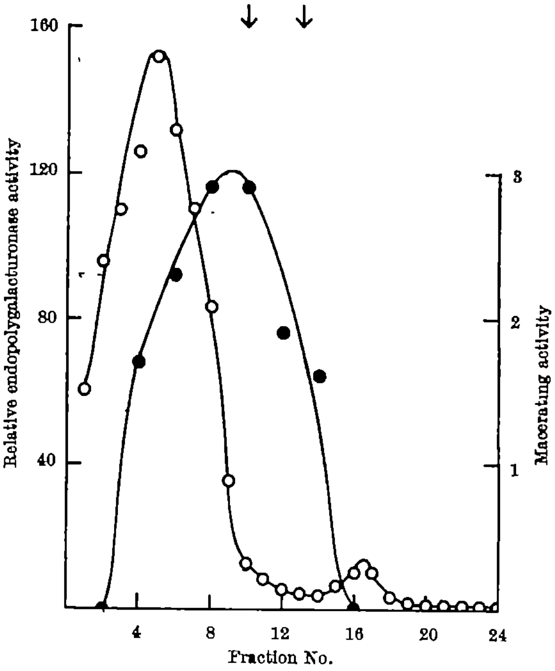

Fig. 1. Gel filtration on 'Sephadex *G*.75' of *S. fructigena* culture filtrate, showing endopolygalacturonase activity (○) relative to pectinol 10 M (1 mg/ml.) as 100. Macerating activity (●) is expressed as 100/*t*, where *t* is the mean time (min) for potato disks to lose coherence. Fractions 10–13 (within arrows) were applied to a second column (see Fig. 2)

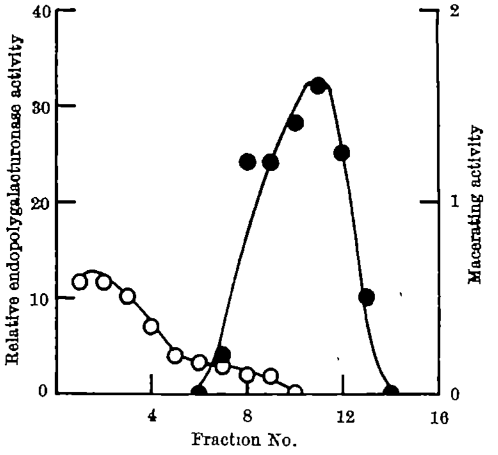

Fig. 2. Second gel filtration of 30 ml. sample. Symbols as in Fig. 1

Fractions 10–13 were re-applied to a shorter 'Sephadex' column, when a further separation of macerating and endopolygalacturonase activity was obtained (Fig. 2). Macerating activity (at *p*H 4·7) free of endopolygalacturonase was also obtained by passage of appropriate salt-free fractions from 'Sephadex' through a column of 'Ecteola'–cellulose[10], which retained the endopolygalacturonase. These preparations showed no polygalacturonase or polymethylgalacturonase activity (at *p*H 4·7), as assessed by the viscosity[11] and reducing group methods[12], while a test for pectinesterase[13] was also negative. The toxicity[14] of the crude culture filtrate to potato and cucumber slices was retained in the fractions showing macerating activity, supporting earlier views on the probable identity of macerating and toxic factors[4,15].

At present, we suggest the use of the term 'maceration factor' for this thermolabile active substance, in preference to 'protopectinase', since the latter term implies an enzymic nature, which we have failed to demonstrate: indeed, the level of protein in actively macerating percolates from 'Ecteola'–cellulose was below the limits of estimation by two methods[16,17]. The retardation of the maceration factor behind the main endopolygalacturonase peak suggests that it may be a smaller molecule[7]; there was also some evidence of adsorption effects between the maceration factor and endopolygalacturonase.

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Plausibility of the Ribonucleic Acid Code

RECENT experimental results indicate that there is a correspondence between RNA trinucleotides and amino-acids. Specifically, for each of the 20 amino-acids, published data list at least one trinucleotide believed to incorporate it in the protein synthesis machinery of *E. coli*. Surprisingly, in each of these published code-words, the symbol U (uracil) occurs in at least one of the three positions.

A careful examination of the experimental procedures reveals that 'random' polynucleotide chains could only be tested if they contained a strong preponderance (at least 65–70 per cent) of the symbol U. Specifically, the trinucleotides containing no U were so much less probable than those containing U that even if some of these were good code-words, and consistently incorporated their specific amino-acids, the frequency of these acids would have been below the significance-level of the experiment. Other experiments have suggested that a very few U-free triplets, such as AAA and CCC, do not code for any amino-acid.

In the face of these results, it is natural to inquire: Is it not overwhelmingly probable that U is needed in most or all of the code-words? An affirmative answer to this question appears to be at variance with the fact that U is not nearly so preponderant in Nature as this would seem to imply.

One hypothesis might be that the 37 triplets containing U include the amino-acid code-words, while the other 27 triplets include the regulatory instructions for the genetic process. However, the facts so far are also consistent with the following model.

Suppose that each amino-acid is 'randomly' assigned three different trinucleotides which code for it. Thus 60 of the 64 triplets are used as code-words. If only 37 of the 64 triplets are 'observable', how many different amino-acids should be represented among the observable triplets?

For a familiar probabilistic formulation one may imagine a pack of 64 cards, including four 'jokers' and three each of the numbers 1–20. If 37 cards are selected at random, how many different numbers are expected to appear? In particular, how likely is it that all 20 numbers be represented at least once each?

The formula for the probability of exactly n different numbers in a hand of 37 cards from this pack of 64 is:

$$P_n = \binom{20}{n} \sum_{j=0}^{n-11} (-1)^j \binom{n}{j} \binom{3(n-j)+4}{37} \Big/ \binom{64}{37}$$

$$\text{for } 11 \leqslant n \leqslant 20$$

Clearly $P_n = 0$ for $n < 11$ or $n > 20$.

To an accuracy of $\pm$ 0·002, this leads to the data in Table 1.

Table 1

n	P_n
20	0·174
19	0·390
18	0·310
17	0·108
16	0·017
15	0·001
14	0·000

Thus, the odds of at least 19 distinct amino-acids being represented in a sample of 37 trinucleotides is better than an even bet; and the probability of at least 17 is a remarkable 98 per cent. The expectation of the distribution is 18·6 distinct amino-acids.

The principal conclusion from this calculation is that it is premature to reach firm conclusions about the role of the U-free trinucleotides.

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Deoxyribonucleic Acid Synthesis or Turn-over in Non-dividing Pollen Cells of Pine

INVESTIGATIONS of deoxyribonucleic acid (DNA) formation *in vivo* have shown that synthesis and replication are completed before separation of chromosomes begins. DNA synthesis, as measured by staining, incorporation of phosphorus-32, or radioactive nucleotide precursors, has generally been thought not to occur in cells unless they are preparing to divide[1]. Research leading to such conclusions has used developing angiosperm pollen cells (microspores) of *Lilium*[2] and *Tradescantia*[3] or bacteria and animal tissues[4].

Other investigations with bacteria[5], nematodes, and animal tissues[6] have indicated that synthesis or turnover of DNA may occur in non-dividing cells. This

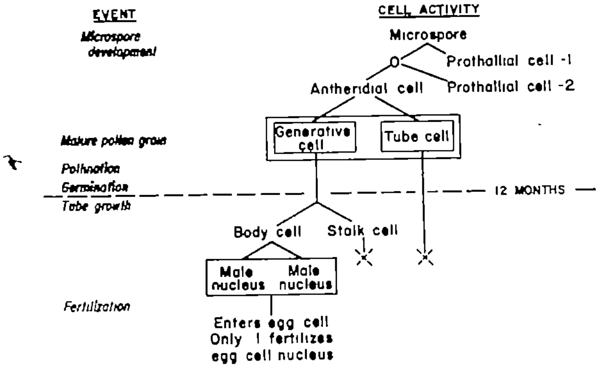

Fig. 1. Sequence of events in developing and germinating pine pollen

communication reports evidence that DNA synthesis or turn-over occurs in the tube nucleus of germinating pine pollen, a non-dividing plant cell.

Mature germinating pine pollen contains two functional nuclei: the tube nucleus, which does not divide, and the generative nucleus, which divides twice before fertilization. Developmental sequences of division in mature pine pollen are shown in Fig. 1. On germination the tube (vegetative) nucleus of the mature pollen moves down the tube. The tube nucleus disintegrates when the tube penetrates or contacts the egg cell. Some workers have claimed—on the basis of decreased staining of nucleic acid in the tube nucleus[7]—that the nucleus disintegrates while still in the tube before reaching the egg. The important fact, relative to this work, is that the tube nucleus is terminal and non-dividing.

We have studied incorporation of thymidine-methyl-^{3}H into germinating pine pollen. The labelling pattern obtained is shown in the autoradiogram in Fig. 2. Note that both the tube nucleus (*T*) and the generative nucleus (*G*) have incorporated tritiated thymidine. Table 1 summarizes experimental evidence that this represents incorporation and not mere absorption.

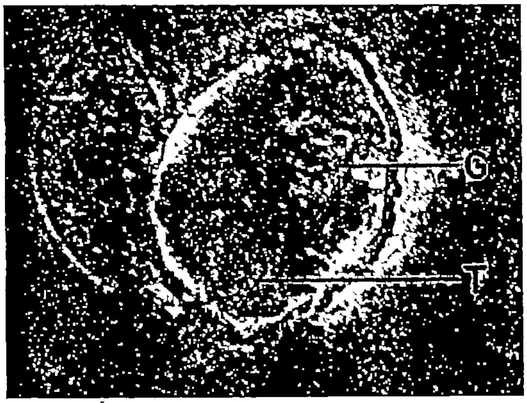

Fig. 2. Tritiated thymidine labelling in germinating pollen, *Pinus ponderosa* Laws. *T*, tube nucleus; *G*, generative nucleus. 100 mg pollen germinated 20 h, 29° C, in 10 ml of 0 002 M calcium phosphate buffer, *p*H 5 8, then for another 27 h in thymidine-methyl-^{3}H, 5 μc /ml culture solution (S A. 8 15 c./mμ) from New England Nuclear Corp, Boston. An equal volume ethanol–acetic acid (3 : 1) was added to each flask to inactivate and fix tissues. After 4 h new alcohol-acid solution was added for 8 h, followed by graded ethanol and butanol dehydration. During germination 'Mycostatin' (Squibb), 15 p p.m., was added as fungicide. Paraffin embedded grains were sectioned serially, about 8μ thick. Autoradiographic stripping film Kodak *AR* 10 was used

Table 1. Effect of DNase and RNase on Thymidine-^{3}H Incorporation in Germinating *Pinus ponderosa* Pollen

Treatment	Average No. of silver grains per section*		
	Generative nucleus	Tube (vegetative) nucleus	Cytoplasm of tube cell
Thymidine-^{3}H + buffer, *p*H 6 0	21	40	18
Thymidine-^{3}H + DNase	12	12	6
Thymidine-^{3}H + RNase	30	40	0

* The average count of 12–15 pollen sections. Pollen germinated and fixed as described under Fig. 2. Deoxyribonuclease and ribonuclease from Nutritional Biochemical Corp. 10 mg/100 ml., adjusted to *p*H 6·0 with sodium hydroxide. DNase solution contained 0 001 M magnesium sulphate. Incubation at 37° C for 1–4 h.

Counts were made of the number of silver grains visible in the emulsion exposed to prepared pollen sections. Results indicate that incorporated nucleoside is hydrolysed and removed by DNase but not by RNase. Washing with 10 per cent perchloric acid at 0° C did not remove activity incorporated in nuclei, although it did reduce the activity in cytoplasm. Cytoplasmic incorporation was also affected by RNase. This probably indicates that the apparent cytoplasmic incorporation actually represents free or entrapped, or merely terminally attached, thymidine-^{3}H rather than incorporation into DNA. Another possible explanation is that thymidine is being metabolized in the cytoplasm and the product is susceptible to hydrolysis by RNase and acids.

Why should the vegetative (tube) cell nucleus of pine pollen retain the capacity to synthesize or replace its DNA? We can only speculate about an answer. One hypothesis is that this daughter nucleus, derived from the same parent cell as the generative nucleus (Fig. 1), retains the ability to synthesize DNA but cannot divide. The sister generative cell, on the other hand, can both replicate its DNA and divide. Coulter and Chamberlain[8] cited a report which claimed to have observed a pine pollen tube nucleus which divided. The original paper reporting on a single preparation does not appear to convey this interpretation. Nevertheless, in terms of evolution and ontogenetic development, it is conceivable that the pine pollen tube nucleus may have the totipotency, under certain conditions, to divide as its sister generative cell does. Tulecke[9] has shown that the tube cell of *Ginkgo* pollen can divide and proliferate to produce a cell mass. This further supports the hypothesis of an inherent capacity of gymnosperm pollen tube cell and nucleus to divide under certain conditions.

A second hypothesis recognizes that a rapidly growing cell, such as a pollen tube, produces large amounts of enzymes as it develops[10]. This would require a great amount of RNA template. Possibly, such a growing tissue may have to synthesize or replace a certain amount of DNA. A relationship between high levels of DNA and respiration in anthers forming microsporocytes has been reported[11]. Thus in germinating pollen, which must rapidly increase the enzyme-level, it is conceivable that the level of cell DNA must be increased even if the cell will not divide. The tube nucleus appears actively to incorporate nucleotide components before the generative nucleus[12]. It also operates in an entirely different area of the cell, although the tube cell does produce large amounts of enzyme[10]. Thus its DNA-level may possibly have to be increased or replaced to support such activities. Replication of DNA and separation of the chromatids are two separate events. Synthesis or replacement of DNA may, under certain conditions, occur without separation and division following.

Steffenson and Bergeron[13] showed that the tube nucleus and sperm nuclei of *Lilium* pollen incorporated calcium-45 during tube growth. They suggested that the calcium absorbed was involved in DNA, and they interpreted this calcium absorption as indicating that DNA synthesis occurred in the tube nucleus of *Lilium* pollen. Their conclusion is not supported by others[14]. But if Steffenson and Bergeron are correct, their results would be similar to those reported here: an apparent incorporation or turn-over of DNA can occur in the tube nucleus. However, it seems from the investigation with calcium-45 that the tube nucleus is an active site of cell metabolism. Other plant cell nuclei have been shown to contain $4n$ and even $8n$ levels of DNA. This variation also indicates that DNA synthesis and chromosome replication can occur without nuclear or cell division[1,15].

In this work we followed standard criteria and procedures concerning incorporation of tritiated nucleosides into nucleic acids. Conclusions based on interpretation of these autoradiograms are probably valid. But we cannot be certain that what is called incorporation, through the techniques used, is not merely end-group fixation or short polynucleotide synthesis until the DNA of the tube nucleus is actually isolated and degraded, or some other direct critical analytical tool is used to show the location of the tritiated thymidine.

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Cation–Anion Relationships in Italian Ryegrass (*Lolium multiflorum*)

SOIL, climate, time of sampling and other factors influence the mineral composition of plants, and the same species can contain a wide range of inorganic ions. In spite of these variations several workers[1–3], using different plants, claimed that the sum of the cations (expressed in m.equiv./100 g dry material) of a particular plant is constant. McCalla and Woodford[4] found that the ratio of total cations : total anions in wheat was constant and later Bear[5] showed that, for lucerne, at any pH when ions were expressed in m.equiv./100 g:

$$\frac{K + Na + Ca + Mg}{P + Cl + S + Si + N} = \text{a constant}$$

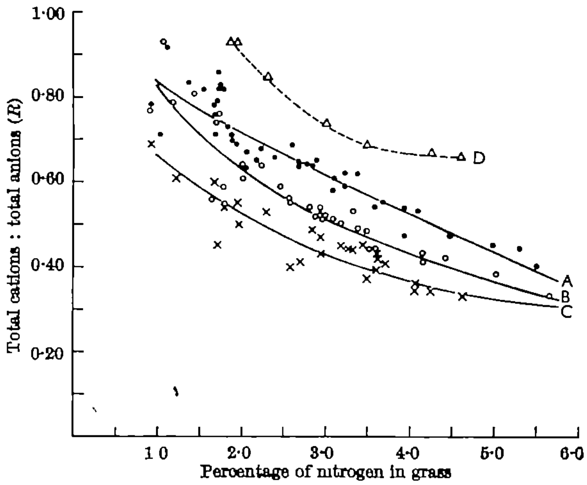

Fig. 1. Relationships between total nitrogen and total cation : anion ratio (R) in Italian ryegrass. ●, Curve A, samples grown in the field with no added nitrogen or with NO_3^- but without NH_4^+; ○, curve B, samples grown in the field with medium amounts of NH_4^+ (for example, 35–70 lb. nitrogen/acre as NH_4NO_3); ×, curve C, samples grown in the field with large amounts of NH_4^+ (for example, 40–160 lb. nitrogen/acre as $(NH_4)_2SO_4$); △, curve D, samples grown in one of the soils in the greenhouse in winter with NO_3^- but without NH_4^+

It is not known if silicon should be included, and Dijkshoorn[6], using perennial ryegrass, obtained a constant = 0·5 when silicon was omitted.

Italian ryegrass, consisting of 6 strains but mainly *S.*22, was grown on 20 soils throughout Britain in five seasons (1957–61); the grass received varying nitrogen–phosphorus–potassium fertilizer treatments and was cut at various stages of growth during the period April–September. More than 100 samples of the grass were analysed for the cations, K^+, Na^+, Ca^{++}, Mg^{++} and the anions, NO_3^- (assumed responsible for the uptake of all N), $H_2PO_4^-$, $SO_4^{=}$ and Cl^-. Silicon was also measured. The results give new information on cation–anion relationships in plants.

(1) The total cations in the grass ranged widely from 80 to 205 m.equiv./100 g dry grass.

(2) The total anions ranged from 130 to 551 m.equiv./100 g (including silicon) and 104 to 496 m.equiv./100 g (omitting silicon). In two groups of samples total cations were more closely correlated with total anions when silicon was omitted ($r = 0\cdot59^{***}$ and $r = 0\cdot69^{***}$ with and without silicon respectively in one group of 45 samples and $r = 0\cdot77^{***}$ and $0\cdot94^{***}$ in another group of 20 samples).

This means that including silicon complicates studies on cation–anion relationships unnecessarily; probably silicon does not enter plants as an ion. Iler[7] showed that there are few silicate ions below pH 9·0, which is above the pH of normal soil.

(3) The ratio of total cations : total anions in all samples was <1 but was not constant (silicon omitted), and ranged from 0·33 to 0·93.

Curves A–C in Fig. 1 show that the ratio of total cations : total anions (silicon omitted) depends on the nitrogen status of the grass and the type of nitrogen (that is, NO_3^- or NH_4^+) taken up by the plant. The relationships between total nitrogen of the grass grown in the field and total cation : total anion ratio (R) are expressed by the following equations:

(A) With no added NH_4^+.

$$R = 0\cdot890 - 0\cdot0936\,N + 0\cdot059/(N+\tfrac{1}{2})$$

(resid. $S.E.$ = ± 0·051)

Grass, receiving no nitrogen but containing much chlorine (> 1·5 per cent), is an exception, and its R value is obtained from equation C.

(B) With medium amount of NH_4^+.

$$R = 0{\cdot}479 - 0{\cdot}0441\ \mathrm{N} + 0{\cdot}606/(\mathrm{N}+\tfrac{1}{2})$$
$$(\text{resid. } S.E. = \pm\ 0{\cdot}053)$$

(C) With large amount of NH_4^+.

$$R = 0{\cdot}444 - 0{\cdot}0382\mathrm{N} + 0{\cdot}382/(\mathrm{N}+\tfrac{1}{2})$$
$$(\text{resid. } S.E. = \pm\ 0{\cdot}038)$$

Curve D shows that the ratio of total cations : total anions for any nitrogen-level is greater in grass grown in the greenhouse during winter than in grass grown in the open between April and September.

I thank M. J. R. Healy, Statistics Department. Rothamsted Experimental Station, for deriving the equations.

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ENTOMOLOGY

Effect of Low Temperatures on Development, Hatching and Survival of the Eggs of *Aedes aegypti* (L)

THE resistance of insect eggs to cold can be an important factor governing the survival and distribution of the species. In the case of the eggs of *A. aegypti*, prolonged exposure to temperatures below 10° C was shown to be lethal[1]. In order to examine more precisely the effect of storage at a low temperature on embryonal development, ability to hatch and viability of the prolarval stage, eggs at various stages of development were transferred to lower temperatures for periods up to 50 days. Respiration-rate and response to hatching stimulus were examined before and after treatment in the cold. Each batch consisted of eggs laid on filter paper during a period of 12 h. 100 mg of air-dried eggs were placed in micro-cups, and the oxygen uptake was measured at 30° C in a conventional Warburg apparatus. Respiration was nearly constant during the time of the experiment for 4–6 h. No fluid was added to the cups, in order to prevent hatching. 0·1 ml. of 20 per cent potassium hydroxide was added to the central well. Hatching was induced by lowering the oxygen concentration in the submersion medium[2]. In order to avoid erratic response, maximum stimulus was applied by adding ascorbic acid (10^{-3} M final concentration) to the hatching vessels and placing them over pyrogallol in jars which were afterwards evacuated.

Water content was determined by the difference in weight of eggs taken directly from storage and after drying at 105° for 24 h. Fat was extracted with alcohol/ether (3 : 1) and esterified fatty acids were determined according to the method of Stern and Shapiro[3]. Respiration and percentage hatching of eggs stored at 28° C was examined daily for the first 9 days following oviposition and at intervals thereafter up to 8 months. During the first four days, the eggs were kept on filter paper saturated with water, in order to allow full embryonal development; thereafter they were transferred to relative humidity 70–90.

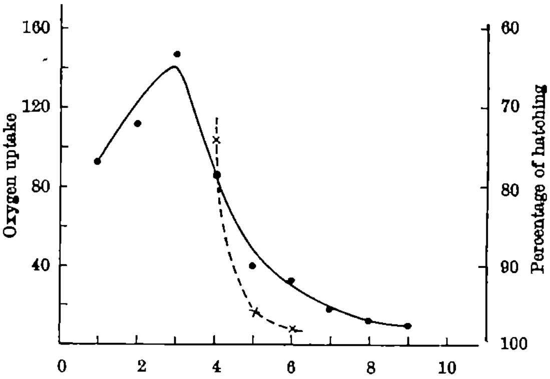

Fig. 1. Oxygen uptake in μl. × 10^{3}/h/mg air-dried eggs during embryonic development (solid line) at 28° C. Percentage of hatching (broken line)

As can be seen from Fig. 1, uptake of oxygen increases during an initial period of 3 days and then falls off steeply for 3 further days and more gradually thereafter. With the decrease in respiration, response to hatching stimulus commences and reaches a maximum after 3 days (90–100 per cent); this high level remains almost constant for about 3 months and then declines (Fig. 2). Thus the time-curve of hatching obtained is in agreement with the observations of Kliewer[4]. In addition, determination of fat and water content during the storage at 28° C and relative humidity 70–90 showed that both constituents decrease continuously; this effect can be correlated with the decrease in the percentage of hatching (Fig. 2). If on the sixth day following oviposition, that is, after the completion of embryonal development, the eggs are transferred to a lower temperature (4°) and then returned to 28° C, the following is observed: (a) Oxygen uptake remains nearly the same (34·4 μl. × 10^{-3}/h/mg after 20 days storage at 4° C as compared with 32·0 μl. × 10^{-3}/h/mg before storage in the cold) while that of eggs from the same batch kept continuously at 28° C drops to a much lower level (9·6 μl. × 10^{-3}/h/mg). (b) Immediately after the storage in the cold, almost no hatching can be induced (1·8 per cent hatching as compared with 95 per cent of eggs not subjected to cold treatment). (c) On further storage at 28°, the respiration

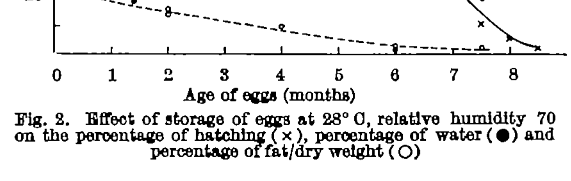

Fig. 2. Effect of storage of eggs at 28° C, relative humidity 70 on the percentage of hatching (×), percentage of water (●) and percentage of fat/dry weight (○)

decreases and after some days reaches almost the same level as that of the control kept constantly at 28° C. At the same time the response to hatching stimulus gradually returns. On the other hand, if the eggs are transferred to a lower temperature during the period of embryonic development, the following differences are observed: The respiration after exposure to cold is lower than before it, but again higher than that of the eggs not subjected to the cold treatment. However, no hatching can be induced even after the eggs have been returned to 28° C for a period of two weeks. The lower respiration at the end of the storage in the cold may be a reflexion of the onset of mortality.

These results indicate that there exists a reverse correlation between respiration of eggs and their hatching ability.

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GENETICS

Incidence on Genetics of Mechanism of Segregation and Disjunction at Meiosis in Man

The explanation of the abnormal numbers of chromosomes observed in man rests on an eventual non-disjunction happening at the first or the second meiotic divisions or at both successively[1,2].

It seems primordial to remember that the two successive divisions of a normal meiosis are of different types: a reductional division which results in the segregation of homologous chromosomes and an equational division leading to the separation of the chromatids, already apparent at the diplotene stage of the first meiosis (tetrad).

So it is clear that non-segregation at the first meiosis (in our opinion erroneously called non-disjunction) must have a repercussion on the development of the second meiotic division.

We have thus computed a table with all the possible karyotypes of the gametes and combinations in the zygotes[3] and obtained the frequencies of the different combinations denoting by p_1 for women and by p_1' for men the probability of non-segregation at first meiotic division, that is, the probability that the two chromosomes of a given homologous pair will be drawn towards the same pole of the spindle, and by p_2 for women and p_2' for men the probability of non-disjunction at second meiosis, that is, the probability that the splitting of the centromere will be postponed until after the division is completed.

If one ignores the eventual degree of lethality of some combinations, the frequency of lethality being yet impossible to estimate, then the relations which link the frequencies of the different abnormalities, ought to be verified simultaneously by the observed frequencies.

The Turner's (*XO*) of paternal origin are twice more frequent than those of maternal origin, if we admit that the probabilities of non-segregation and non-disjunction are equal in both sexes ($p_1=p_1'$, $p_2=p_2'$). In this case, the total frequency of the Turner's is $3/4\ (p_1+p_2)$.

The Klinefelter's, with formula *XXY*, result from three gametic combinations (Table 1).

Table 1

Sperm	Ovum	Origin of abnormal gamete	Frequency
XXY	*O*	paternal and maternal	$\frac{p_1+p_2}{2} \times \frac{p_1' \times p_2'}{4}$ very rare
XY	*X*	paternal	$(1 - p_1 - p_2) \times p_1'/2$
Y	*XX*	maternal	$(1-p_1'-p_2') \times (p_1+p_2)/4$

When $p_1=p_1'$, $p_2=p_2'$, the total frequency of the Klinefelter's is $3/4\ p_1+p_2/4$.

We observe that, theoretically at least, Klinefelter's must be less frequent than Turner's and not equally frequent as stated by some authors[1].

On the other hand, one can easily deduce that the number of Klinefelter's of maternal origin increases with the value of p_2.

Values of p_1 and p_2 may be grossly estimated in the following way. The theoretical frequency of persons *XXY* in the general population is $(3p_1+p_2)/4$.

Observed values of patients with Klinefelter's syndrome (chromatin positive) are 1 : 1,000 males[4] and 1 : 400 males[5]. Admitting that: (1) $p_1=p_1'$ and $p_2=p_2'$; (2) there is no lethality for Klinefelter's syndrome; (3) Klinefelter's with chromatin positive are *XXY*, we obtain two equations:

$$(3p_1+p_2)/4=0{\cdot}0005 \qquad (a)$$

$$(3p_1+p_2)/4=0{\cdot}00125 \qquad (b)$$

These are represented in Fig. 1 by two straight lines (*a*) and (*b*).

The true values of p_1 and p_2 must lie between these lines. When $p_1=p_2$, the estimated values are $p_1=p_2=0{\cdot}0005$ (ref. 4) and $p_1=p_2=0{\cdot}00125$ (ref. 5) respectively, corresponding to the points *P* and *M*, lying on the bisections (Fig. 1).

Data on the frequency of Daltonism[6], the best marker gene for sex chromosomes yet known, will

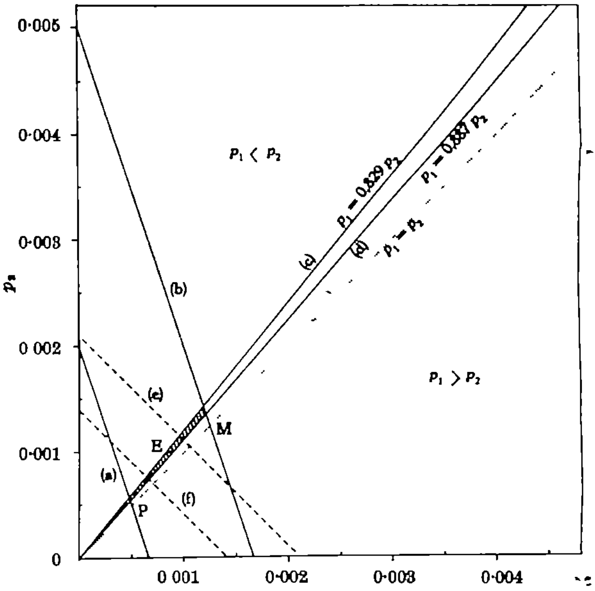

Fig. 1

permit us to give closer estimates of p_1 and p_2 and to decide whether p_1 is larger or smaller than p_2.

p being the frequency of the allele for Daltonism, we can obtain the probability for a Klinefelter to be of maternal or paternal origin and to have normal or abnormal colour vision.

Comparing the ratios:

$$\frac{\text{Klinefelter's of paternal origin}}{\text{Klinefelter's of maternal origin}} = \frac{2p_1}{p_1+p_2} = \frac{A}{B}$$

and

$$\frac{\text{colour-blind Klinefelter's of paternal origin}}{\text{colour-blind Klinefelter's of maternal origin}} = \frac{2p_1 \times p}{p_1 \times p + p_2} = \frac{C}{D}$$

we observe that:

(1) $A \gtrless B$ if $p_1 \gtrless p_2$;

(2) $C \gtrless D$ if $p_1 \times p \gtrless p_2$,

that is to say, if $p_1/p_2 \gtrless 1/p \approx 13$ for $p = 0{\cdot}082$ (Kalmus).

Consequently, if we suppose $p_1 = p_2$, the Klinefelter's of maternal origin must be as frequent as those of paternal origin, but colour-blind Klinefelter's of maternal origin must be 6·6 times more frequent as colour-blind Klinefelter's of paternal origin.

The actual investigations count, on a total of 106 chromatin-positive Klinefelter's (supposed to be XXY), two cases of a colour-blind son of normal parents and one case of a colour-blind son with normal father and colour-blind mother; this last case is of course extremely rare, the observed frequency of colour-blind women being only 0·004.

The values of p_1/p_2 estimated from these data are: 0·887 for the two colour-blind Klinefelter's with normal parents; 0·829 for all three colour-blind Klinefelter's.

We must have $0{\cdot}829 < p_1/p_2 < 0{\cdot}887$.

Combining relations between p_1 and p_2 based on frequencies of Klinefelter's in the population and on frequencies of colour-blind Klinefelter's, we find limits for the values of p_1 and p_2, respectively, the probabilities of non-segregation at first meiosis and non-disjunction at second meiosis. The acceptable values of p_1 and p_2 are the co-ordinates of the points lying on the hatched portion of Fig. 1, between the lines (c) and (d) of equations

$$p_1 = 0{\cdot}829p_2 \qquad (c)$$

$$p_1 = 0{\cdot}887p_2 \qquad (d)$$

E, the central point of this portion, corresponds approximately to:

$$p_1 = 0{\cdot}00085$$

$$p_2 = 0{\cdot}00100$$

which are our final estimates for p_1 and p_2. It must be observed that the real values will be higher than these estimated values if there is some lethality connected with XXY zygotes.

If we now replace p_1 and p_2 by their estimated values, the theoretical frequency of Turner's (XO) is $3/4(p_1+p_2) = 0{\cdot}0014$.

The observed frequency of the Turner's being estimated to 0·0001 in the population[7], we may conclude that at most one XO zygote in 14 survives.

If we use the observed frequencies of mongolism in the same way, and represent by p_1 and p_2 the probabilities of non-segregation and non-disjunction of the 21st pair of chromosomes (in men and in women), we have the relations:

$p_1 + p_2 = 0{\cdot}0021$ for the frequency given by Stevenson[8] (Fig. 1, line e);

$p_1 + p_2 = 0{\cdot}0014$ for the mean of values of frequencies quoted by Penrose[9] (Fig. 1, line f).

Admitting for a moment that the probabilities of non-segregation and non-disjunction are equal to those of the sex chromosomes, and replacing p_1 and p_2 by the estimated values 0·00085 and 0·00100 respectively, we find $p_1 + p_2 = 0{\cdot}00185$, a value situated between the two observed frequencies of mongolism. We wish to direct attention to the fact that the rate of lethality is greater in monosomy than in trisomy: this is true for chromosomes 21 as well as for the sex chromosomes.

We admit the provisional character of the proposed estimates of p_1 and p_2: we think the main interest of our work lies in a trial of quantification of a phenomenon as yet only considered qualitatively. We hope that inquiries in the future will provide data which will help to test the different hypotheses we have felt compelled to introduce in our reasoning.

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PSYCHOLOGY

New Phenomena in Apparent Duration, Distance and Speed

OUR earlier experiments on interdependence in judgments of time, space and movement led to the establishment of a phenomenon, now known as the '*kappa*-effect', in visual and auditory experience[1]. These experiments prompted us to inquire whether analogous phenomena characterize judgments in other contexts when the subject himself undergoes movement, for example, as a passenger in a vehicle on the road.

The subjects travelled in groups of five or ten, in a closed van which precluded all visual cues of the route, or blindfold in a saloon car. A bell was rung at approximately the temporal mid-point of the journey. When the vehicle stopped at the end of the journey, the subjects estimated the pre-bell and post-bell duration, and half of the subjects then estimated the pre-bell and post-bell distance and half of them estimated the pre-bell and post-bell speed. In three trials, at different speeds, the vehicle accelerated when the bell rang; in three corresponding trials it decelerated when the bell rang; and in three trials the speed remained constant for the journey, although it varied from trial to trial. The full journeys lasted 12–30 min., with speeds ranging from 15 to 60 m.p.h. The distance covered was about 12 miles in each journey.

The main results may be grouped as follows:

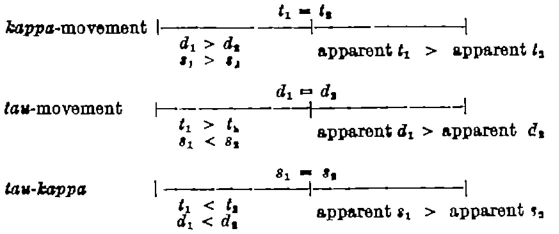
(1) Analysis of the estimates reveals an interdependence in apparent duration, distance and speed such that if two parts of a journey take the same clock time, that part will seem to last longer in which the distance and speed are greater. We propose to designate this the *kappa*-movement effect.

In the same situation, the shorter distance is overestimated and the longer distance, if anything, underestimated. From this, together with the *tau*-effect[2] (which demonstrates the dependence of spatial judgment on temporal factors), we may infer that if two parts of a journey are of equal distance, that part will seem of greater length which is travelled at a slower speed and for a longer time. This we propose to call the *tau*-movement effect.

Finally, in the same situation, the slower speed is over-estimated and the faster speed, if anything, under-estimated. From this, together with the *tau*-effect, we may infer that if two parts of a journey are travelled at the same speed, that part will seem faster in which the distance and time are shorter. This we designate the *kappa-tau*-effect.

The order of magnitude of the *kappa*-movement effect is about 25 per cent, within the range of values studied; the corresponding order of magnitude of the other two effects would seem to be much greater.

The effects may be represented diagrammatically,



(2) In comparing the estimates with the actual durations, distances and speeds, we find that, on the whole, speed is most accurately assessed, distance least accurately, with duration intermediate between the two. The speed which is most accurately estimated, retrospectively, is in the region of 32 m.p.h. At slower speeds, the traveller believes he has travelled faster than he actually has, and at faster speeds he believes he has travelled slower (Fig. 1).

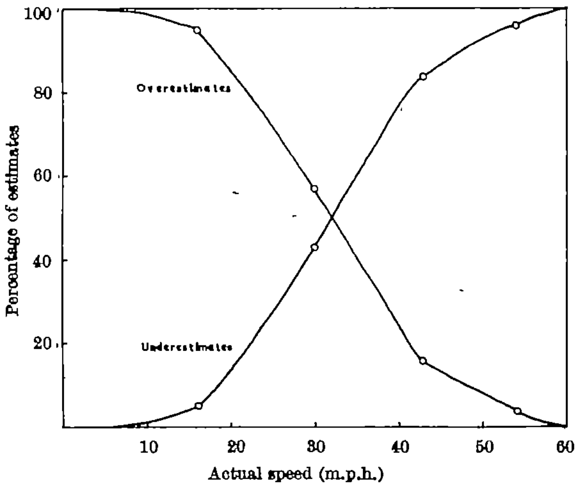


Fig. 1. Percentage of over-estimates and under-estimates of speed

(3) From any two estimates of duration, distance and speed, we can derive the third, and we can compare these derived values with the corresponding direct estimates. On making this comparison, we find that the distance our subjects believe they have travelled is less than that implied by their joint estimates of duration and speed, and conversely they believe they have travelled for a longer time and at a faster speed as judged by the derived estimates of duration and speed respectively.

We thank the Automobile Association for marking out stretches of highway and the Lancashire County and Manchester City Police for providing vehicles and drivers.

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A Regional Difference in Expected Consumption Patterns

DURING the course of a survey of the aspirations and expectations of some 1,300 pupils about to leave school[1] the following questions were asked, in a questionnaire administered in the classroom:

> When you are earning there are probably various things you hope to save up for. What do you hope to own after you have been working for a year?
>
> When you have been working for about 9 or 10 years? What do you hope to own by the time you retire?

The respondents were in their final or penultimate year at school. They were drawn from schools in two main regions, a home county and a county in the south-west of England. Boys and girls came from separate schools. In the home county, secondary modern, technical and grammar schools were visited; in the south-west, modern and grammar schools.

For each of the main sub-samples, the average number of items given in reply to each of these questions was calculated. These figures are shown in two graphs (Fig. 1), illustrating an interesting difference in expected consumption pattern between the two regions.

It can be seen that there is an overall difference in relation to the early years, the home county mentioning a greater number of items. By retirement, there is little difference. The main point of interest, however, is that the south-west England county mentions more items on retirement than at the 10-year point, whereas the home county gives higher figures for the 10-year point than at retirement.

The first period specified, after one year of work, evoked items typical of young people starting work, such as 'a bicycle' or 'clothes'. There is, therefore, less point in comparing these figures with the other two sets, when the items mentioned were very alike for the two periods; predominantly 'a house' and 'a car'. It would seem that the young people in the home county have a greater expectation of accumulating such possessions fairly early (and perhaps paying for them while enjoying them for the remainder of their lives) whereas the expected pattern in the

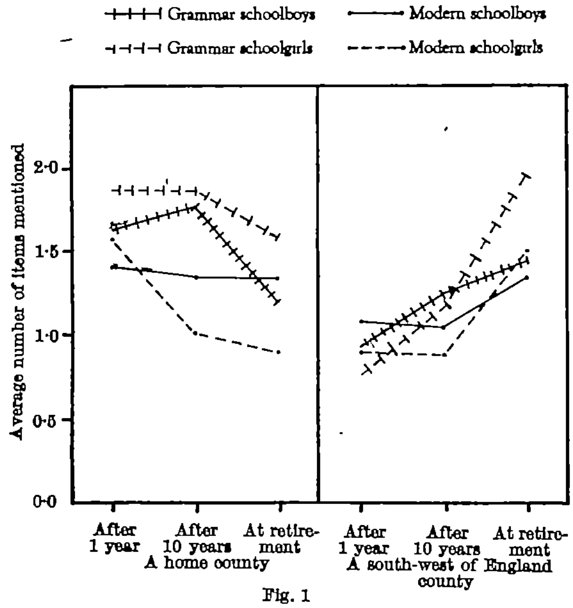

Fig. 1

south-west is to have, and to exercise, relatively greater purchasing power towards the end of the working life.

These findings are not drawn from representative samples. However, they are possibly of interest in that the pattern is repeated independently four times; and the drop from the 10-year period to retirement is repeated twice more, with the technical school boys and girls. These figures are not shown because of the absence of corresponding data for the south-west.

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Traumatically Conditioned Responses acquired during Respiratory Paralysis

THIS communication describes a new technique for conditioning human subjects using a single traumatic stimulus. The need for such a stimulus arises in the conditioned aversive therapy of such behaviours as alcoholism and compulsions. Vomiting has been used frequently as an aversive stimulus[1,2] but suffers from two disadvantages: the onset of vomiting is difficult to predict so that the moment at which the conditioned stimulus must be presented to ensure optimal conditioning is uncertain; also vomiting is accompanied by many undesirable side-effects. An alternative technique, which avoids these disadvantages, is provided by a temporary suppression of respiration, induced by succinylcholine chloride dihydrate ('Scoline').

Experimental traumatic conditioning has been carried out with animal subjects, for example, Solomon and Wynne[3] have shown how conditioned avoidance responses may be formed in dogs by subjecting them to near-tetanizing electric shock. There are no comparable data derived from human subjects. Human subjects are influenced in their judgments of what is painful by adventitious stimuli[4], and in no case has the pain produced the massive autonomic and muscular reactions which have been found in animals. Experiments on 'psychological' trauma encounter similar difficulties: not all the subjects find the treatment traumatic and the physiological arousal is generally slight. The *ex post facto* investigation of individuals who have been in peril of their lives is the only other source of information about the effects of traumata[5].

The consequences of traumatic stimulation have an important place in a number of psychological systems. Some neurotic behaviour is thought to follow from a single traumatic event, and conditioned 'anxiety' responses are utilized in theories of personality though little is known about the generalization and extinction properties of the autonomic events on which 'anxiety' is usually predicated[6]. The technique described in this communication offers a means of providing experimental data relevant to these points.

'Scoline' is a curarizing drug which acts at the motor endplate of the efferent neurones serving the skeletal muscles to cause a nerve-muscle block. For a short period following the injection of 'Scoline' each subject is totally paralysed, unable to move or breathe. During the paralysis the subject remains fully conscious. All the subjects, including some who have themselves given 'Scoline', say that the experience is harrowing to a degree.

The following technique has been used in an investigation of the ability of alcoholic patients to form conditioned responses based on suppression of respiration. The conditioned stimulus was a 600 c/s tone at 70 db. A continuous record of psychogalvanic skin response, respiration, heart-rate and muscle tension was made; these four variables served two purposes: they were used as indices of the conditioned and unconditioned responses, and they were also used to monitor the course of the paralysis. Each subject was first given 1/150 of a grain of 'Atropine' to prevent excess salivation: this dose was injected into a saline drip. The dose of 'Scoline' (20 mg) was given afterwards in the same way. In no case did a subject know from extraneous stimuli that any drug had been given. During the first part of the experiment the subjects were habituated to the tone which they heard through headphones; the tone sounded for five seconds with an average interval of one minute between each presentation. When no response had been made to the tone for five consecutive presentations of the tone the 'Scoline' was given. The tone was presented throughout the period in which no regular respiratory response could be observed on the polygraph. 5 min after the restoration of respiration each subject was given a series of extinction trials. Similar sequences of extinction trials were given one week and three weeks later.

The results of the conditioning procedure indicate that the simultaneous occurrence of respiratory paralysis and tone leads to the formation of conditioned responses. A conditioned response was defined as a change of physiological function following the tone in an interval less than the latency of a 'startle' response. In the extinction trials given immediately after the conditioning trial two out of ten subjects extinguished; in the extinction trials given one week and three weeks later all the ten subjects showed conditioned responses. Table 1 shows the results of 5 subjects who were given identical series of 100 extinction trials. There is very little variation between the subjects in the number of responses given during extinction although, during the initial habituation period, the subjects exhibited marked differences.

Table 1. Number, latency and size of responses given to the conditioned stimulus by 5 male subjects. *a*, during habituation; *b*, in 30 trials immediately following conditioning; *c*, in 30 trials one week later; *d*, in 40 trials three weeks later, *e*, total for 100 trials (latency of response (in seconds), size of responses in ohms)

	a	*b*	*c*	*d*	*e*
Mean No. of responses	13·6	28·0	30·0	38·0	94 0
S.D.	11·0	2 8	0·0	4·5	4·2
Mean latency of responses	2·58	2·3	2·01	1·91	—
S.D	0·28	0 22	0·18	0·17	—
Mean size of responses	658 6	385·8	1,384·0	1,877·2	—
S.D	418·0	131·2	931·4	1,685·3	—

The latency of the conditioned response decreased and its size increased during the interval between the series of extinction trials. These results suggest that conditioning based on a traumatic event leads to responses which exhibit characteristics of an opposite sort to those found in the other conditioning procedures which have been applied to human subjects. In particular the increasing strength of the conditioned response as time passes provides experimental support for the clinically derived notion that 'anxiety' may be a permanent consequence of such a traumatic event.

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STATISTICS

An Iterative Method for obtaining Statistical Frequency Distributions

Nilakantan and Achar[1] have recently described an iterative method for obtaining approximations to frequency distributions from limited data. No attempt was made to provide a theoretical justification of the procedure. In view of the many possible applications of the method it appears worth while to discuss its justification and limitation.

Adopting the notation of Nilakantan and Achar, let x denote discrete variations of a quantity from its arithmetic mean and let F denote the frequency of occurrence of the discrete values of x. Let Y denote the Gaussian probability distribution:

$$Y = \frac{1}{s(2\pi)^{1/2}} \exp(-x^2/2s^2) \qquad (1)$$

where s is a constant.

The probability with which Y predicts a given set of frequencies F is proportional to:

$$p = \frac{1}{(2\pi)^{n/2}s^n} \exp(-\Sigma nFx^2/2s^2) \qquad (2)$$

which is a maximum when:

$$s = (\Sigma Fx^2)^{1/2} = \text{standard deviation } \sigma. \qquad (3)$$

The resulting Y may be denoted by F_1.

Nilakantan and Achar then replace F in equation (3) by $(F+F_1)/2$ and hence compute a new s, say σ_1, which maximizes equation (2) in which F is replaced by $(F+F_1)/2$. The σ_1 is substituted for s in equation (1) in order to obtain a further distribution F_2. Replacement of F in equation (3) by $(F_1+F_2)/2$ similarly leads to a quantity σ_2 and a frequency F_3. In the example provided by Nilakantan and Achar a given set of frequencies F is used for deriving curves F_1 and F_{10}, it being claimed that F_{10} forms a close approximation to the frequencies obtained by choosing a larger number of samples. The process converges rather slowly, leading to $\sigma_8 = 1{\cdot}34$ and $\sigma_9 = 1{\cdot}29$. A set of data containing five times as many samples as was used to compute F was found to have a frequency distribution very close to F_4 for which $\sigma_3 = 1{\cdot}54$.

The validity of the procedure may be examined as follows: the Gaussian function which best predicts the observed set of n samples F is F_1. The frequencies of a hypothetical second set of n samples is therefore best predictable by F_1. For a total of $2n$ samples of which the first n are known to be distributed according to F and the second set are assumed to be distributed according to F_1 the frequency distribution would be $(F+F_1)/2$. The Gaussian function which best predicts this is F_2, and so a third set of samples would be best predicted by F_2. For a total of $2n$ samples of which the first n are of the second set and the second n are of the third set the frequency distribution would be $(F_1+F_2)/2$. The Gaussian function which best predicts this is F_3 and so a fourth set of samples would be best predicted by F_3. Continuing the argument leads to F_4, F_5, etc., to predict the distributions of successive sets of n samples.

The functions F_1, F_2, etc., are thus the Gaussian distributions which best predict certain hypothetical sets of data of which only the first set represents observed data. The assumption that the hypothetical sets become increasingly more typical of actual data is difficult to justify. It would appear that either of

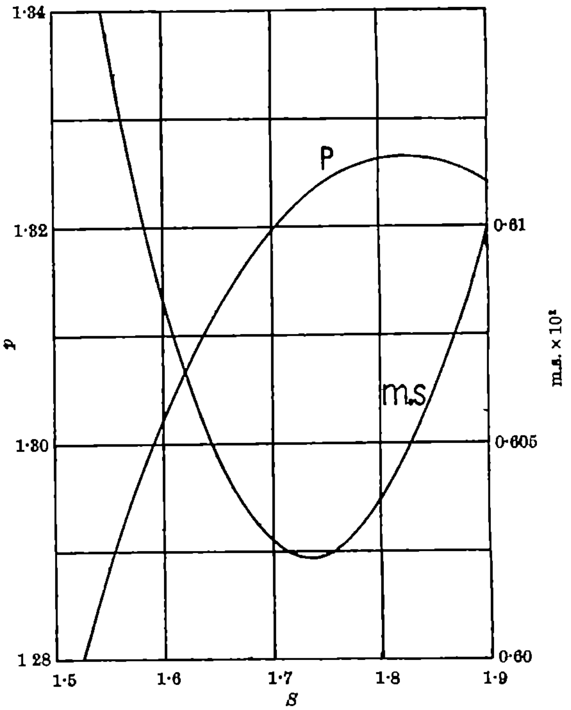

Fig. 1. The probability p according to equation (2) and the mean square (m.s.) difference between Y of equation (1) and the observed frequencies F

the following assumptions are more capable of justification:

Assumption 1: The derived probability distribution, in this instance Gaussian, must have its parameters chosen to give the highest probability to the observed data F.

Assumption 2: The derived distribution must have its parameters chosen to best approximate, in the sense of mean square error, the observed data F.

For different values of s the quantity p of equation (2) is plotted in Fig. 1 for the data of Nilakantan and Achar. Assumption 1 is of course best satisfied when $s = \sigma = 1{\cdot}83$. The mean square difference between Y of equation (1) and F is also shown as a function of s in Fig. 1. It follows that assumption 2 is best satisfied when $s = 1{\cdot}73$.

It is clear from Fig. 1 that the method of Nilakantan and Achar, which leads to $\sigma_9 = 1{\cdot}29$, produces a probability distribution in very poor agreement with either of assumptions 1 and 2. This result, together with the fact that Nilakantan and Achar obtained experimental agreement with σ_3 rather than σ_9, leads me to conclude that their method should be used with considerable caution and only when there are sufficient samples available to permit a check on the validity of the assumed limiting process.

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[1] Nilakantan, P., and Achar, B. N., *Nature*, 193, 1005 (1962).

Since when two samples from a population are combined we get a larger and more representative sample, the iterative process could, in effect, be considered as a means of obtaining a larger sample starting from a small one. As the distribution curve F_2 is derived from the mean values of the frequencies in F and F_1, that is, $(F+F_1)/2$, the result of the combination of two sets of data, namely, the actual curve obtained from n experiments and its Gaussian counterpart, may be regarded as being representative of $2n$ experimental observations (Fig. 1). On the same basis, we may regard F_3 (derived from F_2 and F_1) as representing $3n$ experimental observations and F_4, $5n$ experimental observations. Thus the sample size may be considered as increasing in the following sequence, namely, $n, n, 2n, 3n, 5n, \ldots 89n, \ldots$ corresponding to $F, F_1, F_2, F_3, F_4, \ldots, F_{10}$ and so forth. The $(n+1)$th term in this sequence is given by

$$n\left[\frac{1}{2}\left\{1+\frac{1}{\sqrt{5}}\right\}\left\{\frac{1+\sqrt{5}}{2}\right\}^n+\frac{1}{2}\left\{1-\frac{1}{\sqrt{5}}\right\}\left\{\frac{1-\sqrt{5}}{2}\right\}^n\right]$$

We have since found that a more rational procedure would be to use $\frac{nF+nF_1}{2n}$ instead of $\frac{F+F_1}{2}$ to get F_2, $\frac{nF_1+2nF_2}{3n}$ instead of $\frac{F_1+F_2}{2}$ to get F_3, and so forth. The results obtained by this modification are, however, not significantly different from those obtained previously. But the latter procedure may be considered more logical as indicative of the increase in equivalent sample size.

The iterative process is seen to converge slowly at first, but more rapidly later as may be expected, since the 'equivalent' or effective sample size also increases.

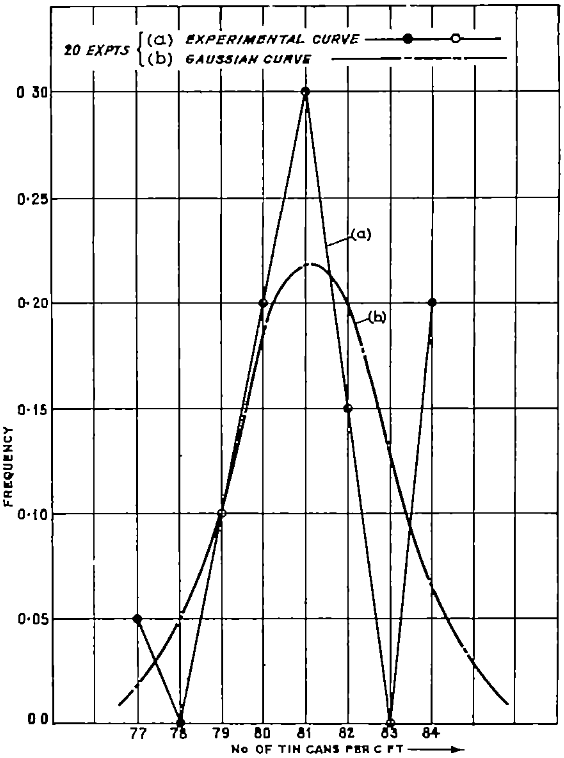

Fig. 1. Frequency distribution curves

Prof. Heaps has assumed that the parameters of the Gaussian distribution curve must be so chosen that they should either give the highest sample probability to the observed data or form the best approximation to the observed data in the sense of mean square difference. He finds that in the case of the experiments carried out by us, $s = 1{\cdot}83$ would give the highest sample probability for the observed data for 20 experiments, whereas $s = 1{\cdot}73$ would give the least mean square difference. As σ_9 obtained from the iterative method has a value 1·29, which is different from either of these, he concludes that the method should be used with caution.

As the Gaussian distribution curve, F_1, has been fitted to the observed data, F, by obtaining the standard deviation $\sigma = 1{\cdot}83$ from the observed data, the first assumption made by Heaps is self-evident, as this value maximizes the sample probability function 'p' which is a characteristic of the size of the sample considered, that is, 20 in the present case. However, in the iterative method the sample size should be regarded as increasing with successive iterations and different 'p' functions would have to be used. Hence $\sigma_9 = 1{\cdot}29$, which would maximize the 'p' function corresponding to an 'effective' sample size of 1,780, is not inconsistent with $\sigma = 1{\cdot}83$ maximizing 'p' for a sample size of 20. The difficulty experienced by Heaps in fitting the results of the iterative method with his assumptions is thus understandable since the variation of the effective sample size was not taken into account by him.

We agree that a considerable amount of experimental investigation will be necessary to establish the general applicability of the method and its possible limitations. We are continuing our investigations on these aspects.

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Generalized Hartley–Ross Unbiased Ratio-Type Estimator

RATIO method of estimation is frequently used in sample surveys to estimate the population mean of the variable under investigation. Several ratio-type estimates can be formed. All these estimates are unsatisfactory in the sense that they are biased. This difficulty was overcome by Hartley and Ross[1], who proposed an unbiased ratio-type estimator for uni-stage sampling designs. In practice, however, we are generally faced with multi-stage sampling designs. This communication gives a generalized form of Hartley–Ross unbiased ratio-type estimator for multi-stage designs. Let N = number of first-stage units in the population, M = number of second-stage units in each of the first-stage units, P = number of third-stage units in each of the second-stage units, $Yijk$ = the value of the variable under investigations for the k-th unit in the j-th second-stage unit of the i-th first-stage unit, and

$$\bar{Y}ij. = \frac{1}{P}\sum_{1}^{P} Yijk,\ \bar{Y}i.. = \frac{1}{MP}\sum_{1}^{M}\sum_{1}^{P} Yijk \text{ and}$$

$$\bar{Y}... = \frac{1}{NMP}\sum_{1}^{N}\sum_{1}^{M}\sum_{1}^{P} Yijk$$

To estimate the population mean $\bar{Y}...$, a simple random sample of n first-stage units is selected. From each of the selected first-stage units, a simple random sample of m second-stage units is selected while from each of the selected second-stage units, a simple random sample of p third-stage units is drawn. Let $Xijk$ be the value of the auxiliary variable corresponding to $Yijk$, $Vijk = \dfrac{Yijk}{Xijk}$ and the quantities $\bar{X}ij.$, $\bar{X}i..$, $\bar{X}...$, $\bar{V}ij.$, $\bar{V}i..$ and $\bar{V}...$ be defined similarly.

Further, let

$$Sijrx = \frac{1}{p-1}\sum_{1}^{p}(Vijk - \bar{V}ij(p))\,(Xijk - \bar{X}ij(p))$$

$$Sirx = \frac{1}{m-1}\sum_{1}^{m}(\bar{V}ij(p) - \bar{V}i(mp)\,(\bar{X}ij(p) - \bar{X}i(mp))$$

$$Sbrx = \frac{1}{n-1}\sum_{1}^{n}(\bar{V}i(mp) - \bar{V}nmp)\,(\bar{X}i(mp) - \bar{X}nmp)$$

$$\bar{S}wrx = \frac{1}{n}\sum_{1}^{n} Sirx$$

$$\bar{\bar{S}}wrx = \frac{1}{nm}\sum_{1}^{n}\sum_{1}^{m} Sijrx$$

where $\bar{V}ij(p)$, $\bar{V}i(mp)$, $\bar{V}nmp$, $\bar{X}ij(p)$, $\bar{X}i(mp)$ and $\bar{X}nmp$ are the corresponding sample means.

Then an unbiased ratio-type estimate of $\bar{Y}...$ is given by

$$\text{Est } \bar{Y}... = \bar{V}nmp\,\bar{X}... + \frac{N-1}{N}Sbrx + \left[\frac{m-1}{m} + \frac{M-m}{Mm}\cdot\frac{1}{N}\right]\bar{S}wrx + \left[\frac{p-1}{p} + \frac{P-p}{Pp}\cdot\frac{1}{NM}\right]\bar{\bar{S}}wrx$$

If all the third-stage units in a second-stage unit have the same value, that is, $Yijn = \bar{Y}ij.$, we obtain the unbiased ratio-type estimate for two-stage designs, namely:

$$\text{Est } \bar{Y}.. = \bar{V}nm\,\bar{X}.. + \frac{N-1}{N}Sbrx + \left[\frac{m-1}{m} + \frac{M-m}{Mm}\cdot\frac{1}{N}\right]\bar{S}wrx$$

If, in addition, all the second-stage units in a first-stage unit have the same value, say, $\bar{Y}ij. = \bar{Y}i..$, we obtain the unbiased ratio-type estimate given by Hartley and Ross[1], namely,

$$\text{Est } \bar{Y}. = \bar{V}n\,\bar{X}. + \frac{N-1}{N}Sbrx$$

The general form of an unbiased ratio-type estimator for a t-stage design is now obvious and can be written down easily by induction.

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[*Note*.—It is understood that results similar to those recorded above have already been obtained by Dr. Alan Ross, and are to be published in a more comprehensive paper in due course.—EDITOR.]

[1] Hartley, M. U., and Ross, A., *Nature*, **174**, 270 (1954).

MISCELLANEOUS

'Absolute' Age: a Meaningless Term

I WISH to appeal to my fellow geologists and workers in the rapidly growing subject of geochronology to discontinue a habit or fashion which is both unnecessary and misleading. I refer to the habit of calling radiometric ages 'absolute' ages. An age does not become 'absolute' by virtue of being expressed in units of time such as a year. If 'absolute' means anything at all, it implies complete independence of all events and relationships. But a year is a relationship and, moreover, our conviction that one year equals another is merely a convenient pragmatic hypothesis. As St. Augustine recognized long ago, there can be no real standard of time as there is of length or space, since the 'time' taken by an event passes away and cannot be brought back to be measured, as a metre rod can be measured by placing it alongside a standard metre rod. Yet we do not go out of our way to say that the 'absolute' length of the rod is one metre. Nor if you ask a man of forty-five how old he is, does he reply "My absolute age is forty-five years".

The term is not only redundant and both philosophically and scientifically without meaning: it is also misleading in its psychological suggestion of a higher degree of accuracy than can be justified. A good example has recently appeared in *Nature*[1], where the title of a particularly welcome contribution begins "Confirmation of the Absolute Age . . .". Now how, if an age is 'absolute', can it require confirmation?

If any of the culprits claim that they are in good company with Newton and Kelvin, who both wrote of 'absolute time', they have only to remember Einstein and the coming of relativity. When it is desirable, as it sometimes is, to distinguish between geological age and the age expressed in years, the latter (except when measured by counting varves) can appropriately be called the 'radiometric age', a term that has already appeared occasionally in the literature of geochronology.

ARTHUR HOLMES

6 Albany, St. John's Avenue,
Putney, London, S.W.15.

[1] *Nature*, **196**, 665 (1962).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

CHEMIST, Principal Scientific Officer or Senior Scientific Officer grade (with a first- or second-class honours degree, or equivalent, in chemistry and several years postgraduate experience in an appropriate field) at Headquarters, London, to initiate and progress research programmes to provide aircraft lubricants, fuels and hydraulic fluids of improved performance; to undertake liaison work in the related research and development fields (including test methods) with the aeronautical and oil industries; and to prepare and maintain specifications for the materials concerned—Ministry of Aviation, Est. 2/3.A.3, Room 516, Adelphi, John Adam Street, London, W.C.2, quoting Ref. HC/2D/852 (December 31).

CYTOLOGIST (with a good honours degree in science or medicine, and preferably experience in tissue culture and modern mammalian chromosome techniques)—The Director, Population Genetics Research Unit, Medical Research Council, Old Road, Headington, Oxford (December 31).

SPECIALIST MICROSCOPIST (with a first- or second-class honours degree in botany or an equivalent research qualification, a sound training in general chemistry and experienced in the application of modern microscopic techniques), to supervise a small team engaged in routine study of botanical and biological materials, and to take up the study of the microscopy of man-made and other fibres, etc.—The Government Chemist, 13 Clement's Inn Passage, London, W.C.2, quoting E/AR/010 (December 31).

RESEARCH FELLOW (with an honours degree in botany or agriculture majoring in pasture ecology, crop husbandry or agricultural botany) IN PASTURE ECOLOGY (Agrostology) at the University College of Rhodesia and Nyasaland, to initiate and carry out studies on grasses used in sown pastures in Rhodesia, as a member of a pasture ecology research team—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 10).

ASSISTANT LECTURER and a LECTURER IN APPLIED MATHEMATICS—The Registrar, The University, Nottingham (January 12).

CHAIR OF CLINICAL CHEMISTRY—The Secretary, The University, Edinburgh (January 14).

LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS, NUMERICAL ANALYSIS AND COMPUTING, OR STATISTICS—The Registrar, The University, Nottingham (January 14).

PROFESSOR AND HEAD OF THE ELECTRICAL ENGINEERING DEPARTMENT—The Assistant Registrar, Kwame Nkrumah University of Science and Technology, 15 Gordon Square, London, W.C.1; or The Registrar, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (January 15).

PROFESSOR AND HEAD OF THE MECHANICAL ENGINEERING DEPARTMENT—The Assistant Registrar, Kwame Nkrumah University of Science and Technology, 15 Gordon Square, London, W.C.1; or The Registrar, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (January 15).

SCIENTIFIC INFORMATION OFFICER (with a botanical or agricultural degree, the ability to write correct English and a knowledge of German or other foreign language), for work which includes the abstracting and reviewing of biological literature—The Director, Commonwealth Bureau of Plant Breeding and Genetics, School of Agriculture, Cambridge (January 15).

ASSISTANT IN CHEMISTRY, with duties primarily in organic chemistry, in St. Salvator's College—The Secretary, The University, College Gate, St. Andrews (January 19).

ASSOCIATE PROFESSOR IN EXPERIMENTAL PSYCHOLOGY; and a LECTURER IN PSYCHOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 25).

DEPUTY LIBRARIAN (with a good honours degree and experience of work in a university library, and preferably professional qualifications) OF THE UNIVERSITY LIBRARY, Newcastle upon Tyne—The Secretary, King's College (University of Durham), Newcastle upon Tyne (January 31).

LECTURER or SENIOR LECTURER (preferably with experience in the physico-chemical study of macromolecules) IN PHYSICAL CHEMISTRY at the University of Melbourne—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

LECTURER (qualified in mathematical statistics) IN STATISTICS—The Secretary of University Court, The University, Glasgow (January 31)

RESEARCH FELLOW (with a degree in science, medicine or veterinary science, postgraduate experience in research, and a working knowledge of the parasitic arthropods) IN PARASITOLOGY—The Registrar, University of Queensland, Brisbane, Queensland, Australia (January 31).

SENIOR LECTURER; and LECTURERS (2) IN THE SCHOOL OF PHYSICS, University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

SENIOR LECTURER IN GEOLOGY (Petroleum Geologist) (January 31); a LECTURER IN CHEMISTRY (January 11); a LECTURER IN PHYSICS (January 4); and a LECTURER IN ZOOLOGY (January 4) at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (applications close on the dates shown in parentheses).

SENIOR LECTURER or LECTURER (with an honours, or higher degree, or other approved higher qualifications, and preferably some teaching experience) IN MATHEMATICS at the South Australian Institute of Technology, Adelaide—The Agent-General for South Australia, Strand, London, W.C.2 (January 31).

SENIOR TUTOR/DEMONSTRATOR (with a degree in science or engineering and some teaching and/or computer experience) IN THE BASSER COMPUTING DEPARTMENT of the School of Physics, University of Sydney—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 31).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, BIOLOGICAL CHEMISTRY, ENGINEERING, PHARMACOLOGY, PHYSICS or in a related subject—The Registrar, The University, Bristol 8 (February 1).

PROFESSOR AND HEAD OF THE DEPARTMENT OF PHYSICS—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (February 8).

PROFESSOR OF PSYCHOLOGY—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (February 8).

ASSISTANT (preferably with an interest in the biochemistry of micro-organisms) IN BIOCHEMISTRY—The Secretary of University Court, The University, Glasgow (February 15).

LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Liverpool 3, quoting Ref. CV/211/N (March 1).

ASSISTANT PROFESSORS; ASSOCIATE PROFESSORS and PROFESSORS IN THE DEPARTMENT OF MATHEMATICS—Prof. M. Wyman, Mathematics Department, University of Alberta, Edmonton, Alberta, Canada.

ASSISTANT SENIOR CHEMIST (with a degree in chemistry or equivalent qualification, experience in modern analytical techniques and preferably experience in the treatment and disposal of effluents) in the Central Laboratories of the West Midlands Gas Board, Nechells, Birmingham—The Personnel Manager, West Midlands Gas Board, 5 Wharf Lane, Solihull, Warwickshire.

ASSOCIATE PROFESSOR IN MATHEMATICS; an ASSISTANT PROFESSOR IN PHYSICS; and an ASSISTANT PROFESSOR IN BIOCHEMISTRY—The Chairman, Division of Natural Sciences, University of Saskatchewan, Regina Campus, Regina, Saskatchewan, Canada.

LECTURER (with an interest in physical metallurgy) IN CHEMICAL ENGINEERING at the University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

MATHEMATICS MASTER (with a first-class degree)—The Head Master, Westminster School, 17 Dean's Yard, Westminster, London, S.W.1.

READER (with substantial research experience) IN CONTROL SYSTEMS ENGINEERING IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—Dr. J. H. Calderwood, Royal College of Advanced Technology, Salford 5, Lancs.

RESEARCH ASSISTANT (with a medical or science degree) IN THE SURGICAL UNIT, to work on human tumours in tissue-culture and the effects on them of chemotherapeutic agents—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2.

RESEARCH ASSISTANTS and GRADUATE TEACHING ASSISTANTS (students studying for degrees of Ph.D. and M.Sc. in chemistry)—Dr. H. E. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

RESEARCH ASSISTANT (B.Sc., preferably with experience of radioactive isotope tracer work) IN THE MEDICAL UNIT, to engage in studies of calcium metabolism—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

REPORTS and other PUBLICATIONS

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APARTHEID IN SOUTH AFRICAN LEARNED SOCIETIES

MINERVA *, a review of science learning and policy, which replaces *Science* and *Freedom*, aims at improving our understanding of the effect of contemporary developments in every sphere of life on the pursuit of truth through science and scholarship, and of what science and scholarship are doing to themselves and to the rest of their society and the world beyond it. By thus improving understanding it hopes to make scientific and academic policy more reasonable and more realistic. The comparison and criticism of experience should promote more daring and judicious policy, stimulate the imagination and discourage efforts doomed to failure. The protection of the creative powers of science and scholarship generally is assisted when the conditions of their vitality are analysed and defined.

These aims are set forth and discussed at some length by the editor in an opening article which displays the way in which Government and science and scholarship interact in the world to-day and how their very growth is largely responsible for the pressures which arise and which, on the side of the university or the research institute, for example, may endanger the very conditions which have made scientific advance so rapid and fertile. Even the brief list of the problems arising in the relations between Government and science given in this opening article indicates how wide is the field with which it is proposed to deal in the new quarterly. The political relations of research and higher learning have undoubtedly become more sensitive in recent years, and there is ample room for such a forum as *Minerva* hopes to provide in which these problems can be analysed critically and seriously.

It is a strange coincidence that the first issue of *Minerva*, which includes the text of Sir Eric Ashby's Chancellor's Lecture to the University of the Witwatersrand, should have been published almost simultaneously with a most disturbing development of the South African Government's policy of apartheid (see *Nature*, 196, 201; 1962). It was announced on December 5 by the Minister of the Interior and of Education, Arts and Science, at a conference of librarians in Pretoria, that the South African Cabinet has decided that there is to be no mixed membership in scientific and professional organizations; where this exists separation must take place immediately. Non-European associations must be linked with national bodies by way of affiliation, naming one or two representatives to attend periodic and defined executive meetings called by the national associations of European organizations. This, the Minister naïvely suggested, would establish channels of contact for the exchange of ideas.

No other remark could better display the South African Government's indifference to the conditions on which the progress of science and learning generally depends or its steadfast repudiation of the very basis of a civilized community. To that attitude Sir Eric Ashby's lecture, for all that he carefully abstained from direct comment, gave unqualified condemnation. What he said of the university is largely relevant to the learned society and the professional association. The maintenance of standards of professional competence and learning is their responsibility; the conditions of admittance to membership are for them, and for them alone, to determine.

Some highly pertinent passages in this opening article in *Minerva* put the real issue admirably. "The claims which government makes on scientists and scholars to contribute to the general welfare and progress must be honoured, but the world of learning would serve neither society nor itself if it fails to scrutinize those claims with the same measure of critical discrimination that it applies to its own internal problems. The claims of society must always be examined with regard to their impact on the primary aims of science and scholarship. The claims of government must be examined to see whether they really are conducive to the welfare and progress of society, and scientists and scholars must not allow their judgment to be dominated by the thought of the public honour or material advantage which comes from the service of their earthly rules."

That is timely and wisely written, for this latest deviation of the South African Government strikes at the very root of the scientific society or professional association and their ability to serve the community. The idea of the Royal Society, the Royal Institute of Chemistry, the British Association, for example, divided into two or more bodies according to race, is as preposterous as it is absurd, and yet this is the natural effect of the policy of the South African Government. Well might *Minerva* add that the demands of Government are illegitimate where they would interfere with the discovery and communication of truth and would offer only the dubious benefits of uniformity of opinion. There the stand should be made and professional men and women should do what they can to remedy the situation through publicity and representation.

Minerva's unhesitating advocacy of this firm stand against the politics of national prestige and partisan advantage, the politics of demagogy and of ideology, where they infringe academic or professional auton-

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[*] *Minerva*, a Review of Science, Learning and Policy, 1, No. 1 (Autumn, 1962). Edited by Prof. Edward Shils. Published under the auspices of the Committee on Science and Freedom. Pp. 1–144. Subscription-rate: 20*s*. or 5 dollars a year. Single issues, 5*s*. (London: C.S.F. Publications, Ltd., 185 Oxford Street, 1962.) [See also p. 1269 of this issue of *Nature*.]

omy or attempt interference with intellectual work with no acceptable justification is the more impressive in that it is fully recognized that restrictions on such autonomy may sometimes reasonably arise from the necessities of financial provision and public policy. It is reasonable, for example, for a Government to wish to have the culture of the people it rules studied systematically and to be made more accessible to intelligent appreciation. Again, it is reasonable for a Government to provide for the creation of more linguists and students of foreign cultures because it foresees a need for such qualifications, or to provide for the training of more engineers or chemists for a like reason. The legitimacy of such proposals or their value to purely intellectual development is not questioned, but it is none the less desirable that the principles on which the legitimacy of Government influence depends and the way in which that influence it exercised should be clarified through the secreting of experience. It is a healthy sign when the recommendations of a Scarbrough Commission or a Hayter Committee are not accepted without question or examination by those affected.

This latest intrusion of the South African Government's ideology into the work of professional bodies could be described as the pressure group in reverse. It is as clearly illegitimate as the demand that scientists or scholars be in agreement with the policies generally of any particular Government, and, failing that, be excluded or degraded, nor does it need this admirable article in *Minerva* to show librarians and other professional men and women, whether in South Africa or elsewhere, where their duty lies. Reports from Pretoria indicate that the Library Association there has already yielded to the Government's decree and that the Association was to discuss at a general meeting resolutions for the establishment of separate associations for different racial groups. Quite apart, however, from the damage done to professional bodies themselves, such steps put further difficulties in the development of scholarship and of professional standards among the peoples of Africa and hinder the emergence of the wise leadership that may well be the strongest hope of avoiding violence.

This latest decision of the South African Government is in fact as untoward from the point of view of the communication of ideas as it is from that of professional standards and competence. It is poles asunder from the vision that has inspired, for example, the recent establishment of the Thomson Foundation. Dr. Voerward and his colleagues seem determined to leave nothing undone which could engender bitterness and inflame the racialism which to-day is such a grave and growing threat to the future of Africa. There has been indication enough of lack of both wisdom and moderation in the proceedings at the General Assembly of the United Nations calling for sanctions against South Africa to give substance to Sir Hugh Foot's stern warning of the danger of conflict in general. This decision of the South African Government could well provoke moves to terminate South Africa's membership of such bodies as the Scientific Council for Africa South of the Sahara. Certainly scientific bodies and professional organizations will need to consider very carefully the implications of all official relations with the South African Government.

Beyond that, however, professional bodies generally have both the right and the duty to protest clearly and emphatically against the infringement of professional responsibility and efficiency which is represented by the South African Government's action. That action can no longer be represented as domestic; it impairs the structure of the republic of science itself. Nor is it less the duty of the professional body to protest in that such protest or representation may be ignored by the South African Government, for that it is made at all cannot fail to encourage their colleagues in South Africa. This, however, is only a first step. With it must go the most careful thought as to what other measures are possible. Here the overriding consideration should be that nothing is done which either offers further hindrances to the ease of communication and the development of full professional responsibility and competence, or which would of itself spread further the intolerance and intransigence which characterize the South African Government's policy, and thus endanger further the advance of science and learning and the conditions in which they flourish.

THE STRATEGY OF WAR

Studies of War

Nuclear and Conventional. By Prof. P. M. S. Blackett. Pp. viii + 242. (Edinburgh and London: Oliver and Boyd, Ltd., 1962.) 21*s*.

PROF. P. M. S. BLACKETT, who was awarded the U.S. Medal for Merit for teaching the Service Chiefs numerical thinking and so helping "to avoid running the war by gusts of emotion" has, in *Studies of War*, extended the methods of operational research analysis to the post-War politico-military situation. Never were emotions, excited by nuclear fears, so gusty and never, as he shows, was numerical thinking so perverted. His "Critique of Some Contemporary Defence Thinking" (Chapter 10) is relentless in its exposure of the numerical methods and the conclusions of some of the American analysts whose influence on nuclear strategy has been profound. He exposes "The Theory of Games" as the basis of conclusions which he demonstrates as false.

He anatomizes the arguments which, posing as numerical, hardened out as the "Great Deterrent", "Massive Retaliation", "Graduated Deterrents", "Limited Nuclear War", "The Balance of Terror", etc. He shows how sums were made to add up to suit inter-Service rivalries, and how the theorists ignored the real facts of situations.

In Chapter 11 he says: "A military commander, planning a campaign or battle, attempts, as a matter of course, to envisage the situation as it must appear to his opponent. He must first find out all he can about the material facts of his opponents' military deployment, and, secondly, he has to assess the possible intentions of his opponent for its use. This has been described as guessing what goes on on the opposite side of the hill".

The lessons of Blackett's book have been amply demonstrated in the course of the Cuban crisis when the world was brought to the precipice edge of nuclear decision by the direct confrontation of the United States and the U.S.S.R. The material facts were conspicuously, indeed suspiciously, plain in Cuba—so plain to aerial cameras that Chairman Kruschev might just as well have sent President Kennedy the ground-plans. They showed the clearing of the area, the arrival of the missiles (without the war-heads), the pouring of the concrete for the launching pads, and all the preparations of a vulnerable site, ninety miles from the American coast. No one asked: "How long does concrete take to set?" Because of urgency, the President of the United States took the war-like initiative of declaring a naval blockade, to intercept the war-heads. This action assumed that the threat of attack was so imminent that it left no time to go to the Security Council nor, in the event of a Soviet veto, to the U.N. General Assembly—a matter of, at most, days since both were in session in New York. What was the real time-factor in terms of the hardening of the launching pads? Here was a case where the intentions—on both sides—were political as much as military—a consideration which Prof. Blackett emphasizes throughout the whole of his book.

Apart from his studies of military strategy, Prof. Blackett has included "Tizard and the Science of War" and studies of science and Government. His Part 2 spells out the methods and scope of operational research. As a collection of articles and lectures they have the merit of not having been edited by hindsight. He reproduces them in the order of their first publication to show how he modified and corrected his own thinking.

RITCHIE CALDER

THREE GREAT SCIENTISTS: MANSON, WILLIAM HERSCHEL AND LYELL

Patrick Manson

The Father of Tropical Medicine. By Sir Philip Manson-Bahr. (British Men of Science.) Pp. viii + 192 + 22 plates and 9 figures. 15*s*.

William Herschell

By Dr. Angus Armitage. (British Men of Science.) Pp. xii + 158 + 14 figures. 15*s*.

Charles Lyell

By Sir Edward Bailey. (British Men of Science.) Pp. x + 214 + 24 plates + 20 figures. 15*s*.
(London and New York: Thomas Nelson and Sons, Ltd., 1962.)

IDEALLY there are not two cultures but only one. With the passing of the years since the Renaissance, parts of the great corpus of human knowledge have developed more than others. The growing and expanding stem of knowledge has bifurcated before it has ramified. The problem of an attempt at re-integrating, which so occupies teachers in schools, writers and broadcasters, and is not unknown in some universities, is clearly of fundamental importance in any system worthy of the name of education. An improvement in the scope and method of teaching the history of science will do something to show the scientist the cultural and human background of his endeavours, and the student of the humanities the changes in the thought, behaviour and potentialities of human beings which have been wrought by science and its applications.

This new series of monographs of *British Men of Science* is to be welcomed. Three have appeared, and sixteen more are planned for the future: nearly a score of great names altogether, most of them have been strangely neglected in adequate books in the past. Thus, it is pleasing to think that soon we may expect a work on each of Joseph Black, William Gilbert, John Herschel, William Thomson Kelvin, among others whose lives are better known, even to the layman. The publishers are to be congratulated in having been able to secure authors so well qualified to deal with each subject. The three volumes which have now appeared augur well for the remainder of the series. The production of the books with regard to paper, printing, binding and illustrations is excellent, particularly in view of the low price. They should have a wide vogue. Among other uses, they will prove valuable in background reading for sixth-form science and arts students, elementary texts for university students reading science and its history, and excellent introductions to the history and methods of science for the intelligent adult wishing to understand his world in terms of the progress of science.

Patrick Manson has been called the 'father of tropical medicine' and his son-in-law, Sir Philip Manson-Bahr, presents the personality and work of this great man in the light of his close association with him over a period of thirteen years. A former work by the same author, in association with the late Colonel A. Alcock, on the same subject is more heavily scientific. Here the treatment is simpler and popular in the proper sense of the word. The book shows how Manson set out to investigate the many terrible, mutilating and weakening diseases of the tropics, and how he evolved a new and original conception of the nature of insect-borne diseases. This was to be one of the chief factors in the development of the understanding, treatment and prevention of such diseases and was to lead to the opening up of the tropics to European settlement and to a vast improvement in the health of the native inhabitants. The author describes conditions as they were when Manson started his work, assesses his contribution to the study of tropical medicine and to its establishment as an essential department of medical science, and details progress up to the present.

Although there is a considerable literature on William Herschel, Dr. Angus Armitage has performed a useful task in presenting the astronomer in the context of his time, his discoveries and the development of astronomy on the foundations of his work. The story of the professional German musician, turned into the self-taught astronomer, who rose from obscurity to world-fame almost literally in a night, is worth telling again. (His extraordinary achievements as a musician in England and Germany are often overlooked. Recently, Lady Susi Jeans, the widow of another distinguished astronomer and cosmologist, played a concerto for organ and string orchestra by the same Herschel.)

Herschel opened up a new era in astronomical optics: he invented an improved telescope, hundreds of which he made and sold. By means of this new telescope Herschel was able to look farther into space than anyone before him, and he thus opened up a wide field of speculation as to the construction of the heavens. The astronomical and physical phenomena, problems and procedures with which Herschel was concerned are described, his researches and discoveries are examined and his contributions to astronomy are assessed and placed in their historical context.

Sir Edward Bailey's splendid little book on Charles Lyell is very readable and is doubly welcome because historians of science have unjustifiably tended to neglect the great geologists. Among other things the book is a good and even exciting travel story. Sir Edward shows with great clarity and some delightful light touches how Lyell gained a wide and varied personal knowledge of the features of the landscape of Britain, Europe and North America, and was thus able "to originate just and comprehensive views concerning the structure of this globe". Lyell's chief work, the *Principles of Geology*, practically vanquished all those geologists who believe that changes in the Earth's surface had been brought about by tremendous catastrophes involving the wholesale destruction of the landscape.

The general editor of the series of monographs is Sir Gavin de Beer.

W. L. SUMNER

IN HONOUR OF SPINOZA

Creation, Emanation and Salvation

A Spinozistic Study. By Prof. H. F. Hallett. Pp. xi + 234. (The Hague: Martinus Nijhoff, 1962.) 25 guilders.

Spinoza, Dreihundert Jahre Ewigkeit

Spinoza-Festschrift, 1632–1932. Herausgegeben von Siegfried Hessing. 2 vermehrte auflage. Pp. xliii + 205. (Den Haag: Martinus Nijhoff, 1962.) 25 guilders.

IT might be incorrect to describe these two scholarly books as in praise of Benedictus de (or Baruch) Spinoza (1632–77), but they are assuredly in his honour. The authors accomplish their tasks as leading authorities in this field of philosophy, and that means presenting their readers with an assessment of Spinoza's genius in the light of twentieth-century criticism and historical method. To the possible query whether we have not enough commentaries already in this particular branch of learning, the writers might well reply that one can never delve too deeply into the great themes of human experience—life, morals, death—and more especially in an age wherein a materialistic outlook is widespread. Furthermore, it is worth more than mere formal recollection that Spinoza, alone perhaps with Plotinus, achieved a purity of the concept of truth unequalled in their day, and probably never exceeded in any subsequent period. Scientists reading these two volumes, or for that matter either of them, may care to be reminded that Spinoza's doctrine of truth finds due applications in parts of morphology, which is nowhere better displayed than in the writings of the late Dr. Agnes Arber. To have her observations by one's side in reading the pages of these recent publications is to add pleasure to pleasure.

Prof. H. F. Hallett's work, *Creation, Emanation and Salvation*, is, he tells us, a Spinozistic study, as indeed was his earlier *Aeternitatis*. After two sections of prolegomena come Part 1, "Nature", and Part 2, "Morality", followed by three indexes: (i) References to the works of Spinoza; (ii) Personal names; (iii) Subjects and catchwords. In view of certain contemporary and plausible attempts to limit (in some cases severely) the objectivity of empirical data, the remark that "man is not an extrinsic spectator of nature" is of supreme moment. This is indeed the human predicament, from which it follows that metaphysics has the paramount aim of making sure that error does not creep in as a result of this vital involvement. One can infer from this that our ratiocination must be clothed with humility, if only to avoid the gross mistake of imagining that research can stand, as it were, on the touchline, and watch with bland unconcern what is going on even in the very plane of discourse which the intellect is striving to comprehend. Or, in the manner of the Psalmist, there is for us no *super flumina*; it is always *de profundis*, whether we like it or not.

The present approach to the central problem is activistic, and this in its turn conditions the author's speculations, both ethical and political. It is well known that Spinoza used the geometrical method; yet it was not essentially by means of it that he produced a truth-doctrine akin to the German *Wahrheit*, or at least one that would support a degree of veracity beyond that which the natural scientist can reach in any purely experimental environment. For Spinoza demands an idea which is, in effect, self-certifying.

Prof. Hallett, in discussing morality and salvation, touches on the relationship between man and his tools, or materials; in fact, his position as a craftsman. Thus, technique is a kind of morality. Everybody who deals with substances knows that to do violence to his clay is no part of a sympathetic, let alone an accomplished, potter. Here, our author is almost in the tradition of John Ruskin: the point is made with a delicate touch which is wholly convincing. A portrait of Spinoza on scraper-board by the author provides an appropriate frontispiece.

Spinoza: Dreihundert Jahre Ewigkeit is in the nature of a dedicatory collection of essays in memory of the period 1632–1932. As one would expect, the emphasis is on the aspect of eternity. The present edition, with the help of additional distinguished authors, has emerged triumphant from the trials and frustrations of the original version of 1933. One can only marvel that from the Germany of that year anything of cultural value should have survived. In itself, it is an example of the indestructibility of truth, and how significant it is that its focal point should have been Spinoza.

The series starts with a salute by the editor; the other members cover most facets of this remarkable life. As an example, there are five scenes, depicted by Franz Droop. These are: (i) a room in Spinoza's house at Amsterdam; (ii) in a near-by street; (iii) in a tavern; (iv) in the Synagogue, on July 27, 1656, the day of his 'excommunication"; (v) in his study at The Hague, the year of his death. These are typical conversation-pieces, redolent of seventeenth-century Holland. Other essays deal with Spinoza's political theory, his biblical criticism, the basic dualism of his system, and so forth. The book ends with three short "remarks" by Einstein, Freud and Wassermann, all testifying to the qualities of mind which make Spinoza stand out as a philosopher for all time. His three-fold structure of knowledge—*intuitiva, ratio, imaginatio*—is secure as one of the treasures of the human race.

F. I. G. RAWLINS

DARWIN VINDICATED

The Structure and Distribution of Coral Reefs

By Charles Darwin. Originally published 1842. Pp. xii + 214 + 3 plates. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1962.) 1.95 dollars; 17*s*. net.

It is now more than one hundred years since Darwin published his work on the structure and distribution of coral reefs and it is good to have this classic readily available in a well-printed paper-back.

A very welcome foreword is contributed by Prof. H. W. Menard, who remarks: "It may be unprecedented for an essentially correct hypothesis to be the centre of a raging controversy for more than a hundred years". He goes on to discuss the hypothesis of the origin of atolls by subsidence in the light of more recent work. The Royal Society's attempt to test this theory by drilling on Funafuti Atoll had been inconclusive, but more recent results at Eniwetok Atoll and elsewhere by drilling, supplemented by seismic soundings, have demonstrated that subsidence has predominated for thousands of years. It has been shown, for example, from seismic studies of four atolls that each has a cap of coral several thousand feet thick, and so Darwin's hypothesis may be taken as proved.

Prof. Menard adds: "Sprinkled among the atolls of the Western Pacific are about a hundred and fifty 'guyots' or ancient islands now submerged to depths as great as a mile. They were originally identified as former islands solely because they are truncated volcanic cones and the truncation could only be ascribed to erosion at sea level. This deduction has been amply confirmed by deep-sea dredging of guyots. The tops are covered with rounded cobbles and fossils typical of very shallow water. . . . Former islands in favourable locations have been capped by coral that has grown upward as fast as they subsided, and these are atolls. Other former islands, lacking the coral, are now guyots".

With Darwin vindicated, the controversy has now shifted to the much-debated question of the cause of the subsidence.

W. J. Rees

STAINING TECHNIQUES

Staining Animal Tissues

Practical and Theoretical. By Edward Gurr, with a foreword by Sir Howard W. Florey. Pp. xii + 631 + 1 photograph. (London: Leonard Hill (Books), Ltd., 1962.) 84*s*.

In recent years a number of books devoted to collections of staining techniques has been published, so that one might question the need at the present time for yet another such volume. The author of this work, however, has stated in his preface that he has attempted to bring together a number of such techniques and staining procedures which were previously scattered throughout many books and journals, so this present book does in fact form a welcome addition to the laboratory bookshelves. It is undoubtedly a great convenience to have such compilations readily at hand.

At the same time, Mr. Gurr has devoted some ninety pages to a discussion of various aspects of the mechanism and theory of staining reactions. Such observations are essential to a proper understanding of the rationale of microtechnique, but one must question the advisability of treating them in a practical text. Of necessity this section must be subordinate to the main part of the book, devoted to the actual techniques themselves, and this leads to a rather limited treatment. For myself, I would like to see such theoretical aspects dealt with at greater length in a separate publication.

The selection of techniques given is very wide (more than 300 staining procedures and 700 recipes are included) and designed to meet the needs of workers in widely separated fields. Each method is given in detail, and notes of special points are appended.

An appendix giving details of the preparation and use of various fixatives, embedding methods and mountants is a useful addition to the main part of the book. There is, one is pleased to note, a very adequate bibliography and a comprehensive author index and general subject index. The book is well produced, indeed, almost too well produced, as its price is rather high and this may well prevent its acquisition by some laboratories. It is welcomed as a valuable source of reference for the busy histologist.

S. Bradbury

SISAL HEMP

Sisal

Twenty-five Years' Sisal Research. By G. W. Lock. (Tropical Science Series.) Pp. xvii + 355 + 62 plates. (London: Longmans, Green and Co., Ltd., 1962.) 60*s*.

Sisal, like so many tropical crops, has reached its greatest stature far from home. This hard fibre, which owes its name to the Mexican port of Sisal whence came the early shipments, is now a major product first of East Africa, with Tanganyika by far the greatest producer, and secondly, of Brazil.

East Africa's lead, not only in bulk of shipments, but also in scientific production, owes much to the far-sighted establishment in 1934 of a research station for sisal in Tanganyika. G. W. Lock was placed in charge, and his direction of the work over a period of twenty-five years has enabled him to write with exceptional experience of the crop.

An introductory chapter outlines the history of sisal in East Africa, going back to seventy years ago, when an article in the *Kew Bulletin* inspired the German agronomist Hindorf to give the plant a trial in Tanganyika where he was employed. The second chapter contains a useful account of the botany and life of the plant. From then on, the author is concerned with the practical handling of the crop, its propagation and raising in the nursery, its establishment in the field, its maintenance, nutrition, and pest-control, its cropping and processing to make fibre. Throughout, the author's recommendations, whether as to choice of planting material or to spacing in the field, are backed by the results of field experiments covering most aspects of cultivation. While forming, therefore, a very adequate manual of the growing and preparation of sisal, the work shows in interesting manner how agricultural research can serve and can shape efficient production.

The author writes clearly and competently, providing a good index and ample references to relevant literature. The book is well produced, with admirable illustrations, and is a welcome addition to the series of works on tropical crop-plants.

T. A. Russell

The Fishes

By Dr. Url Lanham. Pp. 116+24 plates (New York and London: Columbia University Press, 1962.) 5 dollars; 40*s.*

At a lower price this book could fill an empty niche by providing an introduction to fishes, written in the broadest detail, specifically for those without any biological training and not wanting to dig deeply into the subject. The style is simple and lucid, but one cavils at such unnecessary 'simplifications' as "Jaws were invented by one or more stocks of the jawless fish . . .", and "Fishes that evolved the first crude airfloats were, . . . , able to redesign the fins, . . ."

Dr. Lanham touches on most aspects of ichthyology. There is a chapter each on the origin of fishes, on the evolution of the Agnatha, sharks, Dipnoi and bony-fishes, while many facets of anatomy, physiology and ecology are dealt with in five chapters. The two chapters on marine and freshwater fishes are, in effect, summaries of zoogeographical and distributional problems. Speciation is touched on in a chapter devoted to trout and other salmonids. Since all these subjects are covered in one hundred and nine elegantly printed pages, it will be realized that the scope of the book is limited. Nevertheless, it does admirably serve to introduce its subject and even give satisfactory answers to many of the questions asked by the general reader.

The underlying thesis of the book is an unusual one for a popular work, namely the importance of the swimbladder in determining major evolutionary steps and later anatomical and morphological trends within the vertebrates grouped as fishes. Many more advanced text-books have passed too lightly over this fundamental dichotomy in the history of the fishes. It is thus very regrettable that the section dealing with living lungfishes is so misleading, partly by the inclusion of factual errors and partly by loosely phrased statements which result in unsupportable implications.

One final complaint: there is no bibliography.

P. H. Greenwood

The Biology of Malayan Scolytidae and Platypodidae

By F. G. Browne. (Malayan Forest Records, No. 22.) Pp. xi+255+9 plates. (Kuala Lumpur: Government Press, 1961.) 15 Malayan dollars.

This valuable contribution to tropical entomology is the work of a forest officer who spent some thirty years in Malaya and Sarawak. When Mr. Browne began his observations on the bark and ambrosia beetles, less than 10 per cent of the 435 species mentioned in this book were known to occur in Malaya and nearly 250 of them were completely unknown to science.

Part 1 occupies 56 pages and it includes an essential chapter of the habitat and the fauna and another on the general biology of the Scolytidae and Platypodidae. This second chapter explains, with numerous examples, the food and feeding habits, the social organization, the relationship with hosts, the enemies and the economic importance of these bark and ambrosia beetles, as well as the evolution of their general habits. It is very well written and will appeal to any student or worker in forest entomology.

Parts 2 and 3 are devoted to the bionomics of the Malayan species of the Scolytidae and Platypodidae respectively. The descriptions are detailed, well set out and supported by references. There are, of course, some gaps in the information, but with this book now available they should be easier to fill.

The appendixes contain not only a bibliography and an index to the beetles but also a summary of the species of actual or potential importance and an index to the host plants. The plates and line drawings are very good and add appreciably to the usefulness of this *Malayan Forest Record.*

Those who have been aware for many years of Mr. Browne's work in Malaya and Sarawak are very glad that he has recorded in book form his observations and the help he has received from other entomologists on these two families of beetles which are so important to the forester in the tropics. Mr. Browne has now turned his attention to West Africa, where he is in charge of the West African Timber Borer Research Unit.

C. J. Taylor

A Conspectus of Aphids as Vectors of Plant Viruses

By J. S. Kennedy, M. F. Day and V. F. Eastop. Pp. iii+114. (London: Commonwealth Institute of Entomology, 1962.) 25*s.*

Workers in the field of plant virus transmission will welcome the publication of an index of aphid-transmitted viruses and their vectors. This work is a compilation from the literature up to the end of 1960 and consists mainly of two alphabetical indexes. The first lists under each host plant all the recognized viruses that have been tested for transmissibility by aphids. The second is a reciprocal index of all the aphids which have been tested for virus transmission and includes not only the records of successful transmission but also the failures to secure transmission: these latter include many previously unpublished records. There is a bibliography of some 450 titles.

While recognizing that there are obvious gaps in our knowledge and possibly inherent biases in the kinds of information available, the authors have analysed the material they have collected in an attempt to test various hypotheses concerning virus–vector relationships. This work undoubtedly points the way for future investigations and in systematizing this large body of information the authors have produced a work which will be an invaluable reference.

Bruce Johnson

The Classical Theory of Fields

By L. D. Landau and E. M. Lifshitz. Translated from the Russian by Morton Hamermesh. (Course of Theoretical Physics. Vol. 2). Pp. ix+404. (London, Oxford, Paris and Frankfurt: Pergamon Press, 1962 revised second edition.) 80*s.*

This is the second English edition of the book originally published in 1948 in the U.S.S.R. The present edition is substantially the same as that of the earlier English edition published in 1951, although there has been some re-arrangement of the subject-matter. The principal change is the addition of a chapter on cosmological problems which is somewhat uncritical and unselective in its approach. Quite properly there is a reference to the solutions of the gravitational equations at first found by Friedmann in 1922, but the names of de Sitter and Lemaitre do not appear.

A number of additional exercises has also been added in the present volume, which gives a very readable introduction to the special and general theories of relativity and to electromagnetic theory and the theory of light.

G. Temple

EXPERIMENTS ON THE PROBLEM OF CONTROLLED THERMONUCLEAR REACTIONS*

By R. S. PEASE

U.K. Atomic Energy Authority, Culham Laboratory, Abingdon, Berks.

THE object of our research is to devise a practical method of releasing the nuclear energy of light elements in a controlled fashion for generation of power. The only method at present under extensive investigation aims at releasing this energy by using thermonuclear reactions in a deuterium and tritium gas at temperatures in the range 5×10^7 °K – 5×10^8 °K. To achieve a net output of energy, the gas must be confined adiabatically for times exceeding τ, where τ is given by the Lawson criterion[1]:

$$n\tau \geqslant 10^{14} \text{ cm}^{-3} \text{ sec.}$$

Here n is the number of nuclei per c.c. in the gas. The rate of energy release, which is proportional to n^2, has to be in a practicable range; and so n is envisaged as being in the range 10^{13}–10^{17} cm^{-3}, with corresponding minimum confinement times of between 10 and 10^{-3} sec. At these densities and temperature, the gas is fully ionized, the laws of motion of the ions and electrons are predominantly classical, and the forces on the particles are predominantly collective. Such a gas is often called 'plasma', following Langmuir's terminology.

The plasma is to be confined with magnetic fields, so that all envisaged thermonuclear reactors consist essentially of a volume of plasma isolated from the surrounding walls of a containing vessel by a region containing only magnetic fields. The confining forces arise from electric currents j flowing in the plasma which interact with the magnetic field B, so that in equilibrium the familiar Ampere law of force becomes: as

$$\boldsymbol{j} \wedge \boldsymbol{B} + \textbf{grad}\, P = 0$$

where P is the plasma pressure. On integration, this equation shows that, in simple geometrical situations, the magnetic pressure $B^2/8\pi$ outside the plasma must balance the plasma pressure P plus the pressure of any magnetic field inside the plasma. The current density j (and the corresponding pressure gradient) decays with time due to electron–ion collisions, unless it is continually regenerated from an external source; but the collision-rates (and the corresponding plasma resistivity) decrease as the temperature increases; and even without an active maintenance of the current, the characteristic decay time due to binary collisions can easily be made long enough to satisfy the Lawson criterion.

However, the total situation inside the containing vessel is not one of thermodynamic equilibrium, and the potential energy of the system may well be able to relax by other mechanisms, and thus destroy the confinement in too short a time. Such other mechanisms are usually referred to as instabilities, and involve the collective motion of the gas. Instability can also occur during the generation and heating of the plasma.

The nature and growth-time of instabilities and their effects on plasma confinement is the crucial point of uncertainty in all present-day envisaged thermonuclear reactors. The collective forces in a plasma depend on the velocities and positions of all the particles in the system; it is thus impossible to calculate them rigorously. Indeed, the theoreticians have to adopt drastic simplifications to make any headway at all. Consequently, we have to proceed as in other fields of research, by making approximations in the calculations and by doing experiments both to check the approximations and to suggest new ones.

This framework to our research remains as it was when last discussed at the British Association in 1958. There are now many laboratories working in this field, and some hundreds of papers are published every year. I shall limit my report mainly to work at Harwell, where our progress has been broadly indicative of the advance of the subject as a whole. (This work has now been transferred to the Culham Laboratory of the U.K. Atomic Energy Authority.)

Experiments on *Zeta*

Experiments on *Zeta* have been carried out to obtain a picture of the main phenomena occurring in a magnetic confinement system[2], and particularly over the past two or three years to get quantitative measurements of the confinement properties[3].

It will be recalled that the plasma in *Zeta* is confined by the self-magnetic field B_θ of a pulsed high-current toroidal discharge. The currents also generate and heat the plasma. There is a d.-c. longitudinal magnetic field B_z generated by solenoidal windings on the torus, called a stabilizing field, because its purpose is to eliminate the ordinary electrodynamic instabilities of a flexible current-carrying conductor. The discharge lasts for about 3 msec, and the initial gas is usually deuterium at a pressure of between 0·1 and 10 m torr.

The discharge should behave in the following way if the plasma were a well-behaved resistive medium. Because of the well-known skin effect, the current should initially flow largely on the surface of the plasma, and then diffuse throughout the plasma during a time of about σR^2, when σ is the plasma conductivity and R the plasma radius. The same skin-effect should also trap the stabilizing B_z field within the plasma. In the initial stages of the discharge, the field configuration should thus look like Fig. 1*a*. After the time σR^2, which is estimated to be between 100 μsec and 1 msec, depending on exact conditions, the configuration should have degenerated to the configuration of Fig. 1*b* with the fields B_θ and B_z intermixed. The details of the degeneration process are complicated by the variation of plasma conductivity with temperature. The important relations are that the conductivity σ is proportional to (temperature)$^{3/2}$; and the temperature should increase as the power fed in j^2/σ and as the initial gas pressure decreases. The degeneration is closely analogous to the above-mentioned resistive decay of a confinement system; but in *Zeta* the total pinch current is sus-

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* Substance of a lecture delivered to Section A (Physics) of the British Association for the Advancement of Science September 4, 1962.

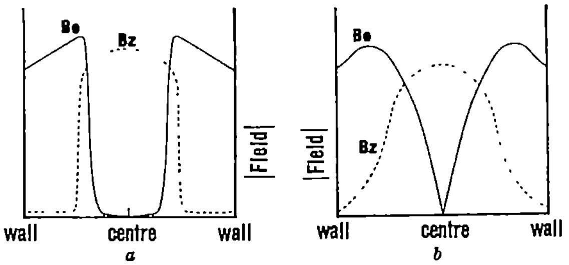

Fig. 1. *Zeta* configuration; magnetic field distributions across the tube bore
a, During initial transient phase; *b*, after diffusion of the skin current

tained by the applied electric field, and the mixed field system of Fig. 1*b* should also confine plasma. However, the sustained currents feed energy into the plasma by ohmic dissipation; and unless this energy is radiated, which should not occur in pure hydrogen plasma at the currents used, the plasma pressure will eventually exceed the confining magnetic pressure and confinement will cease. The confinement time of the mixed-field configuration is thus limited to a value which comes out to be also about σR^2.

Observations have been made on both phases of the *Zeta* discharge. Early observations on the transient configuration at the beginning of the discharge, both by inductance measurements and by search coils, suggested that at the low gas pressures then used, there is virtually no skin effect at all. This matter has been further examined in a detailed series of search coil and Langmuir probe observations by Lees and Rusbridge[4]. They have found a skin effect, and have measured the duration time. They have obtained the conductivity σ from classical binary collision theory using measured values of the electron temperature. Their results on the observed and calculated values of the 'skin-duration time' (defined in an arbitrary but explicit manner) are shown in Fig. 2. The three important results are:

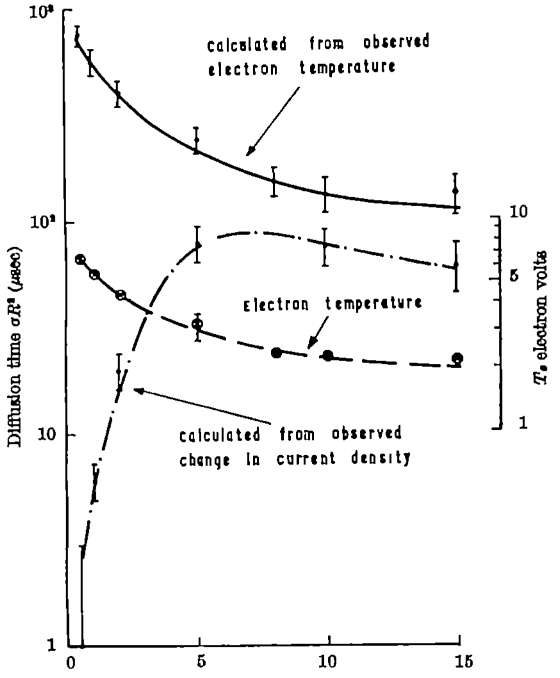

Fig. 2. Duration of the skin effect in *Zeta* from measurements of magnetic search coils compared with the theoretical time σR^2, as a function of initial gas pressure. (By permission of D. J. Lees)

(1) There is adequate agreement between experiment and theory at high initial gas pressures and low plasma temperature (about 2 eV), with a marked skin effect the presence of which is confirmed by inductance measurements.

(2) At lower initial pressures, the electron temperature is higher (as expected) and reaches values of about 10 eV, and the conductivity is calculated to improve; but the skin duration time is observed to decrease instead of increasing—a result which is in qualitative disagreement with the expectations already outlined.

(3) In the final limit of low pressure, the skin disappears about one hundred times faster than it should by ordinary resistive diffusion.

We thus have a clear example of a configuration decaying by some process other than that of binary collisions between the electrons and the ions.

Let us now turn to the steady-state phase after the skin currents have diffused. First, we must distinguish between conditions where the plasma is radiation cooled and where it is not. Observations[7] show that at high pressures or in strongly radiating gases such as argon, the discharge is largely radiation cooled, but at low pressures in hydrogen and deuterium the radiation can be neglected. In these low-pressure conditions, the plasma ought to get hot and we expect a confinement time of at least 500 μsec which increases with the electron temperature, again depending on the detailed conditions assumed.

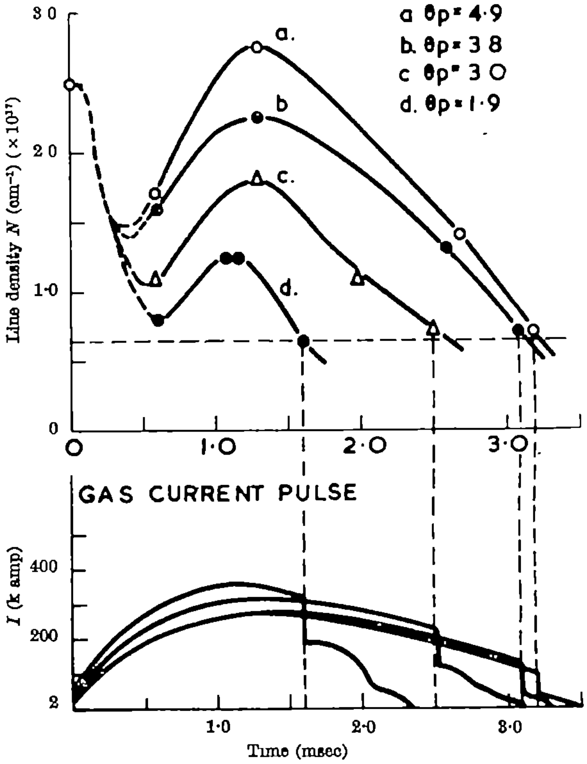

Fig. 3. Gas content (atoms per unit length) of the *Zeta* discharge, as a function of time, deduced from ultra-violet line radiation. The current wave-forms of the discharges concerned are shown in the lower part of the diagram. The parameter θ_p is the ratio of B_θ at maximum current to the initial stabilizing B_z. (From Burton, W. M., and Wilson, R., *Proceedings of the Physical Society*, 78, Pt 6, by permission of the authors and the Institute of Physics and the Physical Society)

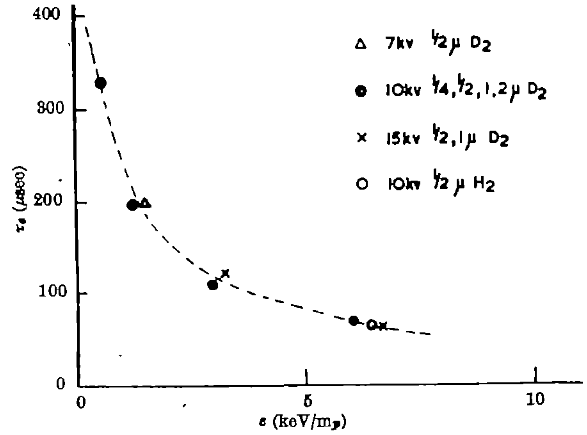

Fig. 4. Plasma confinement time τ_e in *Zeta* as a function of energy supplied per unit mass of initial gas (ϵ = keV per proton mass), for various discharge conditions (from Burton and Wilson (ref. 5))

The actual confinement time has been measured by extensive analysis of the duration of ultra-violet spectral line emission from added trace elements[5]. Emission from highly ionized states, such as argon VIII, should last essentially as long as the tracer element is present in the plasma. A short duration of such emission is in fact observed and shows that the trace element is escaping. All trace elements give the same result—a confinement time typically about 100 μsec. Light from the low states of ionization or from neutral gas should only last a very short time, because these species are ionized rapidly. In this case, however, a relatively long duration of light emission is observed which shows that fresh gas is injected during the discharge. We believe that it is dislodged from the inner surface of the metal torus.

These measurements have been made fully quantitative, and give a plot of the amount of plasma in the discharge as a function of time (Fig. 3). We see that towards the end of the pulse the net loss of plasma reaches a point at which there is a spectacular drop in the discharge current of about 100 k amp in 2–5 μsec. Plausible theory suggests that at this point the discharge begins to behave rather like an accelerator. Strong beams of electrons accelerated to about 10 keV are observed in the gas; they appear to interact violently, and they dissipate the energy of the magnetic configuration by striking the walls of the torus.

These results on the plasma loss and injection have been checked by another novel technique, the intensity of infra-red continuum radiation. For this work, very sensitive and rapid detectors have been developed at the Royal Radar Establishment, Great Malvern, together with the spectroscopes. They enable us to measure plasma density during the pulse without using added tracer elements or probes. The plasma density observations[6] fully confirm the ultra-violet analysis of loss and injection of plasma shown in Fig. 3.

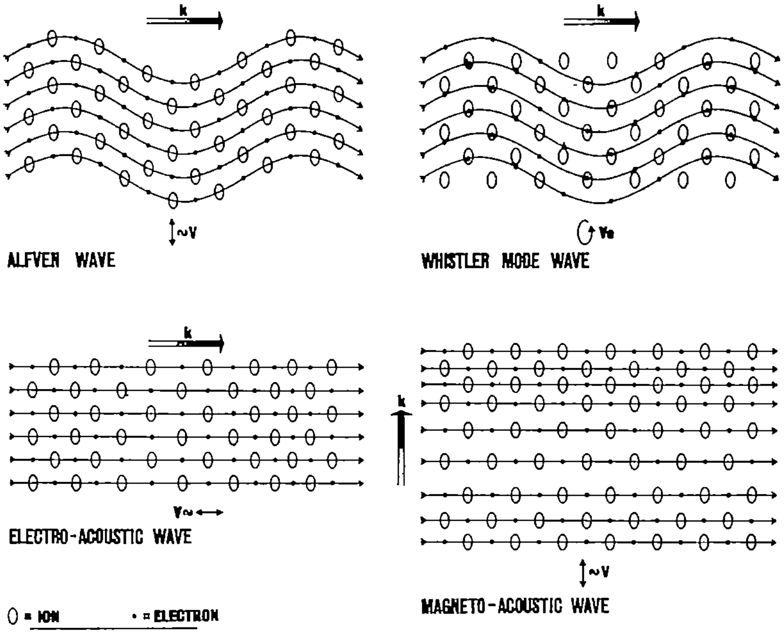

Fig. 5. Some simplified wave motions possible in a magnetized plasma
a, Alfvén waves; *b*, Whistler mode waves; *c*, electro-acoustic waves parallel to the magnetic field; *d*, magneto-acoustic waves perpendicular to the field

Measurements of fluctuating electric and magnetic fields in the plasma[7], and precise quantitative analysis of the Doppler widths of vacuum ultra-violet line spectra[8], strongly suggest that the plasma gets to the walls in a coarse random walk process, each step of which consists of the plasma moving about 10 cm at 10^6 cm/sec. It takes 10 steps to get to the walls—and hence takes about 100 μsec to lose the plasma—in rough quantitative agreement with the ultra-violet and infra-red measurements.

The loss of plasma at low initial gas pressures is thus fully documented. It is at least five times more rapid than one would expect. Moreover, the confinement time, just like the skin diffusion time, should increase with increasing energy per particle (temperature). In fact, measurements show that the opposite occurs (Fig. 4); and only by using dense plasmas that are relatively cold, can we approach confinement times that agree with the σR^2 times of ~ 500 μsec. It is possible that in radiation-cooled conditions, plasma confinement time is longer, but fluctuations of the magnetic fields are still observed and indicate instabilities.

The anomalously rapid skin diffusion and plasma loss can be interpreted as an anomalously low plasma conductivity σ, which appears as the temperature is raised. Indeed, the corresponding 'excess resistance' of the discharge has been directly observed and examined[3]. However, all these anomalous effects are observed to depend on the mass of plasma involved (in particular they increase as the plasma mass is decreased); these excess resistance effects are therefore believed to be simply a reflexion of the observed collective motions of the plasma, rather as the excess resistance of a dynamo (back E.M.F.) is a reflexion of the motion of the armature and not of the resistivity of the windings.

Thus it is clear that in *Zeta*, confinement is being destroyed by instabilities, and that so far we can only obtain high temperatures at the cost of poor

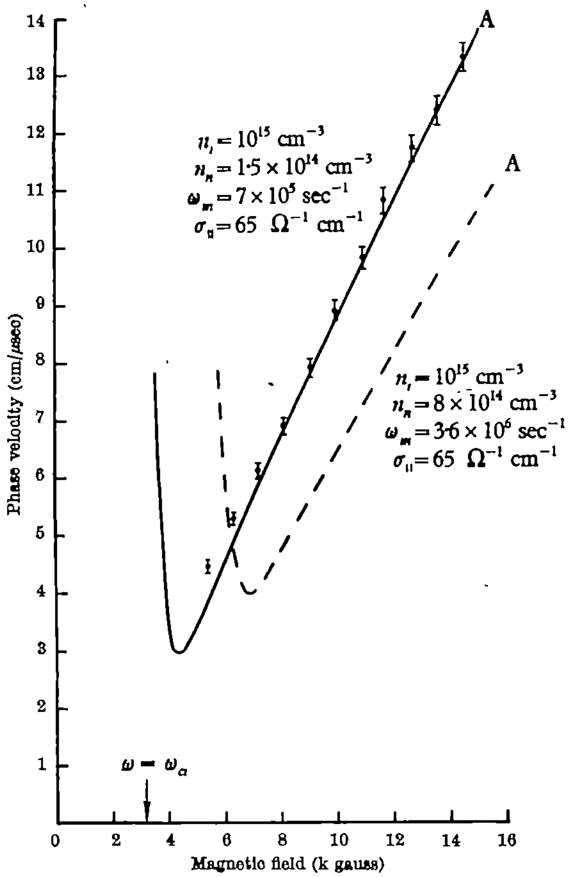

Fig. 6. Phase velocity of Alfvén waves in an argon plasma at fixed frequency as a function of magnetic field. n_i, ion density; n_n, neutral density, ω_{in}, ion-neutral collision frequency; σ = plasma conductivity; – – –, theoretical curves. (From Jephcott, D., and Stocker, P. M., *J. Fluid Mechanics*, 13, Pt. 4. by permission of the authors and the Cambridge University Press)

confinement. The origin of the instabilities is not entirely clear, and I shall describe instead parallel experiments on other apparatus.

Collective Motion of the Plasma

First, we have started controlled experiments on plasma motion for small disturbances. In this case, it is natural to use Fourier analysis, so that the motion can be described as waves. When a plasma system is in thermodynamic equilibrium, all these modes correspond to ordinary oscillatory waves which are damped. When it is not in thermodynamic equilibrium, which is the situation we are trying to achieve, some of the modes may turn out to be exponentially growing disturbances or to be growing waves, driven by the potential energy of the system: that is, the modes are unstable. The basic equations are the same whether the mode is stable or unstable. The experiments aim to show whether or not these equations are really applicable in a plasma by measuring the properties of plasma waves.

A plasma in a magnetic field can, according to theory, propagate many different varieties of waves. Four of these are illustrated in Fig. 5. The Alfvén waves, predicted theoretically in 1944, are transverse displacements of the plasma and magnetic field together, which propagate along the lines of force with a phase velocity of $B/\sqrt{4\pi\rho}$, where ρ is the plasma mass density. These waves are low-frequency waves, because when the frequency exceeds the ion cyclotron frequency the ions no longer move with the lines of force. A second wave mode shown, corresponds to the higher frequency case, when the electrons are stuck to the lines of force, but the ions are not. This is the 'whistler' mode of propagation, predicted by the Appleton–Hartree dispersion relation, and studied in connexion with whistler radio atmospherics. This is the only mode for which much observational data was available in 1958[9]. This mode propagates along the lines of force at frequencies between the electron- and ion-cyclotron frequencies. The other two modes correspond roughly to sound waves along and across the lines of force.

Fig. 6 shows the measured phase velocity of Alfvén waves in a cylindrical argon plasma, obtained by Jephcott[10], as a function of the magnetic field strength. The main predicted features of these waves—phase velocity proportional to magnetic field, and no waves at frequencies higher than the ion cyclotron frequency—are found experimentally. The observed phase velocity and also the damping length agree with precise theory to within 20–30 per cent. The main uncertainty of the experimental measurements is the amount of neutral gas in the plasma, and when this single parameter is adjusted, the observations both of phase velocity and of damping are within a few per cent of theory over a wide range of magnetic field strengths. Similar agreements have been obtained with whistler mode waves by Dellis[11], with longitudinal sound waves by Little[12] and with transverse (magnetic) waves by Niblett *et al.*[13], using 'thetatron' devices at Aldermaston.

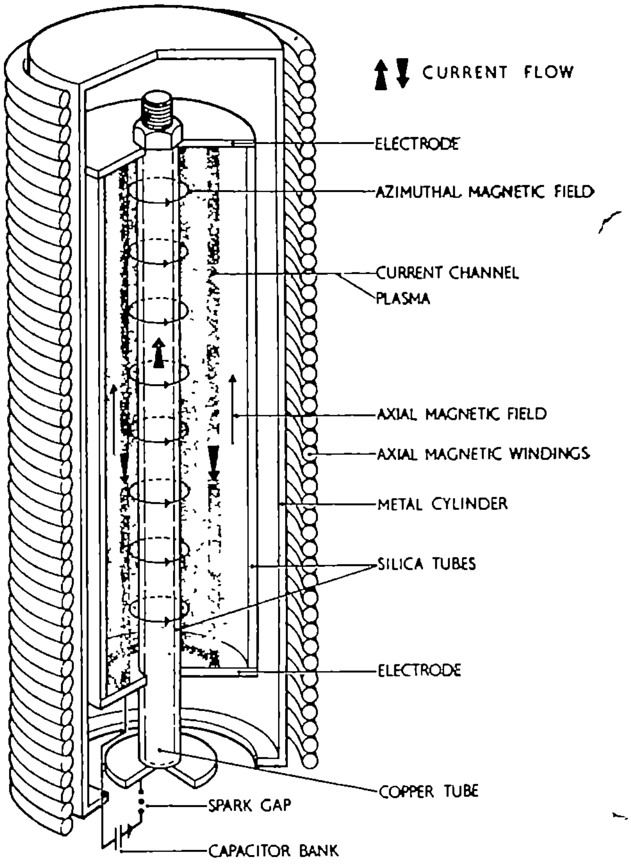

Fig. 7. The hardcore experiment

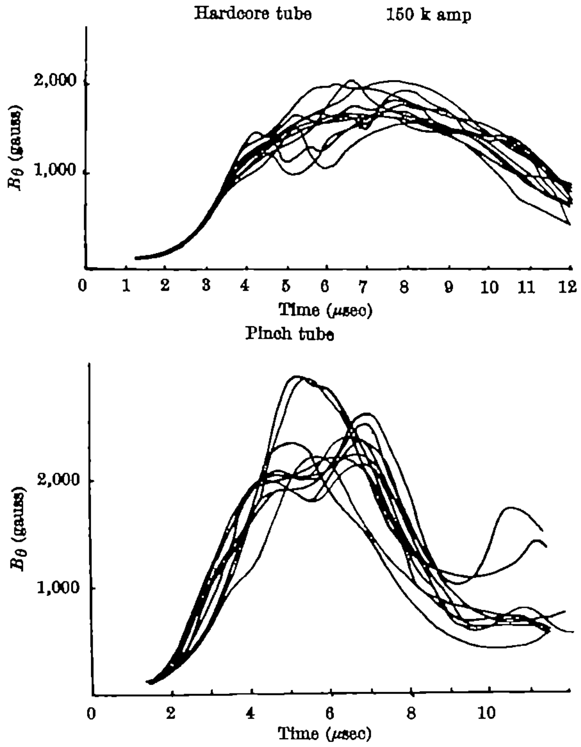

Fig. 8. Overlay of search coil measurements of magnetic field strength, in hardcore and pinch geometries. The search coil was at a radius of 8·5 cm. (By permission of K. Aitken and P. Reynolds)

As a result of these experimental studies, together with similar ones elsewhere, we can have more confidence in the basic equations used to describe the collective motion of plasma. In the case of *Zeta*, the theoretical equation for a straight cylindrical analogue predicts unstable hydromagnetic modes, corresponding roughly to a combination of magnetic sound waves and Alfvén waves, in a rather narrow band of wavelength. Sound waves could also be excited by the relative drift motion of the electrons and ions due to the currents in the plasma, provided the gas pressure is fairly low. The disturbances in *Zeta* have low frequencies, less than the ion cyclotron frequency, and this qualitative result fits the present theoretical predictions.

Stability Studies in the Hard-core Configuration

A second line of experimentation is to investigate more specialized confinement configurations which are predicted to be stable in those modes which might give trouble in the ordinary pinch. This line has been pursued at Harwell by Dr. R. J. Bickerton[14] and his colleagues using a modification of the pinch configuration called 'hard core' (sometimes called 'unpinch') geometry. As shown in Fig. 7, the plasma is confined between a B_θ field generated by a central current carrying rod (the 'hardcore') and an external B_z. It is predicted to be stable by the same theory—infinite-conductivity hydromagnetic theory—which predicts instabilities in *Zeta*.

The observations have been made on straight tubes with discharge currents of up to about 200 k amp, lasting for about 15 μsec. The same discharge tubes have also been operated in the stabilized pinch geometry so that direct comparison can be made. The principal observations have been made with magnetic search coils, which have shown in the first place that the configuration achieved is of the type expected. The important observation made at Harwell and also in the United States and France is that, in the hardcore geometry, there are fluctuations of the magnetic search-coil signals indicative of instabilities (Fig. 8). Indeed, the magnitude of the fluctuations approaches that seen in the pinch in comparable conditions. They correspond to movements of the plasma of about a centimetre or so in a tube of 15 cm radius. The frequencies of the fluctuations correspond to those of hydromagnetic waves. Discharge tubes of varying length have been used to gauge the end-effects inevitably associated with straight tubes—the effects seem to be negligible—and search coils external to the plasma are used to avoid the disturbing effect of the probe in the plasma. It thus seems that simple infinite-conductivity hydromagnetic theory is insufficient to predict stability. The gas pressures in these experiments has been kept high so that drift-excited sound waves should not occur. The penalty has been a relatively cold (5 eV), rather resistive and not fully ionized plasma.

At present it seems quite possible that the finite conductivity of the plasma produces growing Alfvén waves. Normal mode calculations on a rather simplified model using finite instead of infinite conductivity shows that such growing waves should occur in a resistive medium[15], and experiments[16] using mercury colums in place of plasma show that indeed they do. However, some of the detailed predictions of this particular model do not fit with observed properties of the fluctuations, and it seems quite possible that other proposed modes of instability resulting from finite conductivity[17] may be the predominant ones.

Confinement in the Cusp Geometry

A third line of experimentation on magnetic confinement has been carried out by Dr. T. K. Allen *et al.*, using cusp geometry (Fig. 9). We started experiments in this geometry because its stability properties are believed to be radically different from those of the pinch[18]. In cusp geometry, the magnetic field surrounding the plasma everywhere increases as one moves away from the plasma towards the walls. Thus, a plasma protuberance at the surface encounters a stronger magnetic pressure than in the unperturbed state, and this stronger pressure provides a powerful restoring force regardless of the shape or magnitude of the protuberance. This contrasts with the stabilized pinch geometry, where the field decreases outwards from the plasma, and the stability depends on

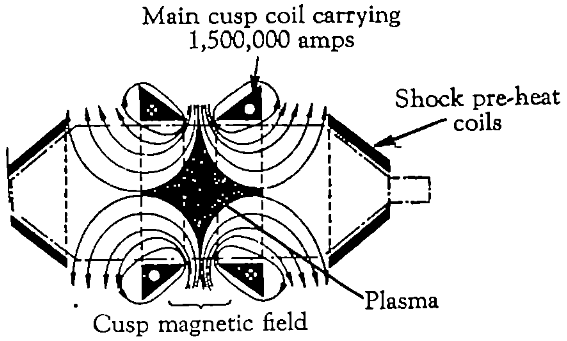

Fig. 9. Cusp geometry experiment

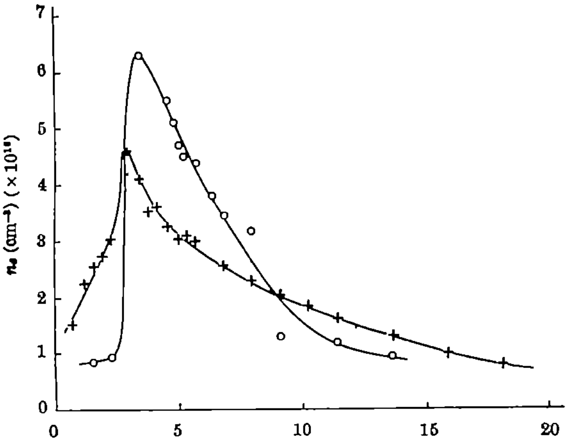

Fig. 10. Spectroscopic measurements of plasma density as a function of time in cusp geometry experiment
O, from Stark width of HeII 3203 Å; +, from intensity of Balmer recombination continuum. Initial gas was hydrogen + 2 per cent helium at 40 m torr. (By permission of McWhirter, R. W. P., to be published)

shear in the magnetic field. However, the simple cusp as used by Dr. Allen is not a complete containment system, because the lines of force are not closed within the vessel, and plasma can leak along the lines of force out along the axis of the system, and also out of the ring leak between the coils.

In these experiments[19], an initial plasma with $n \sim 10^{15}$ cm^{-3} and a temperature of 5×10^4 °K is produced by colliding shock waves. This plasma is then trapped and compressed by the cusp magnetic fields which rise to about 50,000 gauss near the coils in about 10 μsec. High-speed photographs of the plasma have shown that this system appears to be working approximately as planned, although during the compression phase instabilities sometimes appear due to the rapid inward acceleration of the plasma[20]. The final plasma obtained occupies about 100 c.c., has a particle density of about 5×10^{16} cm^{-3} and probably reaches temperatures of about 2×10^5 °K.

The density has been measured from the absolute intensity of the continuum radiation, and also from Stark widths of spectral lines (Fig. 10). After an initial increase during the compression phase the density is found to decrease with time so that most of the plasma escapes in 10–20 μsec. By using small thermocouples and piezo-electric crystal probes, the energy and the momentum of the escaping plasma has been measured in different regions near the wall (Fig. 11). These show that the plasma loss occurs along the lines of force and is limited to a narrow region at the cusp apices where the lines of force penetrate the tube walls. Both the width of the leak region (a few milimetres) and the rate of loss of plasma appear to be roughly in accord with relatively simple considerations of the flow of gas through an orifice the width of which is determined by simple collisional diffusion across the lines of force.

Because of the leaks in this cusp geometry system, the plasma confinement time (~10 μsec) is rather short, and the plasma is not very hot (⩽20 eV). So far as they go, the results suggest that the plasma confinement in this system is behaving according to plan, and does not appear to suffer from the instabilities of the type observed in the pinch system. This experiment is one of the few that gives observational colour to the hope that stable confinement can be achieved.

Discussion

These experiments represent some of the more important ones carried out at Harwell since 1958 on controlled thermonuclear reactions. They have demonstrated the importance of collective motion of the plasma in leading to the breakdown of magnetic confinement systems and provide us with some evidence on the nature of the plasma motion. For this work, novel techniques have been introduced, and we can now make quantitative measurements over a large part of the vacuum ultra-violet spectrum and of the far infra-red spectrum which will greatly help future experiments. Confinement of plasma in accordance with the binary collision processes required to be the major dissipative factor in envisaged thermonuclear reactors has been achieved only with low temperature and rather resistive plasmas, and no good evidence has been obtained either by us or by others that plasma at thermonuclear temperatures can be stably confined.

In specially designed experiments, the observations of small-scale collective motion are in good quantitative agreement with the linearized wave theory of plasmas. This gives us confidence that at least some of the drastic simplifications used in theoretical studies are meaningful. Using such simplifications, confinement systems which avoid some of the instabilities predicted for *Zeta* can be envisaged, including pinch systems with very thin current skins and specially arranged stabilizing fields. However, the results of experiments in the hardcore geometry suggest that infinite conductivity hydromagnetic theory is insufficient to predict stability. The initial experiments in cusp geometry seem to suggest that systems in which the magnetic field increases away from the plasma boundary may turn out to be more

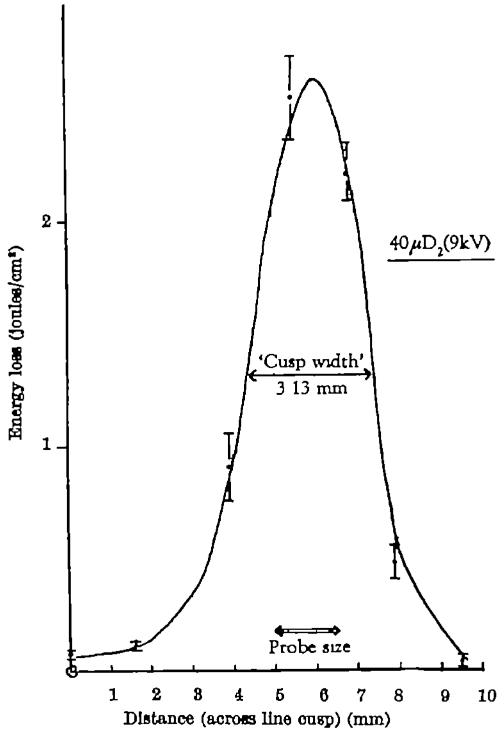

Fig. 11. Energy loss in the cusp geometry trap measured by small thermocouples placed near the ring cusp apex; mean of 10 shots. (By permission of Spalding, I. J., to be published)

promising for stability, a view which has substantial theoretical argument to support it.

It will, however, be clear that our present knowledge of the extreme complexities encountered in our work on magnetic confinement systems does not permit me to make any firm prediction about the ultimate achievement of a working controlled thermonuclear reactor based on the magnetic confinement principle.

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VOICEPRINT IDENTIFICATION

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VOICEPRINT identification is a method by which people can be identified from a spectrographic examination of their voice. Closely analogous to fingerprint identification, which uses the unique features found in people's fingerprints, voiceprint identification uses the unique features found in their utterances. Fingerprinting uses the inked impressions of the ten fingers; voiceprinting uses the spectrographic impressions of the utterances of ten words frequently used in telephone conversations. Thus, when the source material is contextual, the voiceprints for specific cue words are extracted and compared with previously recorded voiceprints of the same cue words.

Fig. 1 illustrates two types of voiceprints of the word 'you' uttered by the same male voice. The voiceprint shown on the left is a conventional bar spectrogram, showing time proceeding from left to right and frequency increasing from bottom to top. The amplitude, or loudness, is indicated by the density of

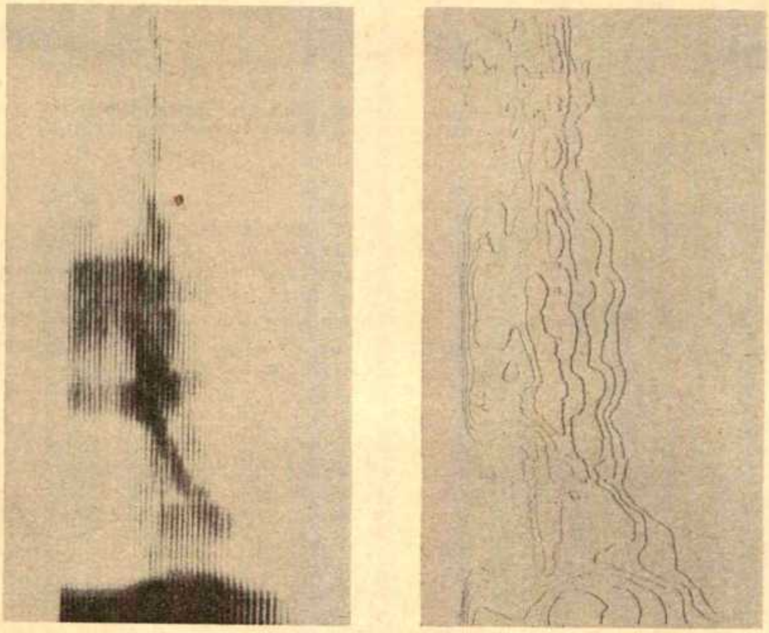

Fig. 1. Male voice uttering 'you'. Bar voiceprint on the left; contour voiceprint on the right

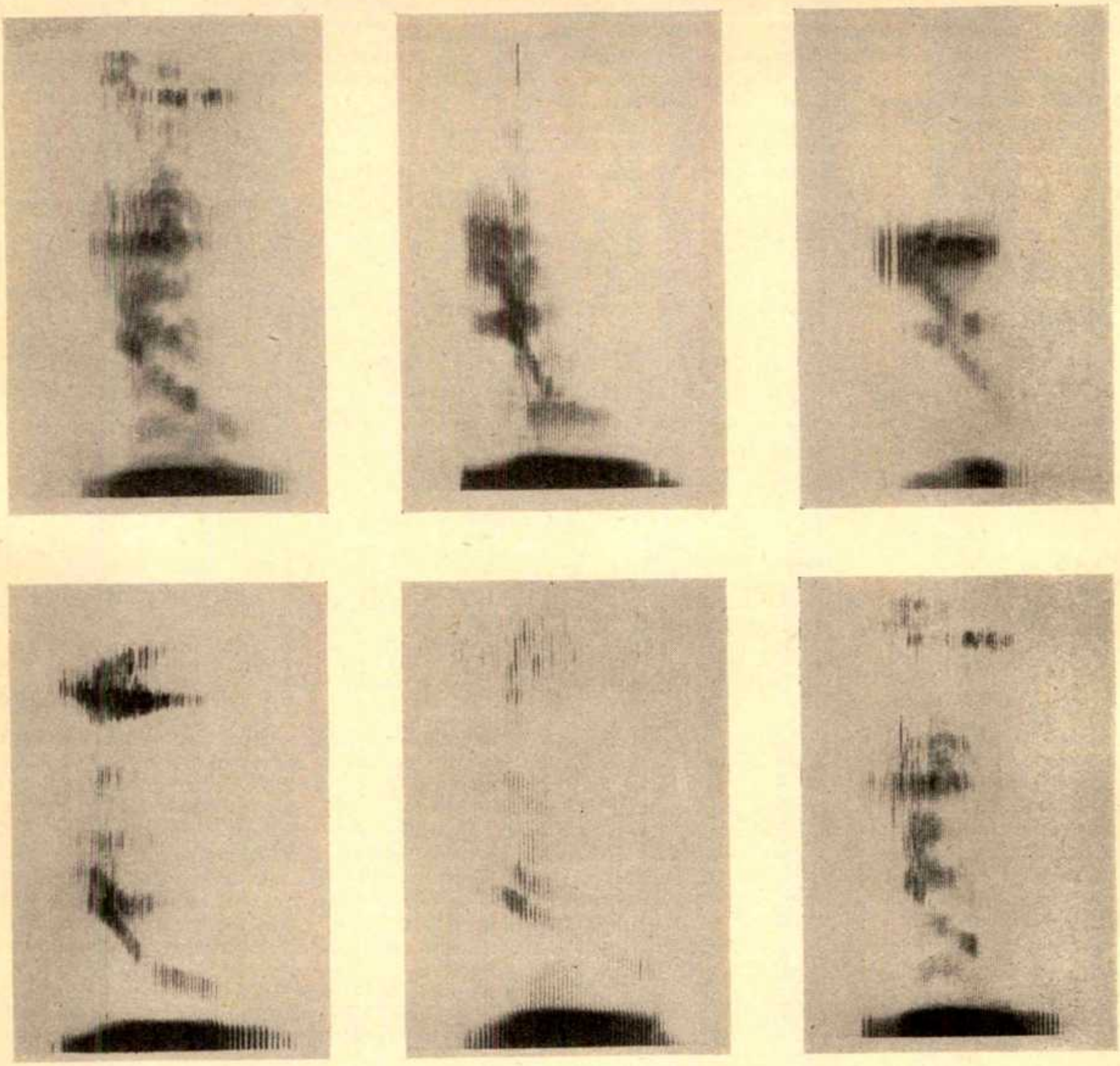

Fig. 2. Bar voiceprints of five male speakers uttering 'you'. One of the speakers uttered 'you' twice. Upper left and lower right were spoken by the same person

the marking. The range of loudness detectable this way is only a little better than 2 to 1 because of the limits imposed by the spectrogram paper characteristics. The contour spectrogram (voiceprint) shown on the right has the same time and frequency dimensions as the bar spectrogram. The amplitude, however, is shown by seven quantized or contour steps. The term 'contour' seemed applicable because the amplitude contours on this type voiceprint are like the altitude contours on a topographical map. With contour portrayals, amplitude doubles with each inward progression from one contour to the next, and we arrive at amplitude peaks as one arrives at altitude peaks on a topographical map.

Subjective experiments were conducted to learn how well subjects could match voiceprints when presented in random matrices, and secondly, how well individual unknown prints could be identified with the correct speaker in a population of previously uttered and filed prints. These results will be presented and discussed later in this article.

Fig. 2 shows a sample array of bar voiceprints of the word 'you'. There are six utterances by five male speakers. Can you detect the two bar prints which were uttered by the same persons ? Similarly, Fig. 3 shows contour voiceprints of the word 'you' uttered by the same speakers, but the order has been changed. Can you detect the two contour prints uttered by the same person ?

You probably had no difficulty in selecting the matching prints, so let us now explore the speech mechanism and see why each person's voice is unique. Fig. 4 illustrates the human vocal mechanism. The parts which principally determine voiceprint uniqueness are the vocal cavities and the articulators. The vocal cavities are resonators which, much like organ pipes, cause energy to be reinforced in specific spectrum areas, dependent on their sizes. The major cavities affecting speech are the throat, nasal and two oral cavities formed in the mouth by the setting of the tongue. The contribution of the vocal cavities to voice uniqueness lies in their size and the manner in

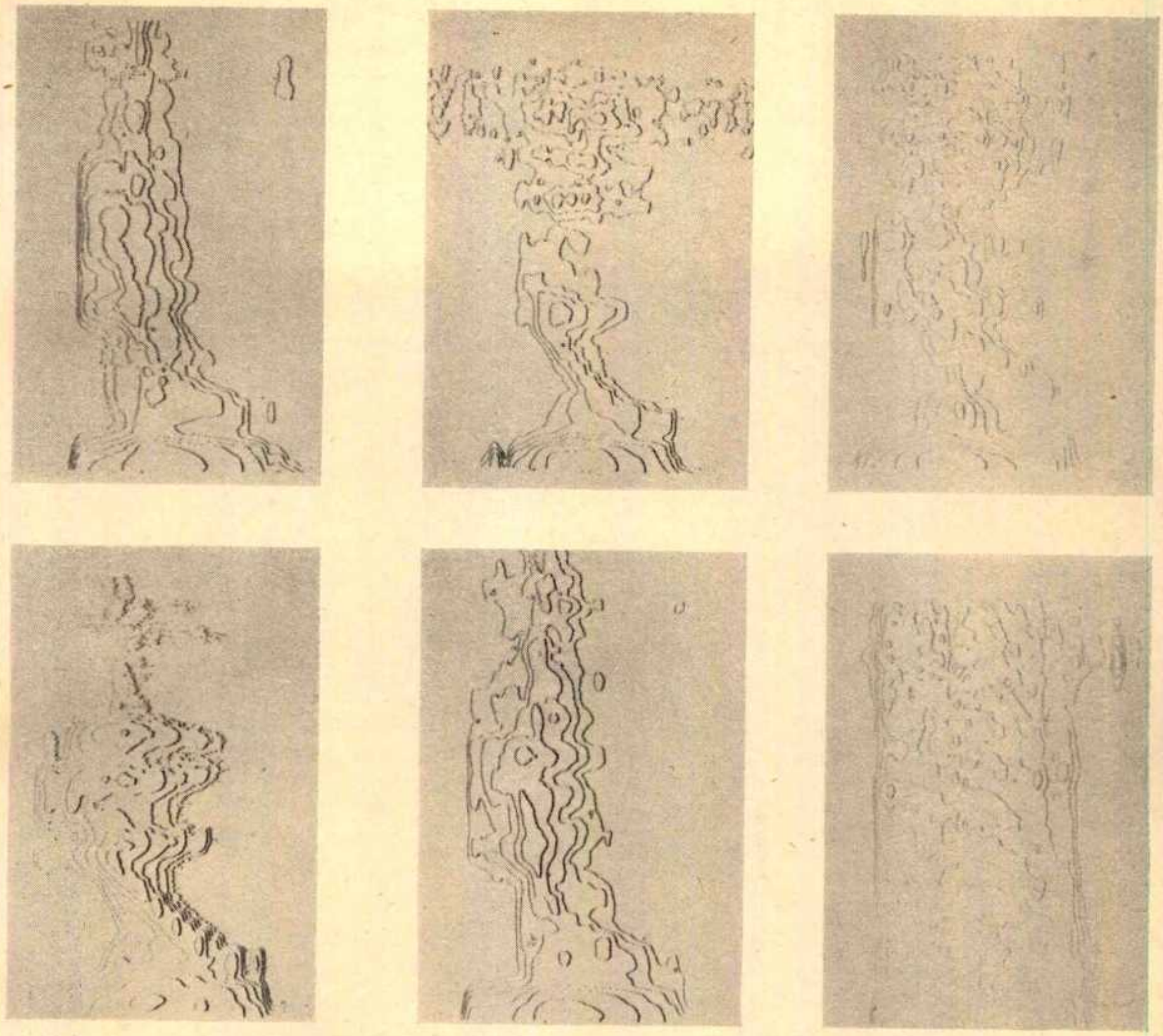

Fig. 3. Contour voiceprints of five male speakers uttering 'you'. One of the speakers uttered 'you' twice. Upper left and lower middle were spoken by the same person

which they are coupled. The likelihood of two people having all vocal cavities the same size and coupled identically seems remote. A still greater factor in determining voice uniqueness is the manner in which the articulators are manipulated during speech. The articulators include the lips, teeth, tongue, soft palate, and jaw muscles, the controlled dynamic interplay of which results in intelligible speech. We well know that intelligible speech is not spontaneously acquired by infants. It is a studied process of the imitation of those around him who are successfully communicating. The desire to communicate causes the infant to accomplish intelligible speech by successive steps of trial and error. Success requires that he learn a dynamic complex manipulation of interrelated muscles controlling the movement of several articulators. Again, the chance that two individuals would have the same dynamic use-patterns for their articulators would be remote. My claim to voice pattern uniqueness then rests on the improbability that two speakers would have vocal cavity dimensions and articulator use-patterns identical enough to confound voiceprint identification methods. Final proof of identification uniqueness depends on a sufficiently large population sample to support the present claims. Recently, when fingerprint identification experts were asked if fingerprinting afforded unique and infallible identification, they replied: "We do not surely know, because we have information for only 93 million people so far".

Let us now consider the identification successes for several subjective experiments. The subjects used in these identification experiments were female high school students 16–17 years old. They were each given about one week of training in voiceprint reading and detection of unique clues in voiceprints. In all, eight girls were used for various periods during the past year. They worked in panels of two because it was found that success scores were markedly better when two panellists were able to consult each other about their identification selections.

Data from an experiment in voiceprint matching are shown in Fig. 5. The voiceprints used here were four different discrete utterances by 5, 9 and 12 male speakers. The words used were 10 frequently used words in English conversation, namely: *the, to, and, me, on, is, you, I, it,* and *a*. The subjects were given a random matrix of voiceprints for the 5, 9 or 12

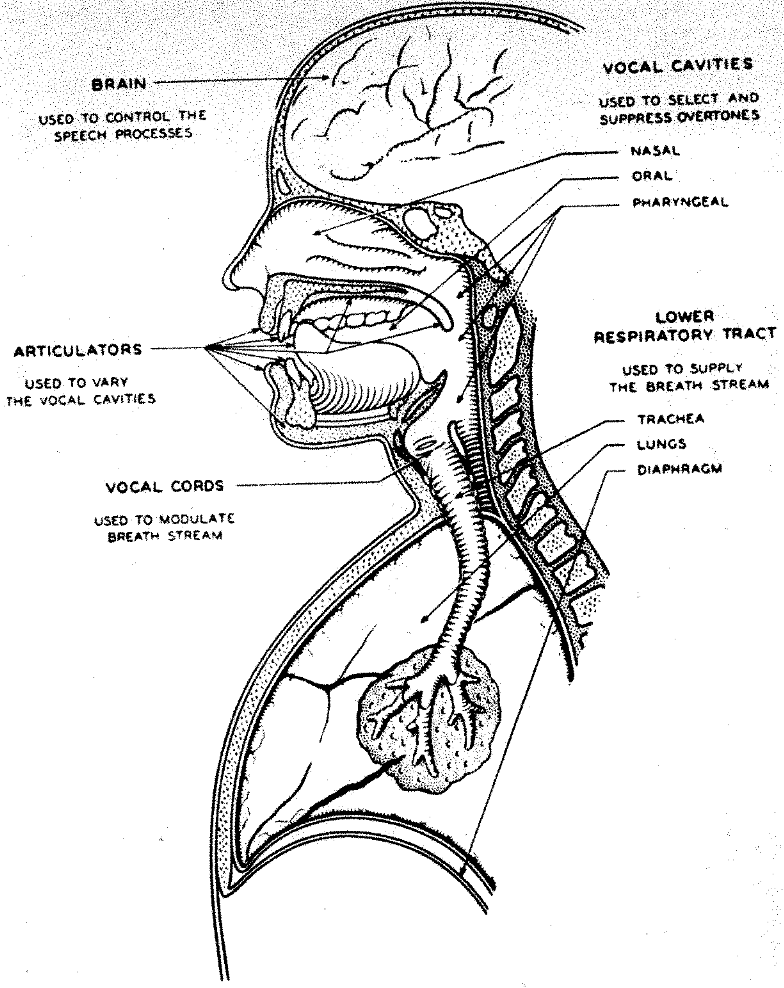
BRAIN
USED TO CONTROL THE SPEECH PROCESSES
VOCAL CAVITIES
USED TO SELECT AND SUPPRESS OVERTONES
NASAL
ORAL
PHARYNGEAL
ARTICULATORS
USED TO VARY THE VOCAL CAVITIES
LOWER RESPIRATORY TRACT
USED TO SUPPLY THE BREATH STREAM
TRACHEA
LUNGS
DIAPHRAGM
VOCAL CORDS
USED TO MODULATE BREATH STREAM

Fig. 4. Diagram of the vocal tract

'talker' groups. Each matrix included four different utterances for each word used. The resultant random matrices included 20, 36 and 48 voiceprints respectively. The experiments were conducted for both bar and contour type voiceprints. As can be seen the lumped errors for all cue words ranged from 0·35 to 1·0 per cent for bar prints, and from 0·37 to 1·5 per cent for contour prints. The number of data for each point is shown in the legend and in all cases was more than 800. Errors recorded for individual words ranged from 1·8 per cent for the word *to* to no errors for the word *a*.

Data in Fig. 6 illustrates the success of voiceprint matching when the cue words were extracted from contextual material. These results show that the lumped error for all cue words is approximately 1·0 per cent. It will be remembered that comparable data (Fig. 5) for discretely spoken words showed a lumped error of 0·8 per cent. The error for these results as shown (Fig. 6) range from 2·0 per cent for the word *is* to no errors for the word *you*.

In the following experiments an identification game was played. It was presumed that one had on file a number of voiceprints identified with a group of people. It was further presumed that there were some unidentified voiceprints for some of the same population. The object of the game was to identify the unknown prints with one of the group of file prints. The file population with which the unknown had to be identified varied between 9 and 15. The results of one such experiment are shown in Fig. 7. These data indicate that in 2,000 identification attempts, the lumped error of misidentifications when using all cue words was 1·0 per cent. I would like to make it clear that the errors shown are for identifications made with single cue words. This is analogous to attempting fingerprint identification with the print of only one finger. In practice, a voiceprint identification would generally be made using several cue words. The identification error in such a case would be the product of the errors for each word. The resultant percentage error would, of course, be very small. It will be noted that the cue words are shown on the abscissa and the error index for each word on the ordinate. The range is from 3·0 per cent for the word *is* to no errors recorded for words *I*, *on* and *it*.

As one may suspect, some voices are more difficult to identify than others. Fortunately, no voices used so far can be classed as difficult to identify. Results in Fig. 8 show the subjective errors committed in identifying a group of 11 male talkers. The identification errors range from 2·4 per cent for two talkers to no errors for three talkers. Each datum point indicated contains at least 163 identification attempts.

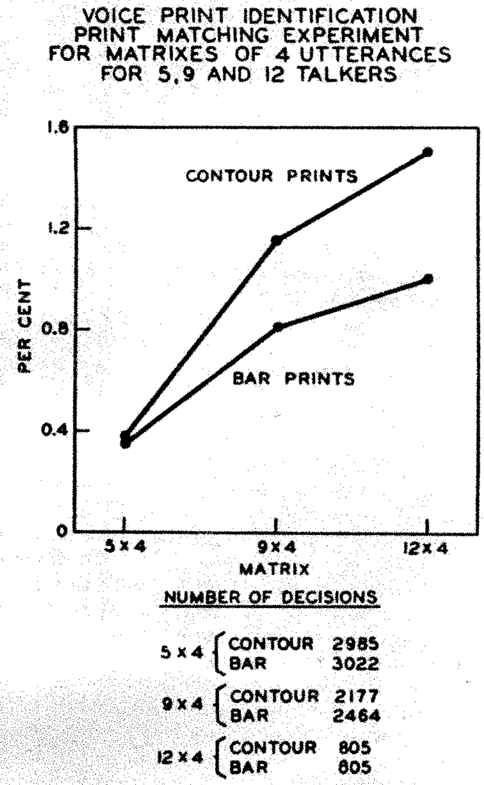

Fig. 5. Lumped data errors for voiceprint matching experiments with random matrices

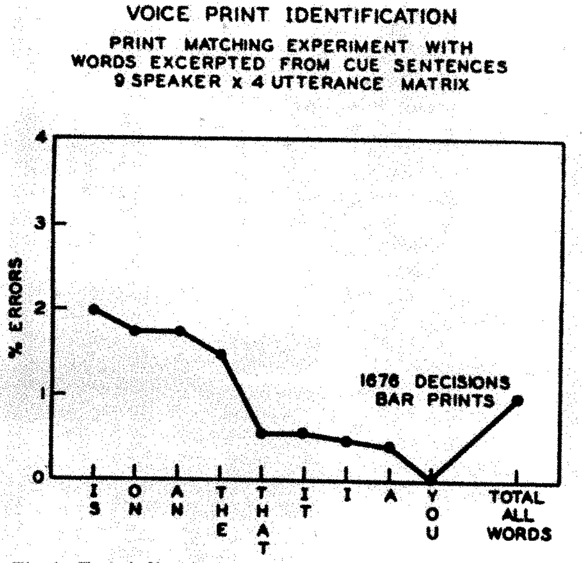

Fig. 6. Error indices for cue words in print matching experiment

The talkers, incidentally, were chosen as normal male voices with no marked dialectal influences.

Experiments similar to those described previously were also conducted for female voices. There was no evidence of complicating difficulties and the data are comparable with those shown for male voices.

A sampling of data has been presented here which was limited in scope because of space restrictions for this article. Other experimental data encourage me to believe that unique identifications from voiceprints can be made.

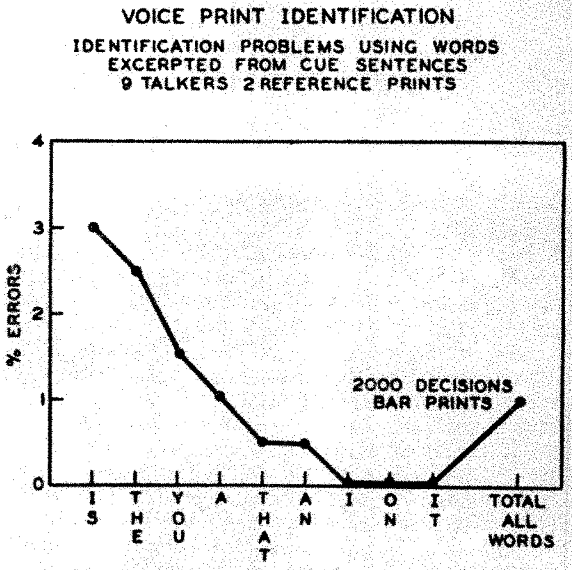

Fig. 7. Identification problem errors for each cue word

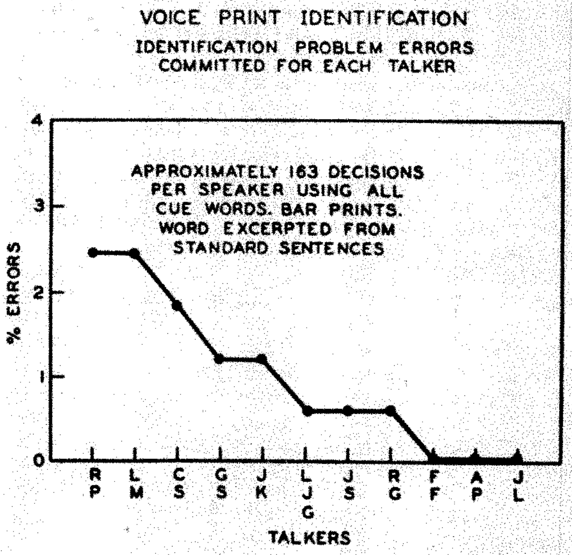

Fig. 8. Identification errors committed for each talker

Work continues, there being questions to answer and problems to solve. Among these are: (1) Is the voice unique enough to afford infallible identifications? This requires sampling the voices of a much larger section of the population. (2) Does the voice of an adult change significantly with time? If so, how? (3) How will attempts to disguise the voice succeed? (4) How successful in disguise are voice mimics? All these questions must be answered by substantiating experimental evidence. It is my opinion, however, that identifiable uniqueness does exist in each voice, and that masking, disguising, or distorting the voice will not defeat identification if the speech is intelligible.

Work is in progress to devise a classification and indexing system for filing voiceprints. The system must afford compactness, rapid access, and reduce the population of filed prints needed for subjective identification to a relatively small number. Several ideas for automation are being examined and seem to hold promise in meeting the foregoing requirements.

BEHAVIOUR OF MONKEYS TOWARDS MIRROR-IMAGES

By PROF. K. R. L. HALL

Department of Psychology, University of Bristol

MIRRORS have been used in field studies of birds to elicit various behaviour-patterns, and it has also long been known that anthropoid apes take a great interest in the perception of their mirror-images. Thus Yerkes and Yerkes[1] described how "in any captive chimpanzee, but especially in an isolated and, therefore, lonesome individual, the self-image commands attention and arouses interest which leads to varied investigative expressions. In a word, the novel object is treated as if it were a fellow being. It may be touched repeatedly with fingers, hands, feet, lips, cheeks, or poked, slapped and struck gently or vigorously with hands, feet or both. Also there may be sudden reaches behind the mirror, if such is used as source of image, in seeming endeavour to surprise the other animal and grasp it. Social interest for a time waxes, as such behaviour runs its course; then it wanes, and presently the image is neglected" (p. 253).

Systematic observational or experimental studies of the behaviour of captive monkeys and apes towards their mirror-images or reflexions or to other mirrored objects or animals are, however, lacking. The present investigation arose as a result of preliminary observations of so-called 'imitation' and 'social facilitation' in four patas monkeys, *Erythrocebus patas*. These monkeys had all been recently captured in Northern Nigeria, and were obtained through the Wellcome Research Laboratories at Beckenham. Until they came to our own laboratories, they had not taken part in any kind of experimental situation. It is reasonable to assume that, in the wild, these animals would not see anything comparable to the mirror-image other than the reflexion of themselves on the horizontal plane such as is provided by stream or pond water. Hence, the initial, and subsequent, behaviour of such experimentally unsophisticated animals to mirrors or mirror-like situations seemed to be worthy of investigation.

Of the four monkeys used in the work, two were full-grown adults, one male and one female, and two were juveniles, also one male and one female. The exact age of none of them was ascertainable. They were normally kept in separate living cages, and, according to the experimental situation, were not allowed to see or have contact with each other. The two young ones were kept in one room, and were let out on most days to be together in a large cage which was divisible into two compartments according to the requirements of the social facilititation and imitation studies. The two adults were in a separate room adjacent to this, and they were also occasionally allowed out into the room. During the latter part of the observations, all four cages were put in the same room while alterations to the other rooms were being made, but appropriate screening of the cages was carried out when experimentally necessary.

Prior to any experimental observations being made, it was noted that whenever the two young monkeys were released into their living room, they showed intense and active interest in the images of themselves that they could see in the glass parts of the partitions dividing, on two walls, their room from the adjacent two rooms. The behaviour seen consisted of a variety of 'observing responses', such as standing on hind-legs, crouching low to the ground with head held low and staring, and of cautious approach towards the partition. In addition, the young female, in particular, frequently made 'gaping' or 'yawning' responses in which, while staring towards the reflexion, she lowered the head while opening the mouth to fullest extent, then raised the head again to the normal horizontal position. This reaction would occur with the animal in the sitting, normal standing, or low-crouched postures, and in partial form (for example, opening wide of the mouth without lowering of the head) as well as in the complete form described. The significance of this reaction, its variation in form and in circumstances of occurrence, and its relations to other behaviour components of the species' repertoire have yet to be fully worked out, so that the term 'yawning', to be used hereafter, is simply a usefully descriptive one. A similar reaction has been described in wild chacma baboon males by Hall[2], and in captive rhesus monkeys by Hinde and Rowell[3]. The interest to the two young animals of these reflexions in the glass partitions delayed any kind of social responses between the two often for several minutes after release from the cages.

The adult male's responses in the same kind of situation were even more prominent, the 'yawning' being often a reaction in which the head was lowered, when the animal was standing, almost to the ground. The adult female, on the contrary, tended to show cautious investigatory and observing responses and rarely, if ever, 'yawned'. This female had been a cage-mate of the adult male for some weeks prior to coming to our laboratories. The adult male has shown a similar, but much briefer, kind of response on seeing his image in a pane of frosted glass standing upright against a wall.

Further indications of the value of this technique came from observing the behaviour of these animals when given access to a small hand-mirror in the cage or lying on the floor of the room. Here the monkey is able to move the mirror about, and the fascination of it to them was strikingly apparent. Indeed, its interest for the young ones in particular was such as frequently to counteract the attraction of preferred food items, such as fruit, in the vicinity of the mirror. Further, the two young animals tended to show as much interest in it when they were together as when they were viewing it individually in their cages. In fact, the interest of one in it tended to be accentuated by the investigatory and playful behaviour towards it of the other, so that social facilitation seemed here, as on many other occasions, to be operating strongly.

The typical behaviour of the two young animals, when given the small hand-mirror separately in their cages, has consisted of a wide variety of investigatory behaviour, such as peering close up to it, licking at its surface, chewing the edge of it, pawing at its surface, picking it up and holding it in one hand or both hands, usually close up to the face, and sometimes putting one hand round to the back of the mirror as though trying to touch the reflected object. Both these animals have also frequently looked obliquely into the mirror, either when holding it up, or when it is lying flat on the ground, and on one occasion the young female 'yawned' while thus looking, and then immediately turned and 'yawned' at the observer whom she had apparently viewed in the mirror.

In a series of trials carried out over several days, and using a variety of different surfaces of the same sized object ranging from completely black to full reflecting, the presentations being randomized to control for serial effects, there is clear evidence of differential stimulating effects according to the degree of mirror-image discernible. It is also clear, however, that there is a gradual habituation to the mirror-image as is shown by the fact that both young animals reduced their investigatory time with the full-reflecting hand-mirror from 100 per cent for each on the first 5-min trial to 60 and 63 per cent respectively on the fifth 5-min trial. There are, however, oscillations with some of the intermediate mirror-surfaces which suggest that the incomplete images may possibly be less subject to habituation than the complete, but these trends need further systematic investigation.

When the hand-mirror has been placed on the floor of the room in which the two young animals have been freed from their cages, it still remains very compelling to them. Thus, the young female immediately on leaving her cage has come straight up to the mirror, peered into it as it lay on the floor, picked it up and held it close to her face, and carried it about with her, even though she was free to climb on the window ledges and look out or explore the rest of the surroundings. The young male also frequently attended closely to the mirror and manipulated it, and often has followed the reactions of the female to it. On one occasion he grabbed the mirror in his hands as she held it to her face and tried to pull it away from her. She then ran away from him on her hindlegs clutching the mirror in both hands. This young female has always had priority of access to preferred food items, such as fruit, when the two young ones have been together. However, when the mirror has also been available to her, she has tended to leave only partly eaten such objects as bananas, and has even tolerated the young male eating a part of a banana very close to her while she has been gazing at her mirror-image.

The reactions of the adult animals, when the hand-mirror has first been placed in their individual cages, have been markedly different from those of the young ones. Thus, when it was placed flat in the food bowl in the adult male's cage, he reacted immediately with startle and jumped away to the back of the cage. The reaction was evidently in response to the mirror-image of himself because it occurred as soon as he stood looking down from directly above it. He then avoided the mirror and went to sit at the back of his cage. Whenever he did glance towards it, his behaviour showed uneasiness in frequent 'yawning' and movements of the lips. It was only after 12 min from the time when the mirror had been put in his cage that he again came and stood looking down on it, and again at once jumped away from it, 'yawned' six times in rather rapid succession, stood on his hindlegs above the mirror, and then gave a series of thirteen 'yawns' at a rate of about one every 15 sec. He continued with this kind of behaviour throughout the whole period of the trial and also climbed around inside his cage very actively. Only after 15 min did he for the first time put one hand out and touch the mirror very gingerly. During the next 6 min he picked up food pellets in his mouth, but without chewing and eating them, merely nibbling at their ends, and he also picked up nutshells that were lying near the mirror.

The adult female's reactions to the hand-mirror likewise showed startle on first perceiving the mirror-image, but then a cautious but more or less continuous investigatory behaviour shown chiefly through peering at the mirror, looking underneath it, twice putting her face right up against its surface, looking briefly into it, but never actually touching it with her hands. For the 5-min period following removal of the mirror from her cage, she continually searched around inside the cage peering several times under the wooden board on the cage floor, looking under the food bowl in which the mirror had been lying, and so on. Thus, in a preliminary way, these observations on these four animals suggested the potential value of a more controlled study with systematic variations of the mirror-stimulus condition.

The first of these studies is described now in outline. A two-sided mirror, 30 in. high by 18 in. wide, was mounted vertically on a wooden four-wheeled carrier, enabling it to be moved readily from one part of the laboratory to another. This mirror, sufficiently large for the animal to perceive the whole of its standing frontal image, was used on experimental trials at varying distances and conditions, while control trials were systematically interspersed with the experimental ones as a check on the effectiveness of the mirror as such rather than of any other conditions surrounding the animals and affecting their behaviour. The control observations were made either when the mirror was set directly in front of the animal in exactly the same way as for an experimental trial except that the mirror-surface was kept covered with a white cloth, or when the mirror was exposed to the direct view of the animal in the next cage but only to the indirect view of the control animal, or when the animal was screened from seeing anything but the usual laboratory environment. Common to all trials, experimental and control, were the presence of the same two observers, and the operation of recording equipment.

The behaviour of the two adult animals, when confronted with the mirror separately and at distances of 3, 6 or 9 ft. from the front of the cage, indicated a high degree of tension in comparison with their behaviour in the control situations. Typically, the adult male stood with body tensed, or sat with his back straight and his head alert, staring into the mirror and 'yawning' repeatedly with the mouth gaping open wide and the head going low towards the ground. The head then tended to jerk back to the normal gazing position. On the first mirror trial with the male, this reaction occurred fifteen times during the first 60 sec, that is, once every 4 sec. On this and subsequent trials on other days, the 'yawning' frequency decreased rapidly to near-zero a minute over the trial period of 10 min. However, as this response waned it gave way to other, and perhaps lower intensity, behaviour patterns, also indicative of tension. Thus, the male began repeatedly to pick up in his fingers the standard food pellets lying in a bowl in his cage and rubbed them repeatedly and for several seconds with a rolling motion between his two hands before nibbling the end of the pellet, without actually chewing any of it off, then finally letting the pellet drop to the floor and picking up another.

From observation of his normal undisturbed feeding behaviour with these pellets, it is clear that the rubbing is far in excess of that which he usually adopts with this food object, and it is also obvious that the nibbling and discarding of the pellets in this way is an unusual procedure for this animal. When engaging in this kind of 'false' feeding behaviour, the male sat sideways on to the front of the cage, so that he avoided seeing the mirror-image. In normal feeding, he tends to face towards the front of the cage.

Another indication of tension in the adult male's behaviour to the mirror seemed to be an increased frequency of self-scratching, as opposed to self-grooming, occurrences. Self-grooming is a leisurely picking-over of fur and skin, while self-scratching has here been a brief episode of rapid, dog-like scratching; for example, of the back of the neck by the hind foot. To what extent the mirror situation has affected the sexual behaviour of this animal is not yet clear; but it is possible that the observed occasions of erection of the penis and of masturbation during the experimental trials were brought on by the conflict nature of the situation.

As a variation, the vertical mirror was once placed at a distance of 9 ft. from the front of the adult male's cage, uncovered, and then moved slowly by the experimenter towards the cage. This elicited a dramatic and highly intense series of reactions. During the first minute, as the mirror was approaching him, he stood close to the front of the cage and 'yawned' 23 times, that is, at a frequency of 1 in about 2·5 sec. Thereafter, the 'yawn' frequency decreased sharply, giving a total for the 10-min trial of 67. When the mirror was right against the front of the cage, the cage door was slid aside so that there was no barrier between the male and the image. The effect of this was instantaneous, the animal at once striking repeatedly at the mirror with both hands, trying to bite at its surface, and pressing hard against it. This hand-scrabbling and pressing of the face towards the mirror, interspersed with occasional 'yawns', continued throughout the trial, except for brief intervals during which he scratched and sometimes picked up food pellets from his bowl but threw them immediately aside. When the mirror was moved away at the end of the trial, he continued to move restlessly about his cage, climbing around it, as though attempting to get out, and standing on hindlegs looking around the room. This approach trial was, as would be expected, much more effective in arousing intense and varied behaviour than the trials where the mirror was static.

The adult female, when shown the mirror at a fixed distance in front of her cage, 'yawned' 14 times only in the first trial. This can be compared with the first trial frequency of 50 for the adult male. 'Yawning' was thus not the most prominent response of this animal to the mirror. Rather was her behaviour on the experimental trials characterized by cautious investigation, as shown by her low crouching postures, peering towards the mirror, looking continually around her cage, around the room, up to the ceiling and towards the cage of the adult male. Her agitation or uncertainty was, however, indicated by an enormous increase in frequency of self-scratching episodes during the experimental, as compared with the control, trials. On one trial, frequency of this response built up to a peak as her 'yawning' frequency waned, and 43 separate scratching episodes were observed in the 10-min trial, as compared with an average of 8 or less in control conditions.

This female's behaviour was markedly oriented much of the time towards the adult male on those experimental trials where the screen between them was removed, enabling them to see each other. She repeatedly looked toward him, and he showed a high degree of alertness, standing repeatedly on hindlegs, gazing out in the direction in which she was looking, and occasionally 'yawning'. It seemed thus that he was responding, not to the 'common stimulus' provided by the mirror-image, for that was not visible to him, but to the agitated and watchful behaviour of his female partner. The mirror technique, used in this way on a pair of animals, seems to offer one simple way of studying in the laboratory what Miller, Murphy and Mirsky[4] have called 'communication of affect'.

The two young animals have only so far been given the vertical mirror when they are free together in their large living cage. The immediate effect of the mirror being placed in this cage was to inhibit their vigorous play-activity, including sexual play such as mounting, biting, etc., and to concentrate the attention of both animals on the mirror itself. Therefore, far from being an attraction only for an isolated animal, as Yerkes and Yerkes[1] have suggested, it has seemed to have an effect perhaps comparable with the first introduction of a new monkey of about the same age as the two others. The young female, in particular, scarcely ever left the vicinity of the mirror while it was in the large cage, and, when she had peered into it, she scraped at its surface with her hands, and put her face right against it. She then continually walked round and round the mirror rather slowly and cautiously, looking at it from all angles. The young male, although also interested in the mirror, as indicated by his gazing full into it and waving his arms towards his own image, tended rather, as in other performances when the two have been together, to follow the female around, and hence to do more or less what she was doing.

The frequency data on the distinctive behaviour patterns, such as 'yawning', scratching, fumbling with food pellets, etc., may all, it seems, be used in full-scale experiments to indicate significant trends in the reactivity of the animals to the many variations made possible by the mirror technique. The study of habituation is itself, in the primates, a neglected aspect of learning. Our own preliminary data, using 'yawning' as the response selected for special attention because of its prominence, show a progressive waning in frequency over successive days, as well as within 10-min trials as from the first to the last minute. When more than one experimental trial has been carried out on the same day, the habituation effect has been even greater, and it is probable that it would turn out to be proportional to the time-interval elapsing between the end of one trial and the start of the next. Two further points are suggested by the results. First, if an interval of 24 h occurs between the end of one experimental trial and the beginning of the next, there is a fairly marked initial increase in the frequency of the 'yawning' response. Secondly, as habituation of the adult male to his mirror image progressed, so his more or less complete lack of response to the control situation of covered mirror or mirror to the side of his direct view has tended to alter. Thus, on the first control session, no 'yawns' were recorded, while 50 were recorded in the first experimental session. On the

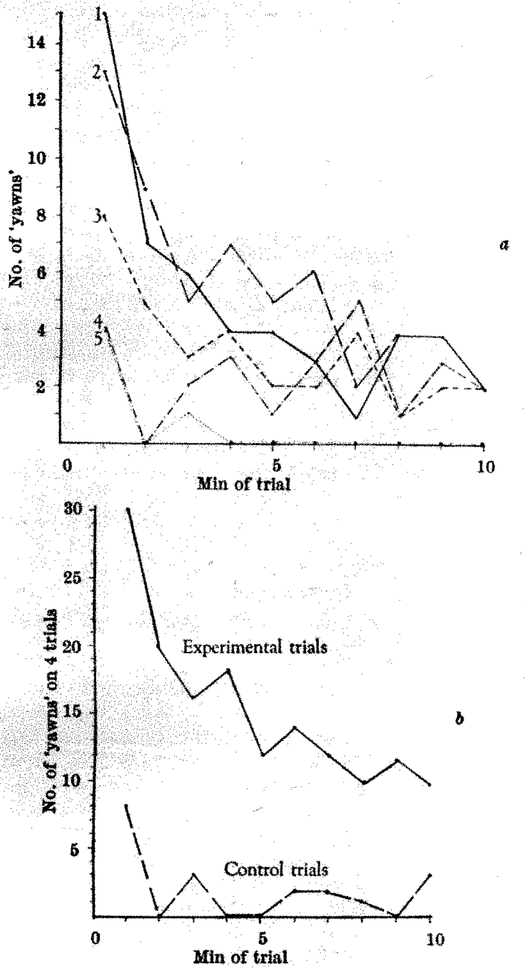

Fig. 1. No. of occurrences of 'yawns' by adult male
(a) Per min of experimental mirror trials on days 1–5, showing habituation trend; b, per min of experimental mirror, compared with control, trials, summed for days 1–4
——, Day 1; — — —, day 2; - - -, day 3; - · -, day 4; · · · ·, day 5

fifth day from the beginning of experiments, the mirror elicited only 5 'yawns', four of them being in the first minute of the trial, and the control situation elicited 13 'yawns', but these were rather evenly spaced throughout the course of the trial. It would thus seem that, with the habituation to the mirror-image, there is a conditioning of agonistic responses to the mirror-when-covered or to the situation in general, which therefore come to elicit a rather greater general level of tension than the normal. These statistical trends are illustrated in Fig. 1, but it is clear that a complex of social and motivational factors must be operating in these animals which may affect all aspects of their behaviour towards the mirror-image. Thus, it is perfectly possible that the œstrous condition of the female may affect her behaviour towards it, as also may her proximity or otherwise to the adult male with whom she is on intimate relationships. Similarly, the reactions of the adult male to an apparent image of another adult male might be expected to vary considerably according to the social context.

These preliminary observations suggest that the mirror technique, with its potentialities for experimental variation and for standardized presentation of 'social' stimuli, may be usefully developed to sample the behaviour of such animals in the restricted setting of laboratory space. Some of the learning processes involved in social approach and avoidance behaviour, highly complex in the natural group situation, can, it seems, with the aid of this technique, be selected for systematic experimental investigations which may help to bridge the gap between field and laboratory findings on non-human primate behaviour. At least in this way realistic situations can apparently be given to the animal so that they elicit a variety of conflict, frustration, and investigatory behaviour-patterns, in circumstances where they can be objectively and quantitatively recorded.

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DYNAMICS OF THE MEMBRANE OF *AMOEBA PROTEUS* STUDIED WITH LABELLED SPECIFIC ANTIBODY

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THE turnover of the surface membrane of cells, in the sense that membrane material is both added to and removed from the surface, is an essential feature of a variety of cellular processes, and is particularly evident in pinocytosis, phagocytosis, vacuolar secretion and cell division[1,2]. Rapid formation of new surface membrane has also been implicated as an essential feature in amoeboid movement[3–5] and it is particularly with this problem that we have been concerned. Controversy exists as to both the behaviour and role of the membrane in amoeboid movement. Goldacre[3,4] has suggested that the main body of the surface is stationary and that the membrane contracts at the tail, passing into the cytoplasm, while new surface is formed at the tip of advancing pseudopods—the cell completely renewing its surface as it moves through its own length. The contraction of the ectoplasmic gel at the tail is supposed to provide the force for amoeboid movement. Bell[5] has suggested that formation of new surface at the advancing tip provides the force for pseudopod extension. On the other hand,

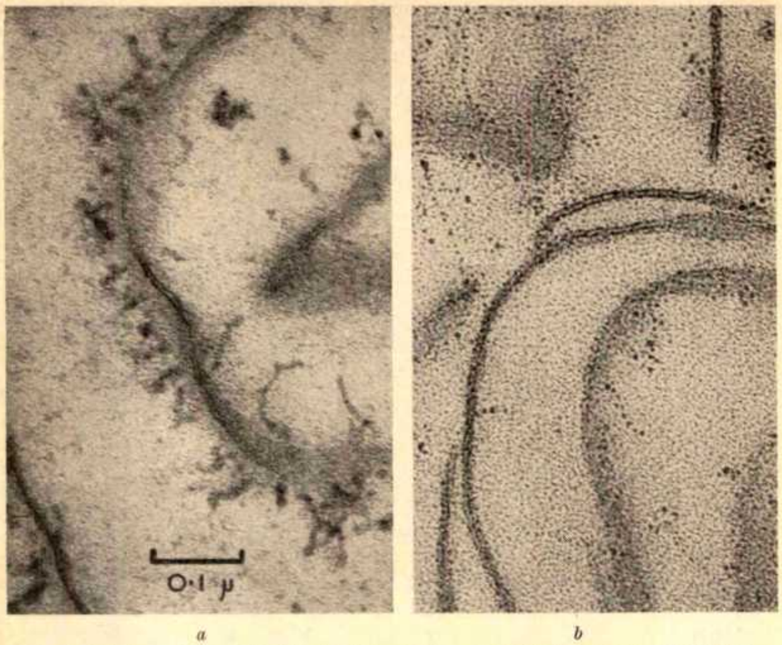

Fig. 1. Electron micrographs of isolated membranes. *a*, Isolated membrane preparation of *Amoeba proteus*, showing the 80 Å double membrane covered with hair-like projections, about 1000 Å long, on its outer surface. Fixed in osmium and stained with lead. *b*, Isolated membrane preparation, treated in the ultrasonic disintegrator for 3 sec. Only the double membrane remains, in 1μ fragments, and the hair-like projections have been dispersed. (*Photo: Dr. E. H. Mercer*)

Mast[21], and more recently Griffin and Allen[6], suggest that the membrane is 'fluid' and that the surface flows forward passively to accommodate changes in shape. Allen has also argued that the force for movement is provided by cytoplasmic contraction at the advancing end[7]. As regards pinocytosis, it is quite clear that membrane leaves the surface[8-10] in the form of pinocytic vesicles and the question is whether there is, during pinocytosis itself, any actual formation of new membrane at the surface. Chapman-Andresen[11] has suggested that no membrane need be formed at the surface itself during the period of pinocytosis, and that the surface area decreases and is the limiting factor.

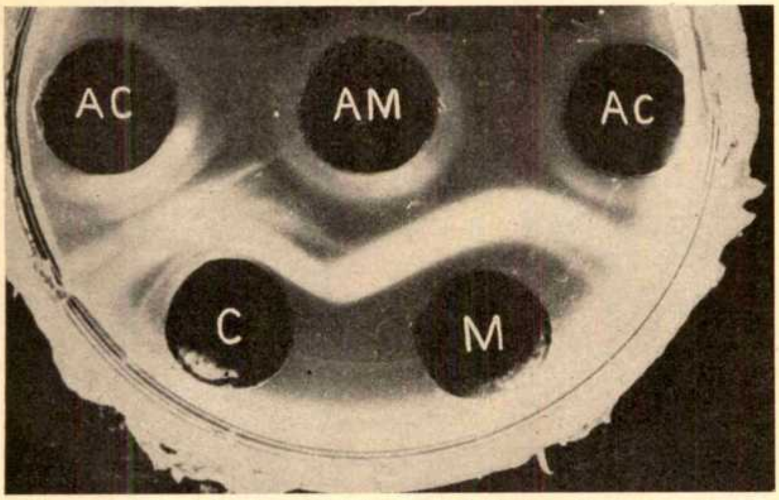

Fig. 2. Precipitin reaction in gel (Ouchterlony plate), showing a single main antigen in the membrane preparation, with traces of two other components *M*, Sonicated membrane; *C*, sonicated amoebae; *AM*, antiserum to membrane; *AC*, antiserum to amoebae

One of the difficulties in investigating turnover of the surface membrane has been to label satisfactorily the cell surface so as to determine the rate and site where new membrane may be formed. Results of investigations of total lipid turnover[1] are difficult to interpret in this connexion, as one cannot distinguish between lipids in the surface membrane and those elsewhere in the cell. Our recent isolation in bulk of the surface membrane of *Amoeba proteus*[12] provides a means of obtaining such data on membrane turnover, and our first approach has been to use a fluorescein-labelled antibody specific to the membrane with which to label the membrane and then to observe its behaviour during locomotion and pinocytosis.

Specificity of the Labelled Antibody

The surface membrane of the amoeba is quite stable and may be recovered from a variety of salt solutions after gentle homogenization of the amoebae. Such membranes are usually contaminated with cytoplasmic materials not easily removed. The method we have used largely avoids this difficulty. Amoebae placed in high concentrations of glycerol or sucrose appear after several days as membranous bags containing a clump of cytoplasm, and the membranes may be separated from the cytoplasmic lump by gentle homogenization followed by differential centrifugation to give a quite pure pellet of membranes[12]. Electron micrographs of isolated membranes (Fig. 1) showed that the membranes are very similar in morphology to those observed in intact cells[12,13]. The membrane comprises a typical lipoprotein or plasma membrane, appearing as a double layer about 80 Å thick, together with a thick coat on its outer surface. This coat shows itself to consist of fine hair-like projections about 1000 Å long and 100 Å thick (Fig. 1*a*). The membrane is periodic acid–Schiff positive[14] and this is ascribed to polysaccharide in the outer coat. Preliminary biochemical studies of the isolated membranes indicate that they contain about 32 per cent lipid, 15 per cent sugar, of which 3·5 per cent is amino-sugar, and 25 per cent protein. A wide range of amino-acids is present but only three sugars—hexoses—have been found, and one of these, as yet unidentified, is the major component. Sialic acids are absent[15].

Antisera to the isolated surface membranes have been prepared by injection into rabbits of supersonic homogenates (5 mg) of lyophilized membrane incorporated in complete Freund adjuvant[16]. Double diffusion in agar gel (Ouchterlony) of the sera obtained indicated the presence of a single reacting system, when the membrane antigen was treated supersonically. Without supersonic treatment the membrane is insoluble and no lines of reaction appeared, and examination of sonicated membranes in the electron microscope showed that the surface coat had been dispersed, leaving only fragments, about 1μ long, of intact plasma membranes (Fig. 1*b*). The reaction of sonicated membranes with sera elicited by whole cells again elicited only one main line, and this showed a reaction of identity with that against membrane antiserum, and with one of the 10 or more lines formed between whole cell serum and whole cell homogenates (Fig. 2). These and other reactions, which will be reported in detail elsewhere, suggest that the antibody to the isolated membranes is elicited mainly by the surface coat, and that the surface coat contains one main antigen which is also found in whole cells.

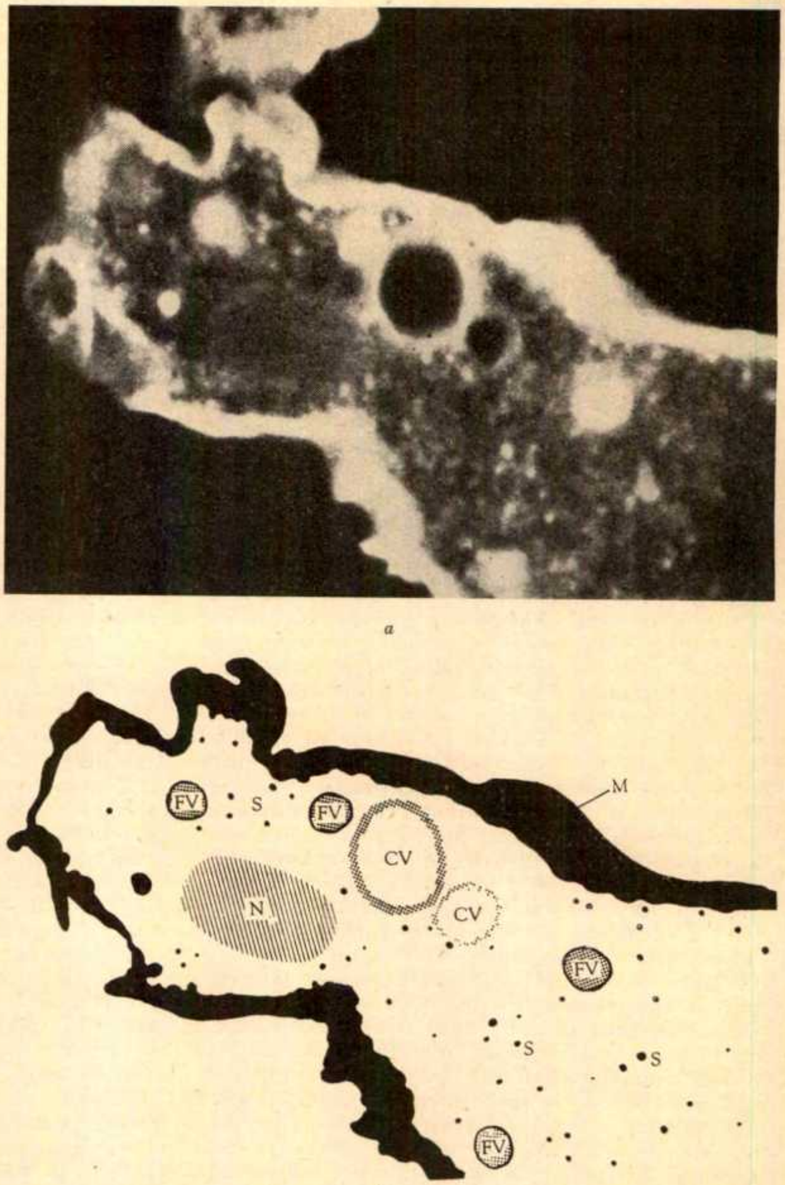

Fig. 3. *a*, Monochrome reproduction of photograph of portion of an amoeba section, treated with fluorescent specific antibody and viewed in ultra-violet light. *b*, Diagram indicating distribution of colour in *a*. Solid areas indicate localization of specific membrane antibody, at the cell surface and at certain sites within the cytoplasm. Shaded areas indicate autofluorescence, particularly strongly emitted by the contents of the food vacuoles

M, Cell surface; *CV*, contractile vacuole; *N*, nucleus; *FV*, food vacuole; *S*, localized sites of antibody staining

The specificity of the antibody was further demonstrated by treating cell sections with fluorescein-labelled antibody. Sera were first fractionated with 50 per cent saturated ammonium sulphate and the globulin fraction thus obtained treated with fluorescein isothiocyanate[17]. Non-specific sera were prepared in parallel. After removal of excess flourescein by dialysis, 0·5 per cent bovine albumen was added to protect the globulin, and the volume of the solution adjusted to a total protein concentration of 1 per cent. The resulting fluorescein-labelled antibody to the surface membrane reacted strongly and mainly with the cell surface, as seen in freeze-substituted sections of whole cells (Fig. 3*a*), and also with certain localized sites within the cytoplasm. The yellow-green fluorescence of the fluorochrome is readily distinguishable from the fainter blue-white autofluorescence of the cell. Some of the fluorescein-stained sites can be identified with food vacuoles, but the majority are much smaller—within the size-range of pinocytic vesicles. Stratification of the cells by centrifugation before fixation causes these smaller sites to form a band at the level of the nucleus, and such cells show no other staining within the cytoplasm. That the cytoplasmic sites are similar, antigenically, to the membrane and that their staining does not indicate cytoplasmic contamination of the isolated membranes is suggested by investigations in which the antibody is previously absorbed with either intact whole cells or cytoplasmic fractions. In both cases the reduction in staining intensity of the membrane was paralleled by that of the cytoplasmic inclusions. No staining whatsoever was obtained in cells treated with the control labelled globulin. Living cells were injected with the specific labelled antibody by Dr. C. Hoskins. The stain was initially uniformly distributed throughout the cytoplasm but could not be detected in the nucleus, food vacuoles or contractile vacuole. At no concentration was there any evidence for the staining of specific sites within the cell, neither at the inner face of the surface membrane nor at any sites within the cytoplasm. Cells maintained apparently normal locomotion, and within a few hours the stain accumulated in vacuoles and was defæcated in a manner similar to food remnants[9]. Cells injected with labelled non-specific antibody behave in a similar manner. The absence of staining of specific sites when the membrane specific antibody is injected into live cells in contrast to the staining shown on sections is possibly due to the main antigenic sites being located on the outside of the surface membrane and on the inside of vesicles, thus making them inaccessible to the antibody.

It is therefore concluded that the antibody prepared in the way described is directed exclusively against macromolecular entities contained within the surface membrane of the amoeba, and also found in certain cytoplasmic vesicles. One major component of the antibody is directed against the surface coat, which is probably a mucoprotein.

It is of interest that five strains of *Amoeba proteus*, and also strains of *Amoeba discoides* and *Pelomyxa carolinensis* reacted positively to the antiserum; strains of *Amoeba dubia*, *Hartmanella rhysodes* and a species of *Acanthamoeba* did not react to the antiserum.

Behaviour of Labelled Cells

If cells are placed in a 1 : 10 dilution of the labelled antibody for 5–7 min, and then washed in culture medium, the cells soon begin to locomote apparently normally. Their surfaces fluoresce brilliantly and there is no evidence for any staining within the cell. Longer exposure of the cells to the specific antibody leads to pinocytosis after about 30 min. Neither control labelled globulin nor fluorescein labels the cell surface. In locomoting labelled cells the surface fluorescence appears to be substantially uniform over the whole of the locomoting cell; an apparently greatly increased intensity at the tail of some cells is largely accounted for by the greatly increased folding and nodulation of the tail surface which is visible using the *N.A.* 0·95 objective. Examination of the tips of advancing pseudopods, in particular, shows a uniform thin surface fluorescence. There is thus no evidence for rapid surface renewal at this point or at any other region of the cell. Cells that had moved through a distance many times their own length still showed uniform labelling over their surface. On occasion pinocytic channels were seen at the tail.

Slowly, over a period of hours, the cells showed a reduction in surface labelling that was paralleled by an increase in cytoplasmic labelling, which appeared as small fluorescent vesicles. This could be seen both in living cells and in whole mounts of freeze-substituted cells, fixed at 2-h intervals after labelling. The stain gradually accumulates in one or more larger vacuoles and after about 12 h all the stain has disappeared from the surface. This phenomenon is well illustrated by restaining such cells, which again show bright surface fluorescence, which again disappears over 24 h and accumulates within vacuoles. This procedure was repeated five times, each time with the same result. The cells survive at least another week during which time the vacuoles are defæcated[9]. While fluorescent labelling is notoriously difficult to quantify, the time required to halve the intensity of the surface stain was estimated to be about 5 h.

These results suggest that there is no rapid turnover of the surface membrane during locomotion such as would be expected if the cell surface were reformed anew each time the cell passed through its own length[3,4], that is about every 2 min. There is, however, a slow turnover with a time-scale of hours. The rate of disappearance of the stain from the surface will be exponential if: (1) there is continual removal and formation of surface; (2) the surface area remains constant; (3) there is complete mixing of old and new surface; (4) stain that leaves the membrane does not enter it again. Our observations suggest that these are reasonable assumptions. Then, if the amount of stain in the membrane is halved after 5 h the rate of membrane renewal and formation is 0·2 per cent/min.

The persistence of the uniform distribution of the labelled antibody on the cell surface as it moves forwards suggests that the surface behaves as a fluid envelope, which flows forwards to accommodate the changes in shape of the pseudopods. This is in complete agreement with the conclusions based on observations of particles attached to the surface[6,21]. This argues strongly against either membrane contraction, accompanied by re-entry at the tail, or formation of new surface membrane at the advancing tip.

Those experiments purporting to demonstrate that the surface is stationary during locomotion and is resorbed at the tail and formed anew at the front are by no means convincing[3,4]. The accumulation of cationic dyes at the tail of the amoeba which has been suggested as showing the resorption of the membrane into the cytoplasm there[3] is open to other interpretations and may be due to the electric potential gradient along the amoeba[18]. Those experiments in which glass fibres or oil drops were applied to the surface and were found not to move forward[4] are also equivocal. These objects only offer local obstruction to membrane flow, and thus cannot show that the whole membrane is stationary.

Normal locomoting amoebae have a well-defined tail, easily recognized by its wrinkled appearance. Evidence was obtained to show that the tail region behaves differently from the rest of the surface. Labelled antibody was applied locally to a small region of the cell surface by drawing the cell into a narrow capillary and briefly immersing its tip in concentrated antibody solution. This resulted in a cell with a local area showing brilliant staining and the remainder of the cell showing no staining. Cells treated in this way recovered at once and performed apparently normal locomotion, and the stained region became the tail in all cases. The staining of the tail persisted over a period of 1 h before it finally dispersed. Observation of the margin of the stained area revealed occasional abrupt separation of small islands of stain within the membrane; these moved quickly forward towards the advancing pseudopod and at the same time rapidly expanded and dispersed. This process was only briefly visible since the intensity of fluorescence decreased rapidly as it spread. These observations, showing relatively little surface flow at the tail compared with the rest of the cell, are supported by our observations on particles attached to the tail. These are carried passively along for periods of many minutes and, unlike those elsewhere on the surface, do not move towards the advancing end. Again, centrifugation of amoebae—at 10,000g for 10 min—sometimes results in the appearance of a small stationary pseudopod at the tail. This remains there without change of shape, during active locomotion, for periods up to 1 h. The tail thus seems to be a region in which the surface does not flow forward and seems to be carried passively along. This view is again in marked contrast to that of Goldacre[4], who suggests that there is both contraction and resorption of membrane at the tail.

Amoebae labelled with specific antibody were also examined as regards pinocytosis and phagocytosis.

In 0·125 M sodium chloride (pH 6·0) and 2 per cent albumen (pH 5·0), pinocytosis of labelled cells was both quantitatively and qualitatively similar to the controls, but in 0·2 mg/ml. ribonuclease pinocytosis seemed to be reduced. Fluorescent pinocytosis channels could be seen. When pinocytosis was ceased —after about 20 min—the surface of the cell was still brightly stained. Labelled cells phagocytosed tetrahymena, and the surface of the food vacuoles was clearly fluorescent. This is in accord with the results of other workers, which show that the surface membrane of the cell gives rise to, and is morphologically very similar to, the membrane of newly formed food or pinocytic vesicles.

Since the cell surface was still stained after membrane had left the surface during pinocytosis and phagocytosis, it seems that no new membrane is formed during these processes, and this is in accord with Chapman-Andresen's[11] conclusions that the availability of membrane is the limiting factor for pinocytosis and that the area of the surface membrane is reduced during pinocytosis by the area of the pinocytic vacuoles that are formed. Moreover, her observation that after pinocytosis the surface area of the membrane is reduced by about half and the cells require a recovery period of about 4 h before they can complete a comparable amount of pinocytosis again, may indicate the time required for the formation of new membrane. This corresponds quite satisfactorily with our figure for membrane turnover of 0·2 per cent/min, since, if re-entry of membrane into the cell ceased, renewal of half the surface area of the membrane would then require 4·2 h. The inability of the cell to form new membrane rapidly is again suggested by the fact that when cells are cut up into about 16 fragments, each shows uniform surface labelling and further attempts at subdivision lead to cytolysis.

Discussion

It thus appears that the surface of the amoeba is fluid, except at the tail, and though there is no rapid formation of new surface membrane, there is a slow turnover. Consideration of the specificity of the antibody suggests that these conclusions may apply to the surface coat only, and one must now consider to what extent its behaviour represents that of the membrane as a whole and, in particular, the plasma membrane.

There appear to be two main possibilities. Either the membrane flows forward as a whole and there is a zone of shear between it and the stationary ectoplasmic gel[7], or the surface coat only is fluid and flows over the plasma membrane which is stationary, and the zone of shear is within the membrane itself. While the first possibility requires no formation of new membrane during locomotion, the second requires that the plasma membrane be formed anew each time the cell passes through its own length as suggested by Goldacre for the membrane as a whole[4].

This second alternative seems improbable for several reasons. If the continuity of the plasma membrane is to be maintained, and the electron microscope evidence suggests that it is, then all the components of the membrane beneath the flowing surface coat must be intussuscepted at the advancing tip and resorbed at the tail. There is no persuasive evidence that this complex series of events is taking place. Also the zone of shear must be localized within a very narrow zone—less than 0·5μ—within the coat, or at the junction between the coat and plasma membrane. This seems improbable, if only in view of the high viscous drag that would result if the zone of shear were restricted to a narrow region in a membrane showing both continuous and radially oriented structure in the electron microscope. We thus prefer the far simpler possibility that the coat and membrane are attached and both flow forward except at the tail. This requires that the zone of shear would be in the hyaline fluid layer that is present between the membrane and the gel[7]. While this fluid may be expected to provide only a low viscous drag, one might expect that one could find evidence for the flow of fluid forwards in the outer region of this layer. We have no evidence for this, but such a flow could well be obscured by the backward movement of the whole hyaline zone[19]. However, it is clear that the membrane moves as a whole during pinocytosis and phagocytosis, since both main components of the membrane are found in food vacuoles and pinocytic channels and vesicles[8–10].

Irrespective of whether the whole membrane or only the outer coat flows forward, the fact that the labelled antibody on the surface enters the cell over a period of hours shows that the membrane slowly both enters the cell and reforms at the surface. Our suggestion is that entry is brought about by pinocytosis at the tail. If pinocytosis goes on slowly at the tail in locomoting amoebae, both the slow turnover of the membrane and the difference in surface behaviour between tail and the rest of the amoeba may be accounted for. Pinocytosis seems the most likely mechanism for the entry of large molecules such as are contained on the surface of the amoeba, and we have observed pinocytosis at the tail of labelled cells, and the gradual appearance within the cytoplasm of numerous small labelled inclusions, probably pinocytic vesicles. At this stage we do not know how much of the pinocytosis at the tail is induced by the application of the labelled antibody itself, but evidence for a similar process in unlabelled cells is suggested by: (1) the presence of small sites, possibly pinocytic vesicles, within the cytoplasm which stain with specific labelled antibody; (2) the highly convoluted surface at the tail; (3) when pinocytosis is induced it usually starts at the tail[8]. All these emphasize the similarity in the state of the membrane at the tail of a locomoting amoeba, and the whole membrane of one which is pinocytosing. It is also pertinent that Bird has observed both pinocytosis and phagocytosis to occur normally at the tail of entamoebae[20].

As regards re-entry of membrane into the surface, we have almost no idea how this occurs nor even in what form—vesicular, micellar, or molecular—the constituents enter the surface membrane. The fate of pinocytic vesicles is unknown. It is possible that the vesicles may fuse directly with the membrane to provide new surface, or they may contribute to the membrane of the contractile vacuole which might provide new surface material since it empties by fusion with the surface membrane. If our conclusions as regards turnover are correct then attention must be directed to slow, rather than fast, formation of new surface membrane, and it would be of great interest to obtain data on other cells and to establish whether fast formation of new surface membrane can occur.

Our results show that those theories of amoeboid movement that require rapid formation of surface at the advancing end, or contraction and entry at the tail, are unsatisfactory. Since the membrane of the amoeba appears to be fluid and flow forward passively, it seems that the forces responsible for amoeboid movement must originate at some site other than the surface membrane. However, the membrane may play an extremely important part as regards the control of movement. This has already been pointed out in relation to the bioelectric potentials across the membrane, which may control the direction of movement[18]. It is possible that the flow properties of the membrane may also be a feature of some importance in locomotion, pinocytosis and phagocytosis. For example, pinocytosis may be associated with reduced membrane flow, and new pseudopod formation may result from an increased fluidity of the membrane.

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OBITUARIES

Sir John Edgell, K.B.E., C.B., F.R.S.

VICE-ADMIRAL SIR JOHN AUGUSTINE EDGELL died in Salisbury on November 14. He was the younger son of the late James Edgell of Teddington, and was born on December 20, 1880. He joined H.M.S. *Britannia* at the age of thirteen and, nine years later, specialized in hydrographic surveying. His first ship, as sub-lieutenant, was H.M.S. *Triton* surveying in the North Sea.

In 1912 he was given his first command, H.M.S. *Mutine*, and served for the next two years on the west and south coasts of Africa. Later, he commanded H.M. Ships *Triton*, *Hearty*, *Endeavour*, *Merlin* and H.M.A.S. *Moresby*. His surveying duties took him to most parts of the world, and he was in charge of surveys in China, Australia, the Red Sea, Mediterranean and the south and west coasts of Africa. He was promoted to commander in December 1915 and two years later became superintendent of charts, which post he held for two years. After a period at sea, he returned to the Admiralty as superintendent of charts during 1923–25. He was promoted to captain in 1923.

Edgell held the appointment of assistant hydrographer during 1928–30 and again during 1931–32; the intervening period he spent in command of H.M.S. *Endeavour* in the Red Sea. He became hydrographer of the Navy in October 1932 and held this post for the next thirteen years, a term only exceeded by Admirals Beaufort and Wharton. He carried the burden of this appointment throughout the Second World War. His service as superintendent of charts during the First World War stood him in good stead, and the experience he gained of the chart production unit in H.M.S. *Endeavour*, during the Dardanelles Campaign, led to similar equipment being installed in H.M.S. *White Bear* for service in the Burma campaign during 1943–45.

Shortly before the outbreak of war in 1939, plans had been made to transfer the Admiralty Chart Establishment at Cricklewood to a specially designed building, equipped with up-to-date rotary offset printing machines, at Taunton. The move did not take place until 1940, and the Cricklewood establishment suffered damage from the early air raids; but output was not affected. In accordance with general policy, the Chart Branch, situated in Cornwall House, London, S.E.1, was transferred to Bath shortly after war broke out and, at the same time, expanded enormously to cope with the extra demand for special charts. When the Production Division was set up at Taunton, the Issues Division also moved to Taunton from London, and, being under the same roof, was able to cope with the enormous increase in demand for charts, now turned out at a far greater speed on the new presses.

Edgell was most energetic throughout the War in meeting all the calls made on the Hydrographic Department and enjoyed great foresight in planning and preparing new types of charts for the ever-growing tempo of the War. Among his most successful innovations were the chart maps prepared originally for the landings in Sicily and which later were invaluable for the Normandy landings.

His surveying ships and detached officers were engaged in all theatres, from Norway to Burma, and carried out their work inspired by his leadership at the Admiralty.

His horizon was wide and he kept himself fully up to date with modern developments, though his service had begun in the days of sail and sounding under oars. He pressed forward with the introduction of echo-sounding equipment so that, in 1939, the new surveying ships he had done so much hard work to obtain were fitted with the latest types capable of obtaining soundings as deep as 1,000 fathoms.

He was promoted to rear-admiral in 1939 and to vice-admiral in 1938 and placed on the retired list; but he continued in this rank for the next seven years, finally relinquishing his post as hydrographer in May 1945.

During his term of office, the need for better knowledge of the oceans was essential for submarine warfare, and an Oceanographic Section was set up within the Hydrographic Department. With his usual foresight, he realized that Great Britain seriously lacked a centre for research in this subject, and in 1944 he opened discussions in the Admiralty which eventually blossomed, six years later, into the National Institute of Oceanography. After retirement from the Navy, he kept himself fully abreast of all developments and served on the National Oceanographic Council and Executive Committee of the Institute, where his counsels were highly respected. He attended his last committee meeting as recently as June of this year, after which his health began to fail.

For his services in command of surveying ships during the First World War he was awarded the O.B.E. in 1919. In 1936 he was made C.B., and was created K.B.E. in 1942.

But his proudest moment was when he was elected to the fellowship of the Royal Society in 1943.

Edgell was, from its beginning, very closely connected with the activities of the International Hydrographic Bureau and attended four of the quinquennial conferences at Monaco, and in 1939 was elected chairman of the Assembly.

Edgell was appointed by the Admiralty to the Board of the Port of London Authority in 1941, and held this post until his seventieth birthday and was nominated as the Authority's representative on the Kent River Board. He was selected to be the acting conservator of the Mersey on retirement as hydrographer until 1951.

Always a keen cricketer, Edgell lived an active life. Many were his tales of hard work and full enjoyment in his sea-going days, when there was no easy substitute for marching through rough country to make a triangulation station or pulling a whaler for weeks on end to sound out the shallow areas of a survey. He took a very personal interest in his officers and men and would always be forthcoming in giving advice and encouragement. His knowledge of hydrography was unlimited, and he had a flair for imparting his skill, while his memory never failed him for even the most precise point of detail.

He was the last of the old generation of surveyors who had been brought up on the tenets of Wharton and Field and the hard life that the early surveyors had endured.

E. G. Irving

Prof. T. M. MacRobert

Thomas Murray MacRobert died at his home in Glasgow on November 1 at the age of seventy-eight. He held the chair of mathematics in the University of Glasgow from 1927 until 1954, and before that had been assistant and lecturer in the same University. He was educated at Irvine Royal Academy and the University of Glasgow, where he graduated in 1905 with first-class honours in mathematics and natural philosophy. From 1906 until 1910 he was a Major Scholar at Trinity College, Cambridge, and was a Wrangler in the Mathematical Tripos.

Prof. MacRobert was the author of numerous papers on the theory of special functions, on which he was an expert; he continued to be active in research until just before he died. Much of his work was concerned with the properties of E-functions; these functions, which were invented by him, contain as special cases several of the well-known functions of mathematical physics, and make it possible to give a unified treatment of the subject. His books on *Functions of a Complex Variable* (Macmillan, 1917) and *Spherical Harmonics* (Methuen, 1927) were widely used by students, and he also collaborated with other writers in books on Bessel functions and trigonometry. He was responsible for the second revised edition of T. J. I'A. Bromwich's *Infinite Series* (Macmillan, 1926). The value and popularity of these works are attested by the number of different editions in which they have appeared.

He was a friend and helper, not only to generations of Glasgow students, but also to many mathematicians from India and the East, who will sorely miss the willing assistance he gave them in their work, without ever expecting any acknowledgment. He was one of the original founders of the Glasgow Mathematical Association, of which he was twice president and latterly honorary president. The flourishing state of the Association and the establishment and reputation of its *Proceedings* are very largely due to him.

His interests outside mathematics included hill-walking and playing the organ, and he took a prominent part in the preparation of the *Congregational Churches' Hymn Book*. The affection and respect in which he was held by friends, colleagues and former students was amply demonstrated when, after his retirement in 1954, they commissioned Mr. Norman Hepple to paint his portrait. This excellent likeness, which the University of Glasgow is fortunate to possess, also conveys something of his character and personality. He will be remembered for his kindliness and unfailing courtesy, but above all for his absolute integrity.

R. A. Rankin

Prof. E. F. Nash

Prof. Eric Nash, professor of agricultural economics in the University of Wales, Aberystwyth, died on October 31, aged fifty-eight. He is survived by a widow, a daughter and a son.

Nash started his academic career with a first in Greats at University College, Oxford, went on to Modern Greats, in which he also took a first, and at one time seemed set for a career in pure economics (in 1930 he published *Machines and Purchasing Power*, which was an essay in monetary theory). But with his appointment to the Ministry of Agriculture in 1934 he turned his attention to agricultural economics, and most of his work was done in that subject either in that Ministry, the Ministry of Food, the Control Commission for Germany, or, since 1946, at Aberystwyth. At the time of his death he was among the two or three best-known agricultural economists in Great Britain. This was a reputation gained not through the volume of his published work (indeed, during his twelve years of Government service he was scarcely able to publish anything) but through the uniformly high quality of his articles, speeches, teaching and contributions to discussions and debates. He was much in demand as a speaker and member of committees (Government and other), and his services were borrowed for quite long periods by the authorities in Jamaica, who were concerned with the low state of agricultural development in that country. He had much to do there with the work of the Institute for Economic and Social Studies.

Nash had no special interest at any time in agriculture as an applied science. It is true that as provincial agricultural economist for Wales he had to devote himself to some extent to the practical problems of Welsh farming, especially on the farm management side, and that as a member of the

National Food Survey Committee he came to have an extensive knowledge of nutrition. But his main interest was in size and structure of British agriculture viewed as a component of the national economy, a subject on which Nash felt that the British agricultural industry was both too large and too expensive. He would not, however, have wished to be remembered only as a critic. Most of his criticisms were accompanied by constructive suggestions; and in particular his sympathy was readily engaged by the difficulties experienced by many small Welsh farmers from their unfavourable environment and insufficient size of farm. Had he lived to ordinary retiring age there is no doubt that he would have done much more writing on this and allied subjects, and that it would have had the same originality and lucidity that were the main features of his work up to the time of his early death.

J. H. KIRK

NEWS and VIEWS

Physics at Sheffield: Prof. G. E. Bacon

DR. G. E. BACON, at present deputy chief scientific officer at the Atomic Energy Research Establishment, Harwell, has been appointed to a chair of physics at the University of Sheffield as from October 1963. The University has decided to experiment with a system in which the headship of the Department of Physics will be held in turn for a period of three years by each of the two professors concerned, and Dr. Bacon will assume the headship on his arrival, under this arrangement. Dr. Bacon was educated at Derby School and Emmanuel College, Cambridge, where he took Firsts in both parts of the Natural Sciences Tripos. In 1939 he joined the Air Ministry Research Establishment and was eventually in charge of a group concerned with ground radar research and development at what was then the Telecommunications Research Establishment, Malvern. He was seconded to Chalk River, Canada, for a period, soon after going to the Atomic Energy Research Establishment, Harwell, and since 1948 has been principally engaged in neutron diffraction experiments. His book on this subject is well known as an important work and he has published numerous contributions in this field. It is expected that Dr. Bacon will continue his work on neutron diffraction with particular reference to magnetic ordering in metals and alloys through arrangements to be made outside the University, and a programme of X-ray experiments designed to elucidate further the electronic structure of molecules and solids will be built up following Dr. Bacon's arrival at Sheffield. Dr. Bacon will bring very wide experience in research on the physics of solids to the chair at Sheffield, and with it energy and enthusiasm for implementing new ideas on the teaching side, particularly on the training of postgraduate students, and it is expected that this aspect of the work of the Department of Physics will build up rapidly over the next few years.

Head of Space Department, Royal Aircraft Establishment: Mr. E. G. C. Burt

MR. E. G. C. BURT has been appointed head of Space Department, Royal Aircraft Establishment, Farnborough, in succession to Dr. A. W. Lines, who has taken up an appointment with the European Preparatory Commission for Space Research. Mr. Burt, who is forty, was educated at Yeovil School and Queen Mary College, University of London, where he graduated with a first class honours degree in electrical engineering in 1943. After serving in the Royal Air Force, he joined the Royal Aircraft Establishment in 1947, where as a scientific officer he worked in the field of computers and servo-mechanisms. He was promoted to senior scientific officer in 1950, to principal scientific officer in 1953, and to senior principal scientific officer in 1955, when he became superintendent of the Dynamic Analysis Division of Guided Weapons Department. During this period he published a number of papers concerned with stochastic processes in control and guidance systems and the theory of optimization, and he was awarded the Navigation Prize for his lecture to the Royal Aeronautical Society on this subject. After the launching of the first Earth satellite in 1957 Mr. Burt took a leading part in the Royal Aircraft Establishment's work on satellite tracking, and the correlation of observation with theory concerning the effects of the Earth's oblateness. On the formation of Space Department at the beginning of this year he was appointed head of its Dynamics Division; he has taken an active part in the Department's work—in particular, on communication satellites, where he has made a number of contributions to attitude control theory and orbital patterns.

British Committee for Biological Education

THE Royal Society and the Institute of Biology have established a Joint Committee to report on desirable changes in the teaching of biology in the United Kingdom. It is hoped that this Committee will, through its representative membership, ensure that the merits of various proposals for changes in syllabuses, examinations and teaching methods can be assessed. The Committee will have close liaison with the group of biologists seconded to the Nuffield Foundation in order to produce texts, teachers' guides, apparatus, films and visual aids for the biological education of children from eleven to sixteen years of age. The membership of the Committee is: Prof. W. O. James and Prof. M. M. Swann (Royal Society); Sir Gerard Thornton and Mr. J. B. Cragg (Institute of Biology); Dr. A. Urie (Ministry of Education—observer); Dr. Lucy Boyd (Scottish Education Department—observer); Dr. H. G. Vevers (Biological Council); Mr. W. H. Dowdeswell (Science Masters' Association); Miss V. J. Evans (Association of Women Science Teachers); Mr. L. C. Comber (Secondary Schools Examination Council). Individuals selected for the Committee from universities, examining boards, technical colleges and teacher training colleges include: Prof. T. A. Bennet-Clark, Mr. D. A. Coult, Prof. G. E. Newell, Dr. T. G. Onions and Mr. L. Wolff. The secretariat for the Committee is being provided by the Institute of Biology (41 Queen's Gate, London, S.W.7).

Research in Cutlery and Stainless Steel

In moving that the Cutlery and Stainless Steel Flatware Industry (Scientific Research Levy) Order, 1962, be approved in the House of Commons on November 22, the Parliamentary Secretary to the Board of Trade, Mr. D. Price, said that the primary purpose of the Order was to increase the industry's contribution from £9,000 a year to just on £14,000. with a concomitant increase in the grant from the Department of Scientific and Industrial Research from £6,000 to £9,000. These resources would be used for research and development projects, a technical inquiry service and the dissemination of scientific and technical information. The main research effort would be devoted to increasing the degree of mechanization in the industry, in which labour represented 50–60 per cent of manufacturing costs, and to reducing the number of manufacturing operations. That programme included research on knife and scissor grinding, on multiple polishing, on abrasive operations and on the metallurgy of the edges of knives and scissors. Only firms with a turnover of £10,000 or more would be called on for an increased contribution, and exemption of the first £3,000 of turnover would reduce the number of firms liable for the levy from 280 to 190. The Cutlery Research Council had been reconstituted, at the request of the Department of Scientific and Industrial Research, as an incorporated body under the title of the Cutlery and Allied Trades Research Association. The annual production of the industry, as defined for the purposes of the Order, was about £10 million and it was exporting about £3·5 million a year.

University of Delhi: Physiology of Reproduction

The Ford Foundation has made a grant of 195,000 dollars to the Department of Zoology, University of Delhi, for expanding the facilities for research and training in the field of physiology of reproduction over a five-year period. Dr. M. R. N. Prasad and Dr. B. R. Seshachar will be the chief investigators in charge of the programme. As part of the activity envisaged in the Ford Foundation grant, the Department of Zoology has organized a training programme consisting of a series of sixteen seminars on physiology of reproduction in collaboration with Dr. Sheldon J. Segal, consultant to the Ford Foundation on reproductive biology. The seminars are given by scientists from India and the United States. The seminar series, which was inaugurated by Dr. C. D. Deshmukh, vice-chancellor, University of Delhi, on October 22, will continue until February 25, 1963.

Scientific Research in France

Increasing expenditure on research in France reflects a growing interest in science. Government assistance on a generous scale is extended to scientific bodies from a special Scientific and Development Fund and, in the field of private enterprise, progressive firms co-operate in setting up research centres. Efforts are also being made to strengthen the ties between the universities and industry. A high-powered Inter-Ministerial Committee on Scientific and Technical Research, under the chairmanship of the Premier, was established in 1958 to advise the Government on the best ways of developing scientific research, implementing scientific equipment programmes and distributing proposed State expenditure on research among the various Government departments. To advise the Committee there is a consultative body of twelve persons (known as the 'Committee of Wise Men') chosen by the Premier. The Centre National de la Recherche Scientifique acts as a co-ordinating body and is available to help all scientists, whatever their speciality and whatever the organization—State or private—for which they work. This body itself administers forty groups of laboratories or research institutes, including low-temperature research at Bellevue, astrophysics at Haute-Provence, atomic synthesis at Ivry, macromolecular physics at Strasbourg, and solar energy utilization at Montlouis. At Beni-Abbès there is a Sahara Research Centre. In the field of medical research, the Pasteur Institute—an independent, non-profit-making body—has about 100 laboratories with 33 divisions covering a wide field. About 18 Pasteur Institutes have been established in Africa, Asia and the West Indies. During the past twenty years, OSTROM (the overseas research organization) has set up many research centres in the tropics, the activities of which range from geophysics and plant physiology to river hydrology and oceanography. The position of scientific research in France is outlined in a publication recently issued by the Secretary-General of the French Government (*France*. Pp. 144. Paris: Secretariat Général du Gouvernement, Direction de la Documentation, 16, rue Lord-Byron, 1962).

Minerva: a Review of Science, Learning and Policy

Besides the text of Sir Eric Ashby's first Chancellor's Lecture to the University of the Witwatersrand, the first issue of *Minerva*, the new quarterly review of science, learning and policy, includes articles by Prof. W. Kornhauser on "Strains and Accommodations in Industrial Research Organizations in the United States"; Dr. A. K. Singh on "The Impact of Foreign Study: the Indian Experience"; and Prof. M. Polanyi on "The Republic of Science: its Political and Economic Theory". There are also summaries of the reports of the Scientific Commission of Pakistan and of the report to the President of the United States on Government Contracting for Research and Development, and the recommendations of the Science Council for the Development of Scientific Institutions in Western Germany (*Minerva*: a Review of Science, Learning and Policy, Vol. 1 (Autumn 1962). Edited by Prof. Edward Shils. Pp. 1–144. (London: C.S.F. Publications, Ltd., 135 Oxford Street, 1962.) Published under the auspices of the Committee on Science and Freedom. Subscription-rate: 20*s*. or 5 dollars a year; single issues, 5*s*.) [See also p. 1241 of this issue of *Nature*.]

Careers in Veterinary Science

A recently published booklet in the series on careers issued by the Ministry of Labour provides a good general account of the prospects offered by a veterinary career (Central Youth Employment Executive. Choice of Careers, No. 111: *The Veterinary Scientist*. Pp. 32 (4 plates). London: H.M.S.O., 1962). The five chapters deal respectively with educational and personal qualities required; training; scope and opportunities; the work of the veterinary scientist; and work with the veterinary scientist. This last chapter concerns the work of the various types of auxiliaries employed privately, in Government services or elsewhere. These are experimental officers, laboratory technicians, animal nurses, dressers, skilled animal attendants, etc. (Booklets

No. 57, *The Medical Laboratory Technician*, and No. 32, *The Civil Service: General Scientific and Technical Posts*, give information about careers other than purely veterinary that are concerned with various aspects of the work.) Good illustrations showing veterinary surgeons engaged in various duties are provided. A breakdown shows the numbers of veterinary surgeons engaged in the various branches of the occupations in the United Kingdom. There are 3,247 in private practice and about 1,000 in salaried occupations. Of the latter, 533 are in the service of the Ministry of Agriculture, 114 in that of Northern Ireland, 213 on the staffs of the veterinary schools, 96 employed by commercial concerns, 27 in the service of the Agricultural Research Council, 27 in municipal veterinary services, 52 on the staffs of various research institutes, and 30 in the Royal Army Veterinary Corps. In addition, there are 315 in the Colonial Veterinary Service serving overseas.

Computer Control in Soviet Steel Mills

THE U.S.S.R.'s most advanced industrial computers are those used for the automation of iron and steel production, according to an article on the application of computers and logical operation devices to the automation of factories in the U.S.S.R. (*Mechanical Engineering*. Academy of Sciences, U.S.S.R., Moscow, 1958. Pp. 383. Order 60-51089 from the Office of Technical Services, U.S. Department of Commerce, Washington 25, D.C. Price 3.75 dollars). The article is one of a collection of seventeen papers on mechanical engineering available in an English translation from the Office of Technical Services, U.S. Department of Commerce. Also included are studies on a vacuum dilatometer for investigating the sintering process of metal powders; a device for studying the characteristics of polymer specimens under tension; and an apparatus for testing the impact hardness of ice under field conditions. Although computers are being used in many ferrous metallurgical processes, the article indicates that their application has not yet been extended to rolling mills. This is explained by "the lack of an adequate study of variable conditions in the mills and the lack of the necessary measuring equipment (tension pickups, pickups for the metal pressure on the rolls, 'floating' micrometers, and strip-speed pickups)". The solution of the problem of automation of rolling mills requires the design of electric models for individual components as well as for the mill as a unit. "Work in designing such electric models is being carried out by several organizations." The report was translated for the U.S. Government through a co-operative Federal agency translation programme co-ordinated by the National Science Foundation.

The Lenin State Library, Moscow

THE library which now bears the name of the Lenin State Library was founded in July 1862 as a part of the Roumyantzev Museum in Moscow. The growth of this library was in no small measure due to the accretion of the 'compulsory' copies of all books published in Russia, for it also possesses a very large number of non-Russian books, and in many ways it can claim to rival the great national libraries in London, Paris and Washington. At the present time, as described by A. Y. Chernyak (*Priroda*, 8, 60; 1962), the Library is well housed in a number of buildings. Chernyak states that there is a staff of 2,000 and that the Library has 200,000 readers using 22 reading rooms, and contains 22 million books, sets of periodicals, microfilms and manuscripts. In 1913 the Library used to exchange books with 90 institutions in 19 countries; at the present time it exchanges books with 2,600 institutions in 80 countries, totalling 250,000 outgoing and 212,000 incoming books. Among the older books the Library possesses very valuable works by Copernicus, Bruno, Pliny and many other famous writers. Its reference section is staffed by a number of specialists and it serves, as does its publication service, a very large community.

Science Museums in Developing Countries

THE International Council of Museums has recently published a small booklet on *Science Museums in Developing Countries* (Pp. vi+66. Paris: International Council of Museums, 6 rue Franklin, 1962. 4 NF.; 7*s*.; 0·80 dollars). It is written by Mr. Frank Greenaway, of the Science Museum, London, with additional chapters by Dr. Torsten Althin, of Sweden, and Messrs. W. T. O'Dea, of London, and W. Stephen Thomas, of the United States. Science is concerned, for museum purposes, with ideas about the nature of the physical universe and those aspects of living things which can be the subject of exact or controlled study. Museums are places where things, both still and in action, are collected together, studied and presented. The handbook is intended to encourage educational authorities in countries which are trying to raise cultural standards to set up museums of science and technology. The main part consists of generalizations and the principles which justify the existence of such museums and regulate their organizations. The second part deals with the application of these principles in three widely separated areas—India, Latin America and Sweden. Finally, actual examples are given of objects which could be usefully incorporated in science museums. This is an excellent handbook and many of the dicta are applicable to all types of museums.

The European Seismological Commission

MEETINGS of the European Seismological Commission were held at Jena during September 24–30. Representatives from twelve European countries, including the United Kingdom, attended the meetings. The meetings were first concerned with the study of reports of work presented by members of the Sub-commissions for: (*a*) Alpine explosions; (*b*) seismo-tectonic map of Europe; (*c*) seismicity of the Carpathian region; (*d*) publications. Secondly, there followed a symposium entitled "The Crust of the Earth in Europe", with data from great explosions, near-earthquakes, dispersion of surface waves, *Lg* and similar waves and microseisms. Communications were also presented on the centenaries of the births of Prof. V. Láska, by Prof. A. Zátopek, and of Prof. B. B. Galitzin, by Prof. E. F. Savarenski. The account of the work on the new seismic scales was presented by Dr. W. Sponheuer. The European Seismological Commission of the Union of Geodesy and Geophysics is indebted to the Deutsche Akademie der Wissenschaften zu Berlin for sponsoring the meetings and to colleagues at Jena under Dr. W. Sponheuer for the admirable local arrangements. The next meetings of the Commission will be held in Budapest in 1964, and the new officers are: *President*, Prof. A. Zátopek (Prague); *Vice-Presidents*, Prof. E. F. Savarenski (Moscow), Dr. E. Vesanen (Helsinki); *Secretary*, Dr. E. Peterschmitt (Strasbourg).

Christmas Lectures for Schoolboys in Chemical Engineering

The Institute of Chemical Engineers has organized two Christmas lectures for schoolboys which will be held at the Lecture Theatre of the Science Museum, South Kensington, London, S.W.7, on the afternoon of January 11. Mr. E. S. Sellars, head of the Petroleum Division of the B.P. Research Centre at Sunbury, and a vice-president of the Institution of Chemical Engineers, will speak on "Stages in the Development of the Industrial Process", and Prof. M. W. Thring, professor of fuel technology and chemical engineering in the University of Sheffield, will speak on "Some Possible Future Developments in Industry". Further information can be obtained from the General Secretary, the Institution of Chemical Engineers, 16 Belgrave Square, London, S.W.1.

The Australian Mathematical Society

The Australian Mathematical Society met in Sydney, at the University of Sydney, during August 15–17; eighty members attended. The new Council elected was as follows: *President*, Prof. E. S. Barnes; *Vice-presidents*, Profs. T. G. Room and A. L. Blakers; *Publications Secretary*, Dr. G. E. Wall; *Treasurer*, Prof. C. S. Davis; *General Secretary*, Prof. H. O. Lancaster; *Councillors for the second term*, Prof. A. Brown, Prof. R. C. T. Smith and Dr. G. Szekeres; *Councillors for the first term*, Prof. L. S. Goddard, Dr. C. F. Moppert and Prof. K. C. Westfold. A summer research institute was held at the Australian National University, Canberra, during January and February, 1962, with Prof. E. S. Barnes as director. A third meeting of the institute will be held in Canberra during January 1963, with Prof. B. H. Neumann as director. The seventh general meeting of the Society will be held at Monash University during May 21–23, 1963. Further information concerning the general meeting can be obtained from the local secretary, G. C. Smith, Department of Mathematics, Monash University, Clayton, Victoria.

The Welsh Soils Discussion Group

The following have been elected officers for the fifth session, 1962–63, of the Welsh Soils Discussion Group: *Chairman*, Dr. R. Williams, regional advisory chemist, National Agricultural Advisory Service, Trawscoed, Cardiganshire; *Secretary*, J. A. Taylor, lecturer in geography, University College of Wales, Aberystwyth; *Assistant Secretary*, Dr. C. B. Crampton, soil surveyor, National Agricultural Advisory Service, Cardiff; *Editor*, D. F. Ball, Nature Conservancy, Bangor. J. R. Hampson, Forestry Commission, North Wales Conservancy, Shrewsbury, was elected an ordinary member. The theme for the fifth session will be "Soil Moisture". A meeting of the Group will be held at Bangor on February 27, when the subject will be "The Biological Role of Soil Water". Further information can be obtained from Mr. J. A. Taylor.

The Night Sky in January

Full moon occurs on Jan. 9d. 23h. 09m. U.T. and new moon on Jan. 25d. 13h. 42m. The following conjunctions with the Moon take place: Jan. 12d. 15h., Mars 2° N.; Jan. 21d. 18h., Venus 0·1° S.; Jan. 28d. 07h., Jupiter 3° N. In addition to these conjunctions with the Moon, Venus is in conjunction with Antares on Jan. 15d. 09h., Venus being 9° N. There will be a penumbral eclipse of the Moon on January 9, visible at Greenwich; Moon enters penumbra on Jan. 9d. 21h. 04m., mid-eclipse is at 23h. 19m. and Moon leaves penumbra at Jan. 10d. 01h. 34m. There will be an annular eclipse of the Sun on January 25, invisible at Greenwich. Mercury is an evening star visible during the first part of the month low in the south-west after sunset. Venus is a morning star, rising at 4h. 15m., 4h. 25m. and 4h. 45m. on Jan. 1, 15 and 31, respectively. Its stellar magnitude is −4·2; its distance increases during the month from 47 to 69 million miles, and the visible portion of the apparent disk increases from 0·365 to 0·545. Mars rises at 19h. 40m., 18h. 30m. and 16h. 55m. on Jan. 1, 15 and 31, respectively. It is at a distance of 66 million miles on January 15, when its stellar magnitude is −0·7; conditions are now favourable for observation. Mars is in Leo, not far from Regulus. Jupiter is an evening star, setting at 21h. 15m., 20h. 35m. and 19h. 50m. at the beginning, middle and end of the month. It is in Aquarius, its stellar magnitude is −1·7 and its distance from the Earth in the middle of the month is 520 million miles. Saturn is too close to the Sun for easy observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Jan. 4d. 19h. 04·5m., ξ^2 Cet. (*D*); Jan. 6d. 20h. 30·7m., 63 Tau. (*D*). *D* refers to disappearance. The Quadrantid meteors are active on January 3, the radiant being near R.A. 15h. 28m., Dec. +50°. The Earth is at perihelion on January 4, its distance from the Sun being 91·4 million miles.

Announcements

Mr. H. Faulkner, director of the Telecommunication Engineering and Manufacturing Association, retires at the end of the year. He was formerly deputy engineer-in-chief of the British Post Office and has held office in the Association for nine years. Mr. Faulkner will be succeeded by Mr. R. A. Moir, until recently a director of Standard Telephones and Cables, Ltd.

The British Biophysical Society is organizing a meeting on "Nucleic Acids and Nucleoproteins" at King's College, Newcastle upon Tyne, during April 2–4. The titles of the symposia will be "Structure and Physicochemical Properties" and "Effects of Radiation". Further information can be obtained from the local secretary, Dr. G. Scholes, Chemistry Department, King's College, Newcastle upon Tyne 1.

The centenary meeting of the British Pharmaceutical Conference will be held in London during September 2–6, 1963. The closing date for the receipt of papers to be delivered at the meeting is May 1. The Conference Lecture is to be given by Dr. J. M. Barnes, head of the Toxicology Research Unit of the Medical Research Council. Further information can be obtained from the honorary local secretary, Dr. K. R. Capper, 17 Bloomsbury Square, London, W.C.1.

The British Ecological Society, which was founded in 1913, will celebrate its fiftieth jubilee with a special meeting, to be held at Queen Elizabeth College, Campden Hill Road, London, W.8, during March 8–31. The meeting will include half-day sessions on various topics, ranging from "The History of Ecology in Britain" and "Quaternary Ecology" to "Production Ecology" and "Conservation and Ecology". Further information can be obtained from P. J. Newbould, Botany Department, University College, Gower Street, London, W.C.1.

THE PRAGUE AEROSOL CONFERENCE

THE tenth anniversary of the Czechoslovak Academy of Sciences was celebrated by a conference on aerosols attended by 163 national and 56 foreign delegates. The meetings were held during October 8–13, in the Academy's castle at Liblice, near Mělník, about twenty miles north of Prague (Fig. 1).

The proceedings opened with a series of formal statements on the importance of aerosols in industry, science, agriculture, medicine and meteorology. Prof. K. Spurný, head of the Aerosol Department of the Institute of Physical Chemistry of the Academy, then outlined the development and organization of aerosol research in Czechoslovakia.

The first scientific paper was to have been given by N. Fuchs, of the Karpov Institute of Physical Chemistry, Moscow, but he and several other Russian scientists, who are well known for their work on aerosols, failed to attend although their papers were announced in the programme and no explanation of their absence was forthcoming. It is probable that this occurrence, which is common on such occasions, reflects merely the inability of the over-centralized organization of Communist countries to deal efficiently with the issue of visas and to relax its control of foreign travel.

The programme, listing more than 90 papers, will not be discussed in detail as it is intended to publish the papers and discussions in English some time in 1963; only contributions of a fundamental nature will be considered individually.

Several such papers dealt with fluid motion. Pančev (University of Sofia) used the Kolmogorov theory of isotropic turbulence to show that the root mean square of the difference between particle and air velocity is given by a formula like that for sedimentation under gravity except that g is replaced by the root mean square acceleration of an element of air which is expressible in terms of the energy of turbulent motion. The extent to which particles follow the fluctuations of air flow can thus be specified.

The formation of deposits when an aerosol flows past a step was examined theoretically by Smolik (Prague), who deduced the flow field by a combination of conformal transformation and boundary layer theories. The characteristic pattern of the deposit was shown to be decided by the re-entrainment of deposited particles.

C. N. Davies (London), in a theoretical paper, showed how the sedimentation-rate and inertia of particles, which are being sucked into a sampling orifice from air which is otherwise at rest, reduce the efficiency of sampling.

Present interest in the kinetic theory of transport phenomena in aerosols was displayed by three papers. Podzimek (Czechoslovak Geophysics Institute) calculated the Stefan flow of aerosol particles, concluding that the deposition of small aerosol particles on a water droplet, during its growth by condensation, was important in some meteorological situations; Goldsmith (Harwell), using Waldmann's theory, concluded that the atmospheric scavenging effect of molecular bombardment by condensing vapour was negligible for growing droplets which were around 10μ diameter. Two basic effects exist when aerosol particles are surrounded by diffusing vapour; a radiometer force on them, due to uneven molecular bombardment, gives rise to diffusiophoresis and a hydrodynamic drag on the particle is set up by a bulk gas flow which is necessary to preserve constant pressure in a mixture of a gas and a vapour which diffuse at unequal rates; the latter is the Stefan flow and can sometimes exceed the effect due to molecular bombardment.

These problems have been examined in great detail by Deryaghin *et al.* in Moscow. In his absence, the paper by himself and Bakanov was described by J. Pich and proved to contain a new formula for the thermal transport of particles which are large compared with the mean free path. This formula purports to explain how it is that thermal precipitators operate some 25 times better than they should, on the old theory, when sampling aerosol particles of high thermal conductivity. In the absence of the author the derivation of the formula could not be explained.

The whole subject is of interest to occupational hygiene because of this and in view of a possible importance in lung deposition of dust where the counterflow of carbon dioxide and oxygen may influence the collection of particles in the alveoli.

The abstracts of the Russian papers which were not delivered showed interest in transport problems. N. Fuchs had a theory of the discontinuities of heat, concentration and velocity, existing in a gas adjacent to an aerosol particle, which covers the difficult range when the particle size is comparable with the mean free path. Prochorov and Leonov were to have described the measurement of the minute force which exists between a pair of evaporating drops and may prevent them from coalescing. This work was also the subject of a paper which was to have been delivered by them at a discussion on the physical chemistry of aerosols held at Bristol in 1960. They were not present and were therefore unable to give a satisfactory account of the remarkable sensitivity that they claimed for their torsion balance when this was queried; the sensitivity claimed at Prague was double the Bristol value.

Another experimental paper, available only in abstract, was by Storozhilova and Deryaghin, and dealt with the lateral displacement of aerosol particles, flowing between parallel plates, due to a cross-flow of water vapour causing diffusiophoresis; confirmation of the Waldmann equation for particles small compared with the wave-length was claimed. A flow apparatus, for studying the evaporation of supercooled mists, should have been described by Dukhin, Berezhnaya and Silaev.

The subject of aerosol filtration was introduced by Pich and Binek (Institute of Physical Chemistry, Prague). Since the Brownian motion of particles increases with rise in temperature, while their stop distance, due to gas viscosity, decreases, it is clear that the minimal efficiency of a fibrous filter should shift to a larger particle size at a higher temperature; this was confirmed.

Interesting experimental work by Raduškevič and Kolganov (Moscow) was unfortunately not presented; according to the abstract this was a study of

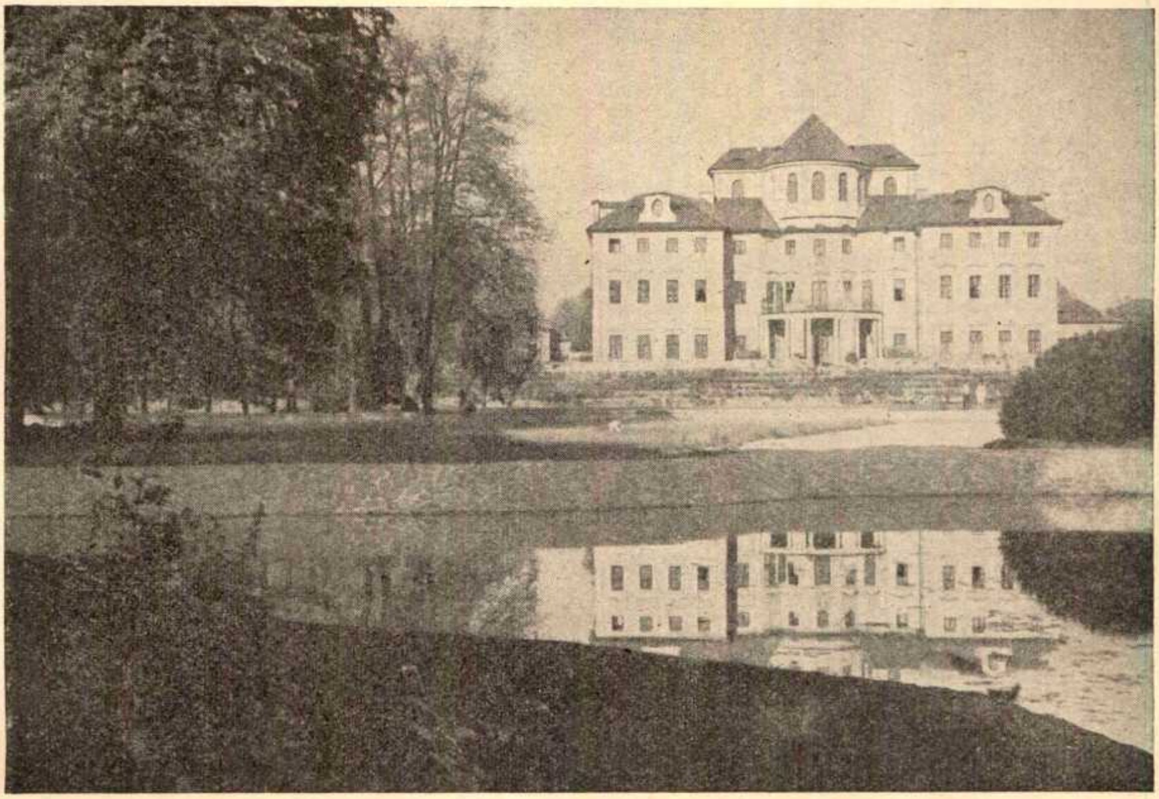

Fig. 1. The castle at Liblice which has been adapted for scientific conferences by the Czechoslovak Academy of Sciences

the deposition of particles between 0·04 and 1μ in size on individual fibres of asbestos 0·06–0·08μ thick. Spurný and Pich (Prague) had a capillary model for membrane filters which was used to explain the capture of particles.

Przyborowski (Institut für Staubforschung und radioaktive Schwebstoffe, Berlin) was concerned with the testing of filters and demonstrated the value of pre-filters in plant handling radioactive dust. Lapáček (Prague) gave an account of attempts to develop highly efficient filters capable of controlling the most dangerous radioactive substances to a level of 10^{-46} g/l. of air.

Electrostatic separation and the use of electric charges to encourage the deposition of aerosols on vegetation were subjects in the paper by Pauthenier and Ponron (Paris). The failure of electrostatic precipitators due to the build-up of an insulating layer of dust and to ionization in the deposit can now be properly accounted for. A new type of centrifugal separator was described by Binek and Pich (Prague).

A whole day was devoted to radioactive aerosols. Results of several series of fall-out measurements were given and various types of recording apparatus were described. An extremely interesting calculation of the dose absorbed, when radon decay products are inhaled was made by J. Thomas (Institute of Hygiene and Occupational Health, Prague), who made use of the best available data on lung anatomy, mucus thickness and rate of movement by ciliary action, particle deposition and the radioactive transformations, taking into account all factors which could possibly influence the dosage rate to the respiratory epithelium; his conclusions differ very much from previous ideas which have been based on global estimates rather than on an analytical approach.

The deposition of radioactivity on atmospheric particles has been examined by Lassen (Heidelberg). This process is accelerated by their electric charge to an increasing extent for particles smaller than 0·1μ. He concludes that 90–95 per cent of natural activity should be deposited on particles less than 0·5μ and 50–75 per cent on those less than 0·05μ. Experiments on the deposition of thoron decay products were described by Rüger (Institut für Staubforschung und radioaktive Schwebstoffe, Berlin).

Spendlove (Maryland, United States) directed attention to the possibility of determining the quantities of radioactive dust deposited experimentally in human subjects on the bronchial epithelium and in the alveoli by comparing the excretion-rates; extrapolated back to the time of administration these rates can be determined for gamma active particles with the subject inside a whole-body scintillation counter.

Another day was given to aerosols in meteorology. Goetz (Pasadena, United States) discussed the use of the aerosol spectrometer as an analytical tool and Mészáros (Institute of Meteorology, Budapest) noted that sodium chloride particles more than 1μ in diameter, which he had measured, could be used to identify the origin of air masses. Several speakers from the Meteorological Laboratory at Hradec Králové were concerned with atmospheric nuclei: Vrkočová and Podzimek had gelatine film methods of identifying chlorine and sulphate and membrane filter techniques for ammonium and nitrate; the latter author with Černoch surveyed flight data; Volfová and Horák found tens of thousands of Aitken nuclei per cm^3 in fogs, high concentrations which may be associated, according to Kauf (Jena), with orographical weather. Three papers dealt with meteoric dust. A gelatine film technique for measuring formaldehyde associated with aerosol particles, the first organic substance to be assessed in this way, was described by Lodge and Frank (National Center for Atmospheric Research, Colorado).

The optics of aerosol particles formed the subject of three speakers. Kubie (Institute of Physical Chemistry, Prague) gave a detailed discussion of recent developments in ultramicroscopy; Gucker, Rowell and Chiu (Indiana, United States) presented the results of Mie theory computations for spheres of dioctyl phthalate (refractive index 1·486) which are of value in determining the particle size of uniform mists from measurements of the angular intensity distributions of scattered light; the range of the parameter α (circumference of particle/wave-length) was 18·0(0·1) 23·9. Hodkinson (Safety in Mines Research Establishment, Sheffield) explained the results of his experiments on polydisperse aerosols of irregular particles for which the narrow-angle extinction coefficient can be taken as constant down to quite small sizes depending, in a simple way, on the refractive index.

Eight papers were associated with industrial aerosols and filtration and 18 with agricultural, horticultural and insecticidal applications. The final session of 11 papers dealt with medical treatment by aerosols, including an account of an ultrasonic generator which was stated to deliver into the lungs up to 1 g of material dispersed as an aerosol in 10 min. Clinical successes in this field undoubtedly require rigorous substantiation.

C. N. DAVIES

HUMAN POPULATIONS

THE three hundredth anniversary of the publication of John Graunt's *Natural and Political Observations made upon the Bills of Mortality*, the first book to be published on demography, provided the occasion for a discussion meeting organized by the Royal Society on November 15 and 16, when workers from the biological, medical and social sciences were brought together to discuss problems of common interest in the study of demography. The symposium was financially assisted by the Nuffield Foundation, which made it possible to invite a number of distinguished foreign scholars. Among those attending were Profs. R. Freedman, F. W. Notestein and E. B. Wilson from the United States, Prof. Bernardo Colombo from Italy, Mr. H. Gille from the United Nations Population Division, and Dr. J. Sutter, deputizing for M. Louis Henry of the French Institut National d'Etudes Démographiques. About fifty participants from Great Britain attended the discussion. Responsibility for the organization of the meeting was shared by Prof. D. V. Glass, Dr. P. B. Medawar and Prof. W. S. J. Morgan. The papers will afterwards be published in the *Proceedings of the Royal Society*.

The discussion began with a paper by Prof. D. V. Glass, dealing with the life and work of John Graunt. Graunt, a haberdasher by trade, was one of the earliest Fellows of the Royal Society. His election was recommended by the King himself, who particularly charged the Society "that if they found any more such tradesmen, they should be sure to admit them all, without any more ado". After sketching Graunt's life, Prof. Glass turned to an assessment of the significance of his work. He was the first to appreciate the principle of statistical regularity in demographic data; among the subjects in which he was interested were the estimation of the population of London and of Romsey, the examination of mortality differences between town and country, the case fatality of malignant fever, the investigation of infant mortality, and the introduction of the concept of the life table, though it was not until Halley wrote, some thirty years later, that the correct principles of constructing life tables were first understood. Throughout his work, Graunt was meticulous in assessing the reliability and the imperfections of his data, and his discussion of whether rickets was a new disease was a case in point. Although the generalizations he was able to make were of varying degrees of validity and universality, Graunt's work laid the foundations of demography, and of empirical work in the social sciences. His life table, though probably inaccurate at later ages, was of the right order of magnitude so far as infant and child mortality were concerned, a figure of 36 per cent dying before the age of six, being consistent with later experience of societies in a similar stage of development.

In the second paper of the session, Dr. B. Benjamin, chief statistician at the General Register Office, traced the development of the life table, and of mortality analysis in general, from Graunt through the work of Halley on deaths in the city of Breslau, to the Northampton and Carlisle tables of Price and Milne in the eighteenth and nineteenth centuries, and its full fruition in the work of William Farr, who served in the General Register Office from 1837 until 1880. Farr applied the methods of mortality analysis to the study of differentials between town and country, between healthy and unhealthy districts, and fashioned the life table into an instrument of research in the field of disease and public health. In the twentieth century came the analysis of generation mortality, following Derrick's paper, which showed a remarkable parallelism in the movement of age-specific mortality-rates among persons born in different periods.

More recently, a similar method had been applied to the mortality from lung cancer, a subject on which Mr. Beard reported in the discussion on Dr. Benjamin's paper. He found that movements in lung cancer mortality could be well described by regarding it as being made up of three components: the first depending on age, the second depending on the period of birth, which gave an indication of the proportion of smokers in the population, and a third factor depending on the intensity of smoking. This hypothesis accounted well for the different behaviour of the male and female death-rates from lung cancer.

Another contributor to the discussion, Mr. R. D. Clarke, commented on the curve of deaths, a concept first studied by Karl Pearson. He suggested that it might be fruitful to approach the analysis of mortality by regarding the maximum potential life-span of an individual as being biologically determined, and that the curve of deaths might be looked at from the point of view of determining the extent to which social and environmental factors enabled this biological potential to be realized.

The afternoon session was opened by Prof. J. N. Morris, with a comprehensive survey of recent trends in public health. He commented on public health problems in different age groups of the population, and pointed out that the declines in the infant mortality rate had been similar in different social groups. In adolescence the increased incidence of venereal disease gave cause for concern, and he showed that among younger men the number killed in motor-cycle accidents had recently experienced an alarming rise. Among the middle-aged the growing gap between the male and the female death-rate was one of the features of the present time, and coronary disease and cancer of the lung had become major public health problems. Duodenal ulcer, too, though less lethal than the former two diseases, needed investigation, but there were indications that this disease was becoming slightly less prevalent. Prof. Morris thought that there were indications that the rising trend in these diseases might not continue much further in the future. Among the elderly the problem of incapacity was becoming more serious. A figure of 10·6 per cent of males, aged 61–63, being off work for more than three months was quoted. Prof. Morris thought that it was possible that some of the survivors from previously untreatable diseases might not regain full working capacity. Among the elderly, there was considerable association between disease and air pollution—a point which was also stressed in a written contribution to the discussion by Dr. Percy Stocks. Prof. Morris concluded his paper by a plea for a closer collaboration between medicine and social statistics. In the discussion Sir Austin Bradford Hill commented on the difficulties encountered in preventive medicine to-day, as prevention of cancer of the lung, and, to some extent, of circulatory disease depended on a change in the way of life of individuals, and could not be brought about by social action, as had been the case in the reduction of mortality from infectious disease in the past.

Turning from the discussion of mortality and disease to human fertility and infertility, the next paper was contributed by Monsieur Louis Henry, who dealt with the contribution that demographers could make to the study of natural fertility. He distinguished between 'natural' and physiological fertility, the former term including certain social behaviour patterns, such as periodic prohibitions on intercourse, or length of lactation which affected fertility, but which were probably not regarded as fertility controls by individuals in these populations. Estimates of the level of natural fertility could be obtained by studying populations in undeveloped countries, or European populations living in periods before birth control had become widespread. Mr. Henry pointed to the different kinds of data that could be used for these studies, and suggested that certain researches indicated that differences in 'natural' fertility might well exist between different populations, as well as between individuals. Thus populations in the north of France seemed to be more fertile than those in the south, and this difference could be accounted for by differences in the spacing of births. Whether these in turn were due to social factors, such as longer periods of lactation, or to physiological factors, such as a longer period of post partum sterility in the southern French population, was still an open question.

The next three papers dealt with problems on the borderline of genetics and demography. Prof. L. S. Penrose dealt with genetical aspects of human infertility, Prof. K. Mather, with 'genetical demography', and Mr. J. Hajnal with concepts of random mating and the frequency of consanguineous marriages. Both Prof. Penrose and Prof. Mather stressed the difficulties of making an exact quantitative assessment of the frequency of certain genes in the population. The true frequency of lethal or sub-lethal genes was particularly difficult to determine, as normally only live births were enumerated and still-births or aborted fœtuses frequently escaped enumeration. Prof. Parkes, in a contribution to the discussion, referred to some recent work on golden hamsters, which showed that the rate of overall mortality of the fœtus within the first few days of impregnation was as high as 30 per cent. Prof. Penrose estimated that about 3 per cent of all births carried a lethal or sub-lethal gene, that a further number, probably less than 2 per cent, were viable but essentially infertile, and that between 1 and 5 per cent were viable and indirectly infertile. Prof. Mather stressed the convenience of man as a species for the study of population genetics and the importance of examining whole populations or very large samples. Penrose mentioned some conditions in which demographic behaviour patterns might influence the frequency of certain defects: thus incompatibility between mother and fœtus rarely occurred in the first-born, the incidence of mongolism was very much higher among the children of older mothers and certain other very rare genetic defects seemed to be associated with the age of the parents.

In the discussion, the problem of the negative correlation between intelligence quotient and family size was mentioned and some solutions to the apparent paradox that there was no apparent deterioration in the intelligence quotients of Scottish schoolchildren surveyed in 1932 and 1947 were put forward. The biologists tended to stress the genetic aspects of the problem: one speaker deplored the fact that in population genetics there were too many plausible explanations for the same phenomenon. Prof. Freedman said that in recent studies in the United States, provided social class was held constant, no correlation between intelligence and size of family was found. A suggestion was made in the discussion that more information of a genetic nature might be obtained in the population census, and that the Royal Society might well put pressure on the Registrar General to include questions on this topic in future censuses.

Mr. Hajnal's paper was concerned with a slightly different topic: the assessment of the frequency of cousin marriages in large populations. He showed that it was necessary to distinguish between different kinds of cousin marriage, and had constructed models showing the theoretical frequency of different kinds of cousin marriage under certain general assumptions. The theoretical frequencies were compared with some large-scale surveys and considerable agreement between theory and observation was shown.

Turning back to human demography proper, Prof. E. Grebenik and Miss G. Rowntree presented a paper dealing with the fall in the age at marriage in the population of Great Britain. Results from a recent survey showed that this fall was spread over all social groups of the population: though there seemed to be some slight indications that middle-class girls experienced a higher fall in marriage ages than others. The removal of marriages in which the bride was pregnant at marriage reduced the differentials somewhat; but left the principal conclusions unchanged.

Sir Aubrey Lewis dealt with demographic aspects of mental disorder and surveyed the statistics of different kinds of mental illness, stressing the increase in the rate for elderly persons. In the discussion, stress was laid on the considerable variation in the geographical incidence of various forms of mental disorder, and Miss E. Brooke, from the General Register Office, surveyed some of the results of the Mental Health Survey undertaken by the Registrar General, and commented on the influence of social environment on the incidence of mental disease.

Prof. R. Freedman, of Michigan University, reported on norms for family size in underdeveloped countries. He reminded the audience that all societies regarded the number of children born to a couple as important both individually and socially, and that even in undeveloped societies potential measures of control were available. Surveys asking for the ideal number of children obtained answers which tended to cluster around modal values without great dispersion. Large numbers of children were not regarded as desirable in many undeveloped societies. He then turned to the problem of changing attitudes and said that one of the most important factors in spreading smaller norms was the enlargement of the social unit within which communication normally took place. The role of education and literacy, in his view, was much more important than had hitherto been recognized.

The symposium concluded with a closing statement by Prof. F. W. Notestein. He stressed the importance of demographic research, and deplored the inadequacy of funds available for its prosecution, both on the social and on the physiological side. More money was spent on the investigation of certain very rare and esoteric diseases than on the physiology of reproduction. While the reduction of fertility in countries which are at present growing rapidly was extremely important, he thought it vital that allocations of economic aid should not be made dependent on acceptance of birth control, as had been suggested in some quarters. The desire to limit family size would have to grow within these societies and any attempt to impose it by force or by certain economic sanctions could only lead to anti-Western political feeling being associated with opposition to reduced fertility. Economic development was a necessary condition before population growth could be controlled. He felt that the symposium had served a useful purpose in bringing together scholars from different disciplines, and in showing how much each of them had to contribute to the study of human populations. The Royal Society was to be congratulated on arranging this meeting and he hoped that it would be followed by others, so that natural and social scientists would be able to collaborate in the broader study of mankind and its problems.

ADAPTIVE TEACHING MACHINES

An adaptive teaching machine is a machine that modifies its training programme to suit the student's individual requirements. Like the real-life teacher, it 'learns' about the student's difficulties and takes steps to remedy those misunderstandings which the student is unable to overcome by his own efforts. To achieve this objective, the adaptive teaching machine presents a sequence of instructional items on some suitably automated display, and a sequence of test items for establishing whether or not the instruction has been understood. Test items usually consist of questions to which the student must respond by pressing, within a given time allowance, an appropriate button or key. The working of the machine and the uses to which it may be put have been described by B. N. Lewis of Birkbeck College, London*.

By noting on internal registers the speed and accuracy with which particular items are handled, the machine can build and maintain an up-to-the-moment account of the student's strengths and weaknesses. Computations performed on these registers then enable the training programmes to be adjusted in ways that seem best suited to the student's special idiosyncrasies. In the simplest case, each register is concerned with detecting the presence of a distinct kind of misunderstanding or difficulty. Thus, if prior investigations have shown that a particular skill or subject-matter is susceptible to five basic misunderstandings (in the sense that five different 'dimensions of confusion' habitually operate to frustrate the learning process), the machine will need at least five error registers on which to evaluate the student's mistakes.

If these registers are called *A*, *B*, *C*, *D* and *E*, and the student's first mistake indicates (according to some pre-established criterion), the possibility of *A*, *B* or *E*-type misunderstanding, and if the second mistake points to misunderstanding *B* or *D*, then the two mistakes collectively suggest that misunderstanding *B* is predominant. This information enables the machine to switch to a 'correctional programme' which explicitly concentrates on the removal of *B*, while at the same time continuing to secure evidence about the other possible misunderstandings.

Alternatively, the machine could continue to accumulate evidence over a much longer sequence of tests before taking corrective action. In either case the machine must contain an overall control mechanism that scans the states of its errors registers, performs the necessary computations, and adjusts its programme according to some built-in decision rule(s). These adjustments (and the decision rules on which they are based) conform to a well-designed plan and are in no way haphazard. Without exception, they are intended to keep the level of tuition within the rather narrow limits that the student himself determines. It is, therefore, essential that instruction should be pitched at just the right level of difficulty and should be continuously adjusted as a function of the student's performance. The most direct way of adjusting difficulty is to vary the standard of the instructional items (by omitting some or modifying others), and to make correlative changes in the content of test items. But it is often more convenient to hold the instructional and test items fairly constant and to operate, on a separate display if necessary, a variable supply of supplementary information and hints. In addition, it is useful to impose limits on the time allowed for assimilating instructions and responding to test questions. The time limits can then be differentially adjusted so that longer time is allowed in respect of items that the subject is likely (according to the machine's computations) to find difficult.

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* *The Science Teacher*, 6, No. 1 (October 1962).

These two gambits have been successfully used in several commercial teaching machines of which the best known is the *SAKI* (Solatron Automatic Keyboard Instructor) device. This was pioneered by Gordon Pask for training operators on the 12-key 'Hollerith' card punch. Briefly, the system consists of a display unit that gives instructions and prompts, a keyboard which the trainee must operate (without looking at the keys), and an adaptive computer which 'senses' the characteristics of the student and adjusts the training programme to suit his needs. The display unit consists of a back-lighted exercise card (containing four lines of 24 figures) and an array of 12 lights laid out in the same spatial pattern as the 12 keys on the keyboard. Since some combinations of figures are harder to punch than others, the exercise cards are changeable and are graded with respect to difficulty.

The figures on the exercise card are illuminated one at a time (moving from left to right) and, for each figure that appears, a corresponding light shines in the 12-light array to indicate the position of the key on the real keyboard. The trainee's task is to press the appropriate key within a given allowance. If he fails to do so, the figure is automatically replaced by the next figure to the right, and the trainee is recorded as having made a mistake. During the early stages of learning the figures move on rather slowly. However, as the trainee gains in speed and accuracy the waiting times become shorter, and the prompting lights (in the 12-light array) diminish in intensity. But this is done differentially, so that the machine continues to wait longer and to give prompts on those keys which persist in giving difficulty. Furthermore, it will slow down and restore prompts in respect of any key on which the trainee has a sudden relapse. Whereas the simpler teaching machines take corrective action on the basis of just one response, *SAKI* adjusts its programme in accordance with an integrated performance measure (based on speed and accuracy) assured over a whole series of responses. *SAKI* also 'paces' the student by adjusting difficulty and time pressure so that he is always working near the limit of his ability.

This machine uses a technique that tends to minimize the amount of useful information gained about the student. For if the student were to be given easy problems that almost everyone could solve, little would be learned about him. Conversely, little is learned by loading him with problems which are beyond his capacity. An optimal 'in-between' strategy is to start with easy problems and to step up their difficulty. But this means the student is most likely to reveal his incipient weaknesses without, at the same time, wanting to withdraw from the situation. By keeping the student around the threshold of his ability, his attention tends to be completely absorbed by the instructional material presented, and this renders him controllable for teaching purposes. As a further refinement the teaching machine may conceal the fact that it has detected a weakness in the student; in this way it can continue to probe for information without losing the student's goodwill.

The teaching principles embodied in *SAKI* are entirely general. Adaptive machines have been built to instruct such diverse skills as typing, fault-finding and equipment maintenance, radar training, pattern recognition, and inductive and deductive reasoning. In every case the same dynamic relationship is established between the student and machine, so that the student is spurred on to greater and greater efficiency under optimally congenial conditions. There are theoretical reasons for supposing that no teaching method could be better than this.

THE COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION (AUSTRALIA)

THE fourteenth annual report of the Commonwealth Scientific and Industrial Research Organization (Australia)*, covering the year ended June 30, 1962, besides the general review and brief survey of progress in research, includes lists of papers published during the year and details of membership of the Advisory Council, State Committees and staff. Of total expenditure of £12,089,389, £1,122,354 was on capital works, and of £10,967,035 spent on investigations, £174,814 went to the Commonwealth Agricultural Bureaux and other outside bodies. Expenditure on the Animal Research Laboratories was £1,190,120; on plant research, £994,727; entomology, £434,183; soils research, £360,048; food preservation, £390,294; forest products research, £426,306; chemical research laboratories, £981,747; the National Standards Laboratory, £836,017; fisheries and oceanography, £257,412; building research, £208,046; radiophysics, £429,664; wool research, £711,331; land research and regional survey, £364,929; dairy research, £165,698; tribophysics, £125,988; fuel research, £299,203; and wildlife survey, £187,254. During the year, 35 junior and 41 senior postgraduate studentships were awarded and 10 overseas studentships. The 210-ft. radio-telescope for the Division of Radiophysics was officially commissioned on October 31, 1961.

Among the legumes and grasses of value to the grass industry as a result of the work of the Tropical Pastures Division are *Lotononis bainesii*, *Siratro* and *Nunbank* buffel grass, while 100 per cent prevention of *Phalaris* staggers was obtained by administration of heavy cobalt pellets along with steel grinders to cattle grazing on *Phalaris tuberosa*. A method of soil analysis has been developed to give a direct assessment of the phosphate fertilizer requirements of wheat for the most economic return, and field experiments indicate that the average rate of application of superphosphate in the southern wheat belt of New South Wales could be increased to give returns of

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* Commonwealth Scientific and Industrial Research Organization, Australia. Fourteenth Annual Report, 1961–62. Pp. iv+153+10 plates. (Melbourne and Sydney: Commonwealth Scientific and Industrial Research Organization, 1962).

£2–3 per acre. A broad-scale field survey of the Tipperary region in the Northern Territory was completed during the year, and inoculation of pelleted seed with very high numbers of nodule bacteria by a multi-step process has produced up to 100 per cent nodulation of subterranean and crimson clovers sown on skeletal hill soils where the standard commercial inoculation is without effect. A technique has been developed permitting separation of composite particles of silica from a plant without damaging the structure or altering the composition of the particles; detailed studies of the silica from the vegetative and reproductive parts of the mature oat have revealed that the walls of all types of epidermal cells except cork cells are impregnated with silica.

Studies with radioactive phosphorus have shown that roots of *Pinus radiata* infected with mycorrhizal fungi take up larger amounts of nutrient and suggest that particularly efficient fungi can be selected for inoculating forestry nurseries. Evidence has been obtained that the cellular organelles (mitochondria) associated with the production of adenosine triphosphate in plant tissues of fruits become heavily labelled by the methyl group of methionine, mostly in the lipid fraction, and, in particular, plant sterols and ubiquinone have been isolated and found to be radioactive. The natural enzymatic formation of iron-porphyrin complexes is being investigated, and the structure of the anti-fungal compound, pisatin, isolated from infected peas, has been established as 3-hydroxypterocarpin.

As little as 0·01 per cent of decontaminating proteins have been detected in the protein hormones that control wool growth by zone electrophoresis on starch gel, and the breakdown of metabolic efficiency incurred by vitamin-B_{12} deficiency in sheep has been located in the chemical transformation through which propionic acid is launched into the energy-producing cycle, that is, in the isomeric conversion of the intermediate methylmalonic acid to succinic acid. An investigation of the pyrrolizidone alkaloids occurring in plants indicates that those causing chronic or acute liver damage when eaten by animals have similarities in the structure of their molecules and that their effects on the cell nuclei resemble the effects of the so-called biological alkylating agents. An investigation of the flight behaviour of the Australian plague locust early in 1962 indicated that the occurrence of numerous flying swarms in the 'outbreak areas' is not necessarily a prelude to a widespread outbreak.

A laboratory has been established at Canberra, where the alarm and distress signals of several species of birds have been recorded and prepared for analysis, and where aggressive, territorial, food finding, mating and other calls are being studied in relation to the behaviour and social organization of birds. An infra-red design of a single-effect solar still with a plastic base, producing about 10 gal. of distilled water on a clear summer day, at an estimated cost of £1 per 1,000 gal., is undergoing tests and a prototype for use in isolated areas is being built. The illumination of pedestrian crossings by floodlighting the pedestrians from the directions of the approaching traffic streams has been successfully tested in two experimental installations. Four protein preparations have now been extracted from wool fibre; the two with a high content of sulphur have much smaller molecules than the two with a low content of sulphur and are basic in nature in the original fibre, whereas the low-sulphur proteins are acidic. Ultra-violet radiation in sunlight splits the disulphide bonds of cystine, and chemical investigations of pure cystine suggest that, in presence of air, it is the bond between the carbon and sulphur atoms that is split in neutral and alkaline solutions, whereas in acid solutions the bond between the two sulphur atoms is split.

By spray drying an emulsion of butter fat in a solution of sodium caseinate and non-fat milk solids a new dairy product, powdered butter, has been produced, containing 80 per cent of fat with 15 per cent of milk protein and some mineral salts, which is more convenient than normal butter for use in cakes, frying batter and ice-cream. A long-term investigation is in progress of the physical factors important in bulk wheat storage, particularly heat and moisture transfers within the bulk. A new humidity-sensitive element, consisting essentially of a thin layer of selenium dioxide, the electrical resistance of which responds to changes in the humidity of the surrounding atmosphere, has been developed with rapid response and good accuracy even at more than 90 per cent relative humidity and near the freezing point. Completion of the first survey of the distribution of ozone in the southern hemisphere has shown that the distribution differs significantly from that in the northern hemisphere. Mathematical methods for removing the effect of the spread of energy and greatly reducing the random scatter in ionization investigations have been devised by utilizing certain redundancies in the measured data. An instrument has been devised which measures simultaneously the atmospheric distortion in measurements of the total light from a star, its position and its image.

UNIVERSITY DEVELOPMENT IN INDIA

THE Statistical Report, 1961–62, on *University Development in India**, prepared by the Statistics Section of the University Grants Commission, New Delhi, is based on returns received from the 53 universities recognized in July 1962; of these 15 are unitary, 2 federal and 36 affiliating, while there are 5 other institutions, including the Indian Agricultural Research Institute, New Delhi, and the Indian Institute of Science, Bangalore, deemed to be universities under the University Grants Commission Act of 1956. The number of teaching departments was 904, including 233 in science, 80 in medicine, 33 in engineering and 17 in technology, and the total number of students enrolled was 980,380, of whom 841,580 were in the 1,676 affiliated colleges and 138,800 in the teaching departments: there were also 169,627 women students; these totals represent increases of 13 per cent and 12 per cent, respectively, on the number of students in 1960–61. The Universities of Agra, Bombay, Calcutta, Kerale, Madras and the Punjab together account for 40·1 per cent of the total compared with 41·8 per cent in 1960–61, and there are now 6 universities instead of 2 with less than 1,000 students.

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* *University Development in India*: A Statistical Report, 1961–62. Pp. vii + 76. (New Delhi: University Grants Commission, 1962.) Rs. 3.50 nP.; 5s.

The total teaching staff was 54,853, together with 8,200 tutors and demonstrators, giving a staff–student ratio of 1 : 15·5; slight improvement is noted at Agra, Calcutta and the Punjab, but at Madras and Kerale the ratio has worsened. Students residing in halls of residence numbered 178,423, or 18·2 per cent compared with 17·6 per cent the previous year. Some 72,859 students were engaged in postgraduate/research work compared with 63,000 the previous year. Of the postgraduate students 14,511 were in science, with a further 1,876 engaged in research, but for engineering and technology the corresponding figures were only 418 and 29 and 243 and 17, respectively, and in medicine, 1,841 and 46. Besides an analysis of enrolment trends generally the report reviews the estimates for the Third Year Plan in comparison with the Second Year Plan, which sets a target of 1,300,000 students in 58 universities in 1965–66 and is expected to provide 21,000 postgraduates in science, 100,000 engineers and technologists, and some 32,000 in agriculture and allied sciences. Expenditure on research is estimated at 130,000 crores of rupees, compared with 7,200 under the Second Plan.

DEMAGNETIZING FIELDS OF INHOMOGENEOUS INDUCED MAGNETIZATION

By Dr. ANDREAS VOGEL

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THE induced magnetization of bodies with constant susceptibility and homogeneous magnetization is described by the following equation:

$$J_e = J_0 - \varkappa N J_e \qquad (1)$$

when J_e = effective magnetization; J_0 = primary magnetization; $\varkappa$ = susceptibility; N = demagnetization factor.

Based on the effect of free magnetic poles on the surface of magnetized bodies N has only been calcul-

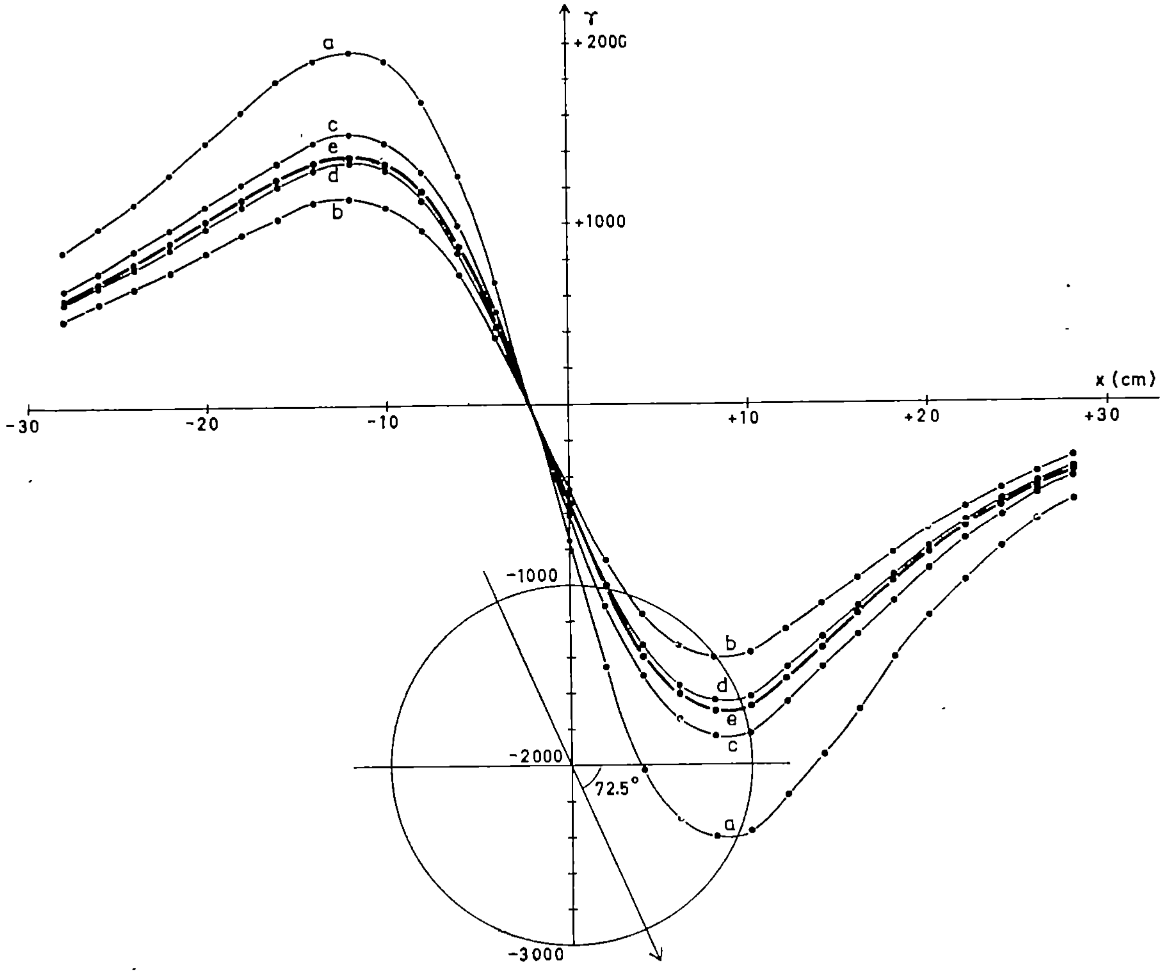

Fig. 1

ated for special bodies. It has been experimentally proved, however, that this formula can only be applied to bodies of second degree surface lying in a homogeneous magnetizing field.

In this case the demagnetizing field is opposite to the definite magnetization so it can be replaced by the factor N. In the case of inhomogeneous magnetization the demagnetization has to be expressed by a vector.

Here it will be shown in which way the definitive magnetization for any bodies can be established. This method is based on iterative calculation of magnetizing fields inside the body. The field beyond a body with the magnetization $J_0 = H_0\varkappa$, where J_0 is the primary magnetization, H_0 the magnetizing field and $\varkappa$ the susceptibility of the body, can be derived from the potential:

$$V = \int \left(J_{0x}\frac{\partial}{\partial_x} + J_{0y}\frac{\partial}{\partial_y} + J_{0z}\frac{\partial}{\partial_z} \right)\frac{1}{r}\,dx\,dy\,dz \qquad (2)$$

J_{0x}, J_{0y}, J_{0z} are the components of J_0 and r is the distance from the element $dx\,dy\,dz$.

This function, when applied inside the body, gives after derivation the inner field H_1 due to the primary magnetization J_0. The field H_1 causes a magnetization J_1. The field which comes from J_1 by integration with equation (2) is H_2 and the magnetization due to H_2 is J_2, etc. The effective magnetization will be the sum of the magnetization fields:

$$J_e = \varkappa(H_0 + H_1 + H_2 + \ldots\ldots) \qquad (3)$$

This method of iterative calculation of magnetization fields can be illustrated by taking the simple case of a homogeneous magnetized sphere. The susceptibility of the sphere may be $\varkappa$ and the magnetizing field H_0. The inner field due to this magnetization is $-\left(\frac{4}{3}\pi\varkappa H_0\right)$, the magnetization by this field is $\varkappa\left(\frac{4}{3}\pi\varkappa H_0\right)$. This magnetization gives an inner field with the intensity $+\left(\frac{4}{3}\pi\varkappa\right)^2 H_0$ and the magnetization $+\varkappa\left(\frac{4}{3}\pi\varkappa\right)^2 H_0$, etc.

The sum of these magnetizations gives the effective magnetization:

$$J_e = \varkappa\left[H_0 - \frac{4}{3}\pi\varkappa H_0 + \left(\frac{4}{3}\pi\varkappa\right)^2 H_0 - \ldots\ldots\right] \qquad (4)$$

If J_e is replaced by $H_0\varkappa_e$, where $\varkappa_e$ is the effective susceptibility, we get after division by H_0:

$$\varkappa_e = \varkappa\left[1 - \frac{4}{3}\pi\varkappa + \left(\frac{4}{3}\pi\varkappa\right)^2 - \ldots\ldots\right] \qquad (5)$$

Formula (1) can be written:

$$\varkappa_e = \frac{\varkappa}{(1 + N\varkappa)} \qquad (6)$$

Or if we replace N by the demagnetization factor of the sphere $\frac{4}{3}\pi$:

$$\varkappa_e = \frac{\varkappa}{\left(1 + \frac{4}{3}\pi\varkappa\right)} \qquad (7)$$

For the case of a sphere the iterative field computation, equation (5), gives a formula which is mathematically a series of equation (7). It can easily be shown that for homogeneously magnetized

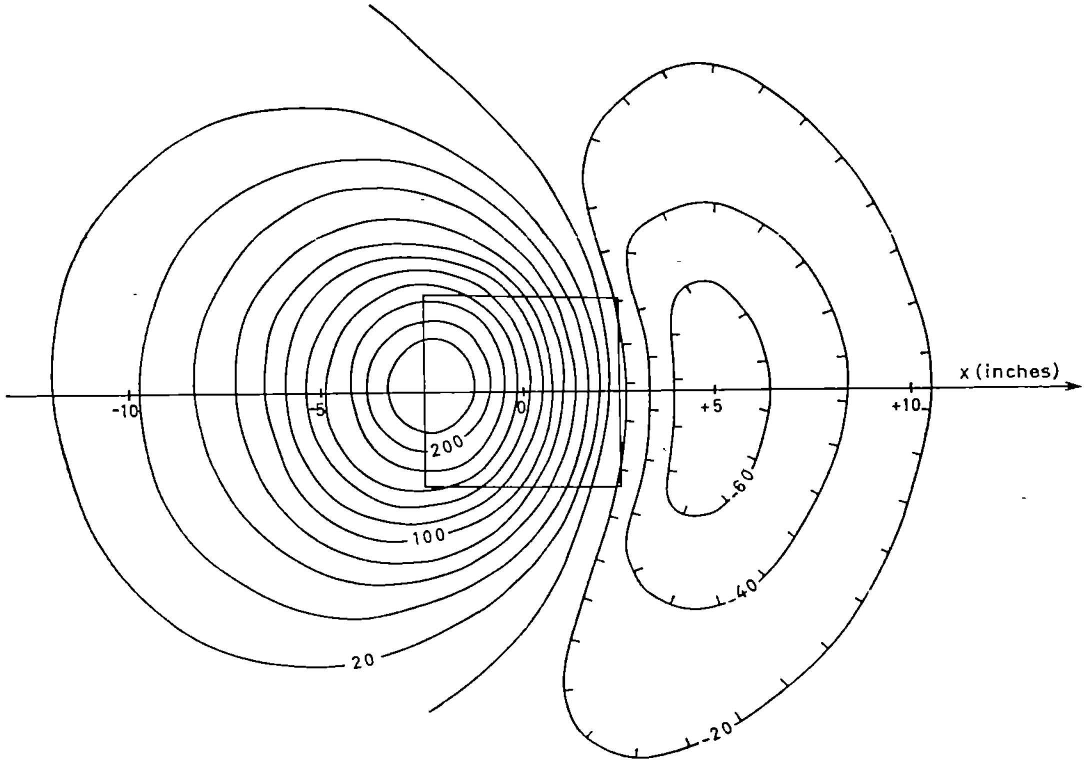

Fig. 2

bodies we always get identical results with equation (6), which has been proved by experiment.

Equation (4) can be written generally for all homogeneously magnetized bodies:

$$J_e = \varkappa[H_0 - N\varkappa H_0 + (N\varkappa^2)H_0 - \ldots\ldots] \quad (8)$$

The example of a sphere with the susceptibility $\varkappa = 0{\cdot}1$ in a field with intensity $H_{0x} = 15{,}000\gamma$ and $H_{0z} = 47{,}500\gamma$ demonstrates practically the way in which the sum of magnetizing fields converges towards the definite magnetization.

Fig. 1 represents the relative horizontal intensity of this sphere with radius $r = 10$ cm in a horizontal profile at a distance of 20 cm from the centre of the sphere.

The curve *a* is the horizontal intensity for the effective magnetization derived from equation (1); the curve *b* is based on the primary magnetization $J_0 = H_0\varkappa$ without considering demagnetization. The curve *c* is based on the magnetization $(H_0 + H_1)\varkappa$, curve *d* on $(H_0 + H_1 + H_2)\varkappa$ and *e* on $(H_0 + H_1 + H_2 + H_3)\varkappa$.

Now let us proceed to a body which is not magnetized homogeneously in a homogeneous field.

Hendersson and Zietz[2] have measured in the laboratory the total field of a horizontal plate with the dimensions 5 × 5 × 0·5 in. in a plane 5 in. above the plate. The magnetizing field was $H = 53{,}400\gamma$, the inclination 60° and the susceptibility of the plate $\varkappa = 0{\cdot}066$. The results are shown in Fig. 2. From these values in the plane the mid-section in north–south direction was taken and the relative total intensity for the same profile was calculated by me.

Before starting the calculation of inner inhomogeneous fields with computers the body has to be divided into elements. The accuracy in the calculation of magnetization increases when the elements are diminished. For the centre of each element the field vector due to the magnetization of all elements of the body has to be calculated. The field calculations of different order were made in 32 points in the plate's interior. In order to avoid too long computing time, when making this first trial, mean values of the components of H from all points were taken starting with H_1. The magnetization from these mean values of H was always taken for the field calculations, which were continued to that order which gave less than 1/100 of the primary field.

In this way the following magnetizing field components were calculated:

$H_{0x} = +\,0{\cdot}26700\gamma$; $H_{1x} = -\,0{\cdot}01650\gamma$.

$H_{0z} = -\,0{\cdot}46250\gamma$; $H_{1z} = +\,0{\cdot}20572\gamma$; $H_{2z} = -\,0{\cdot}08879\gamma$; $H_{3z} = +\,0{\cdot}03836\gamma$; $H_{4z} = -\,0{\cdot}01657\gamma$; $H_{5z} = +\,0{\cdot}00716\gamma$; $H_{6z} = -\,0{\cdot}00309\gamma$.

$\Sigma H_x = +\,0{\cdot}25050\gamma$; $\Sigma H_z = -\,0{\cdot}31971\gamma$.

The effective magnetization is $\varkappa\Sigma H$:

$$J_x = 0{\cdot}0165 \text{ c.g.s.}; \; J_z = 0{\cdot}0211 \text{ c.g.s.}$$

Integration over the whole plate with the above magnetization gives the outer field. For this field in Fig. 3, curve *c* demonstrates the relative total intensity on the same profile as that which has been taken from the measurements of Hendersson and Zietz (curve *b* is the same profile without regarding the demagnetization).

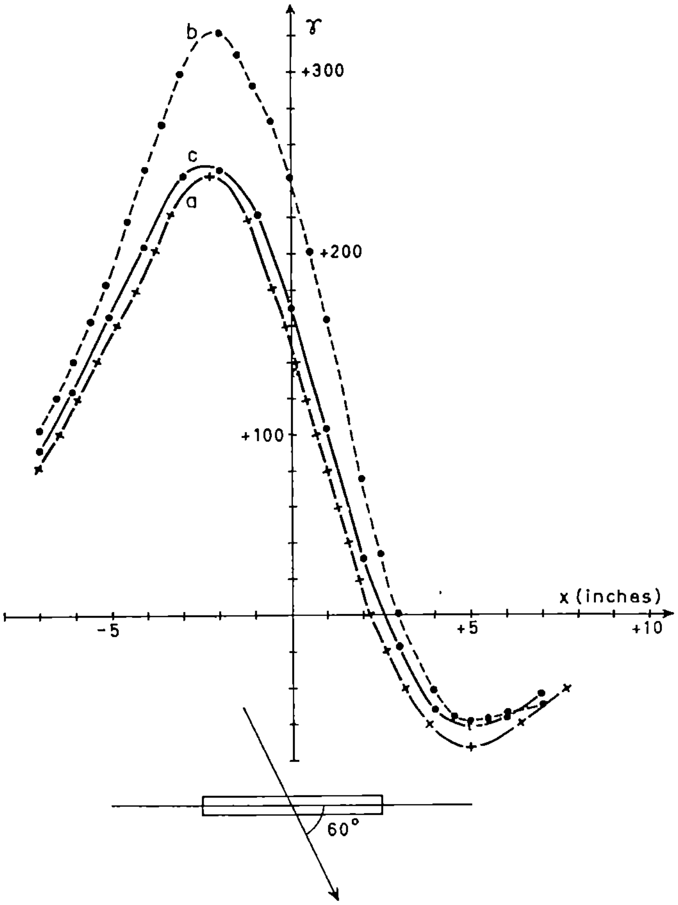

Fig. 3

The graph *c* is, with small deviations, parallel to *a*, the measurements of Hendersson and Zietz. The parallel displacement in vertical direction of about 12γ can only be explained by the fact that the measured total intensity has been reduced to a normal field which is some gammas too high.

When shifting the measured graph by + 12γ the deviation of the two graphs will be less than 2 per cent of the whole amplitude. This error comes mainly from approximation: the number of the points in which the field calculations had been made inside the body is not infinite and in the process of successive calculation the mean value of magnetization has been taken when proceeding to the next order field.

In comparison with the earlier used methods for calculation of demagnetization, this iterative calculation of inner fields is not restricted to special cases or bodies. The theory which has been developed here makes it possible to calculate the demagnetization, that is, the effective magnetization of all bodies however complicated geometrically, when magnetized in a field with given distribution.

I thank Dr. K. Arle from the Swedish Board of Computing Machinery, Stockholm, for her assistance in the programming.

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AURAL RECOGNITION TIME FOR MULTI-DIMENSIONAL SIGNALS

By DR. P. W. NYE
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IT is now well known from the work of Pollack[1] Pollack and Ficks[2], Garner[3], Hayes[4], Eriksen[5], and others that the information per symbol transmitted to a subject can be increased if a multi-dimensional display is utilized. In the majority of these experiments the subjects carried out the discriminations by a check list procedure and no time limits were imposed. It was possible, therefore, that the higher information transfer could be achieved at the expense of time by storing the signal in the immediate memory, or S system, and carrying out serial discriminations on dimensions in the P system (Broadbent[6]). However, the experiment described here indicates that, provided sufficient practice of the association between a multi-dimensional signal and its response is received, the discriminations of these signals can be made at least as rapidly as well as more accurately than corresponding discriminations of signals containing fewer dimensions.

Two codes were constructed (Fig. 1) using the dimensions of frequency, intensity, modulation, noise content and direction, being similar to those used by some earlier workers[2,7]. The intensity-levels used in the $5D$ code were 0 db. and -12 db., and those for the $3D$ code were 0 db., -10 db. and -20 db.; the maximum intensity-level, zero decibels, was adjusted arbitrarily by each subject. All signals were tape-recorded giving a noise-level at about -40 db. relative to the maximum possible signal intensity. The noise dimension was achieved by the addition of a separate noise source raising the background noise level to -12 db. Double side-band modulation giving either a square or sinusoidal envelope formed two states of the modulation dimension while the third was an unmodulated pure wave-form.

5 DIMENSIONAL CODE

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
DIRECTION	R	L	R	R	L	R	R	L	R	L	R	L	R	R	L	R	R	L	R	R	L	R	L	R	L	R
NOISE	+	-		+	-		+	-		+		-		+	-		+	-		+	-		+		-	
MODULATION	+			-			+			-				+			-			+			-			
INTENSITY	H						L							H						L						
FREQUENCY	H (2000 cps)													L (480 cps)												

3 DIMENSIONAL CODE

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
MODULATION	x	y	z	x	y	z	x	y	z	x	y	z	x	y	z	x	y	z	x	y	z	x	y	z	x	y
INTENSITY	H			M			L			H			M			L			H			M			L	
FREQUENCY	H (3000 cps)									M (1200 cps)									L (480 cps)							

KEY

R	RIGHT	M	MEDIUM
L	LEFT	L	LOW
+	PRESENT	x	ABSENT
-	ABSENT	y	SINE
H	HIGH	z	SQUARE

Fig. 1. Structure of the two codes. 'Direction' indicates that sound originated from left or right earphone

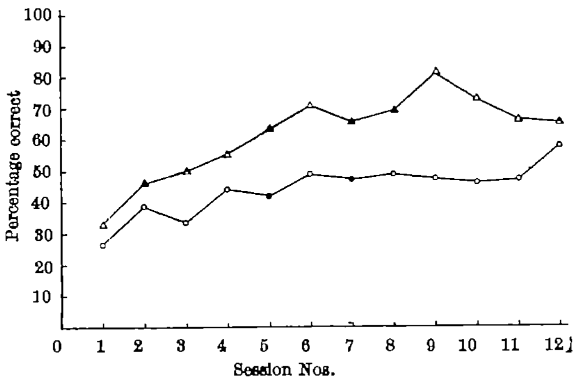

Fig. 2. Learning curves for the two groups of subjects using the full alphabet. ○, $3D$; △, $5D$

Two groups of four subjects, aged 18–25, were recruited and matched for academic ability. Each group began a course of training at letter recognition using one of the two codes. The training-method involved the division of the alphabet into three approximately equal sections which were learnt separately, the transition to the next being made when the slowest member of the two groups had reached the 60 per cent correct level. When each section had been studied in this way the training on the complete alphabet was begun. The average performance of the two groups is shown in Fig. 2 for the nine sessions immediately prior to the final testing and also of the three final test sessions. Each session lasted for 15–20 min and consisted of: (a) earphone balance and adjustment; (b) a short refresher course; (c) practice under test conditions; (d) test recognition of forty symbols. A total of 5 h of subject participation was accumulated from regular sessions during a period of nearly two months. The signals were always 0·6 sec in length and were followed by a 3-sec interval during which the subject made his verbal response. The time was measured from the beginning of the signal to the beginning of the response in each case for a total of 120 symbols selected randomly from the full alphabet. All these

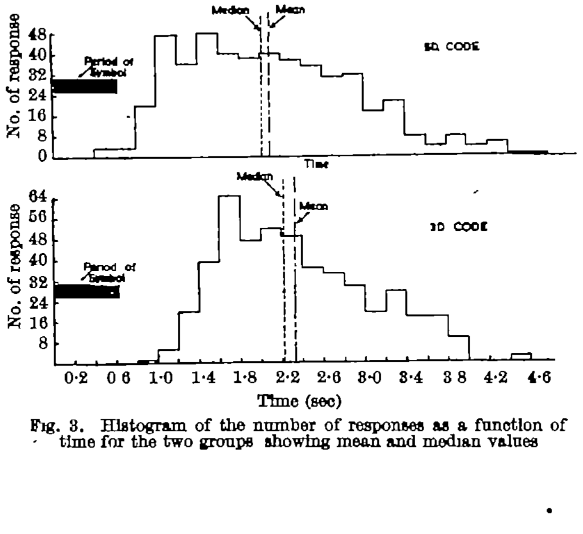

Fig. 3. Histogram of the number of responses as a function of time for the two groups showing mean and median values

Table 1

Group	Subject	Variance	Mean	Group variance	Group mean	't'
3*D*	K. F.	0·84	2·24	0·48	2·34	
	A. C.	0·25	1·95			
	M. W.	0·39	2·53			
	P. P.	0·61	2·63			4·9
5*D*	R. C.-H.	0·18	1·32	0·64	2·10	
	J. K.	0·22	2·16			
	R. H.	0·57	2·72			
	K. H.	0·61	2·18			

Table 2

Group	Dimensions	Input information	Transmitted information
3*D*	Frequency	1·58 bits	0·74 bits
	Intensity	1·58 bits	0·43 bits
	Modulation	1·58 bits	0·81 bits
5*D*	Frequency	1·00 bits	0·47 bits
	Intensity	1·00 bits	0·55 bits
	Modulation	1·00 bits	0·55 bits
	Noise	0·93 bits	0·65 bits
	Direction	0·97 bits	0·61 bits

results have been histogrammed in Fig. 3. The distributions are not significantly changed if only the correct responses are considered. The complete set of response times were therefore pooled for each subject and group and the mean and variances calculated in Table 1.

An information analysis[8] of the three test sessions revealed an average transmission of 3·30 bits/symbol from a possible 4·57 bits/symbol for the 5*D* code compared with 2·60 bits/symbol from a possible 4·62 bits/symbol for the 3*D* code. The information contributed by individual dimensions is shown in Table 2.

The average response time to the 5*D* code was shorter by 0·24 sec than the corresponding time for the 3*D* code, this difference being significant to better than the 0·001 level of probability. However, an insufficient number of subjects were available to permit the elimination of individual variations from the results and hence it is not possible to show directly that the difference between the performance of the two groups did not arise from individual variations. Nevertheless, there is no indication that the 5*D* code gives the slower performance expected from a 'check list' discrimination procedure.

The five dimensional symbols were also identified more accurately. The plateau in the learning curve of Fig. 2 for the 5*D* code occurs at 70 per cent while the corresponding plateau for the 3*D* code is at 49 per cent.

Hick's[9] results indicate that in a signal-response situation, speed and accuracy can be exchanged, and if, therefore, the amount of information transmitted by the two codes were made equal, response times to the 5*D* code could be decreased by an amount proportional to the difference in information transmitted by the two codes (that is, α (3·30–2·60) bits). This would result in a further increase of the difference in response time between the two codes.

Pollack and Ficks[2] and Miller[10] have discussed the significance of multi-dimensional signalling for the study of language structure. Speech can be regarded as a multi-dimensional display medium, although the dimensions of speech are certainly not those of Pollack and Ficks's experiments and are more likely to resemble the discriminant features of Cherry, Halle and Jakobson[11]. It has previously been uncertain, however, whether the multi-dimensional structure of speech is the significant factor on which the speed of recognition depends. The present results indicate a confirmation of the role of dimensionality in speech and suggest the conclusion that when rapid discriminations are demanded it is the capacity of the discrimination or *P* system that is ultimately dependent on the dimensionality of the input signals.

This work is part of a research programme of the St. Dunstans Scientific Committee and is published with its permission. I thank the Director of the National Physical Laboratory and the Superintendent of Autonomics Division for making available facilities for this work. I also thank Mr. R. Rengger for assistance in the experimental work.

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TERTIARY MAMMALIAN FAUNAS AND SEDIMENTS IN KARAMOJA AND KAVIRONDO, EAST AFRICA

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IN August and September 1961 we investigated a series of fossiliferous strata occurring at or near the base of deposits associated with several Tertiary volcanic centres in East Africa. The areas visited in Uganda were south-west of the dissected volcanic cone of Napak (34° 17′ E., 2° 5′ N.), and to the north of Moroto Mountain (34° 45′ E., 2° 32′ N.). In Kenya, the principal localities were at Songhor (35° 13′ E., 0° 3′ S.), Koru (35° 16′ E., 0° 11′ S.) and Fort Ternan (35° 20′ E., 0° 14′ S.), situated to the west and south of the volcanic centre of Tinderet. Brief visits were also made to the sites of Ombo and Mariwa twelve miles west of Kisumu[1].

At Napak, in addition to the sites (Napal I–III) previously described by Bishop[2,3], four new faunal localities were investigated (Napak IV, VI, VII and VIII) which were discovered in 1960 by Dr. A. F. Trendall of the Geological Survey of Uganda during routine sheet mapping. We were fortunate in finding another new site (Napak V) during the expedition.

The first fossils from the vicinity of Moroto Mountain (site Moroto I) were found in 1959 by J. G. Wilson of the Uganda Department of Agriculture, who also discovered the original Napak site, and who kindly described to us the location of his new discovery[4]. Again we were fortunate in finding a second site two miles farther north (Moroto II).

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We thank Dr. L. S. B. Leakey, who kindly invited us to make observations on the geology of the recently discovered Lower Pliocene or Late Miocene site at Fort Ternan[5].

Geological mapping was undertaken at each of the fossil localities using air-photographs as base-maps. A level and staff were used in conjunction with a pair of surveying aneroids to construct levelled profiles on which geological observations were plotted. In some instances additional large-scale maps were prepared by use of a plane-table. The main object of the work was to study the nature of the sediments in relation to the total fauna at each of the fossiliferous localities. Palæo-ecological reconstructions could then be attempted as a background against which to assess observed differences in preservation or in the composition of the various faunal assemblages.

Sediments

The Koru, Songhor, Ombo and Mariwa sites, like those of Karungu, Rusinga and Mfwanganu associated with the Rangwa volcano, have been known for many years, and their faunas are well documented as a result of various British–Kenya Miocene Expeditions[6]. Their geology has been described by Kent[7], Shackleton[8] and officers of the Kenya Geological Survey[9–11]. Although the detailed observations, sections and maps made in all the areas visited during the recent expedition will be published later, it is proposed to outline here preliminary notes on the new exposures at Napak, Moroto and Fort Ternan.

Napak. The eight fossil sites can be broadly divided into two groups on the basis of their lithology: (1) Sands, gravels and tuffs occurring at the base of the volcanics and resting directly on the basement rocks (Sites II, III, VI, VII and VIII); (2) bedded tuffs and agglomerates within the volcanic pile, and some 500 ft. above the basement surface (Sites I, IV and V).

The basal sediments are principally coarse sands, grits and gravels with abundant basement rock fragments, and a few tuffaceous horizons. They appear to be of fluviatile origin and were formed before and during the earliest stages of the volcanic activity. They resemble closely the similarly situated deposits at Moroto which are described here.

The higher sites within the pyroclastic sequence are situated as described by Bishop in the original note on Napak I (refs. 2 and 3), and recently they have been mapped more extensively by Trendall[12]. The fossiliferous series occurs between two coarse agglomerate horizons, and consists of 130 ft. of predominantly grey horizontally bedded fine to coarse tuffs. These are composed largely of volcanic rock fragments and individual crystals, with a calcite cement. Some layers are rich in biotite, and several of the horizons can be classified as crystal-tuffs. Reddened zones and layers of concretionary calcareous nodules suggest time-gaps, as does the abundance of fossil wood at certain levels. However, no definite weathered profiles were observed on top of any particular bed. The deposits mainly represent sub-aerial accumulations of primary volcanic detritus, perhaps locally sorted by water in the form of surface run-off.

Moroto. At the two Moroto localities, which are some 55 miles north-east of the Napak sites and approximately 12 miles north-west of the summit of Moroto Mountain, the fossil-bearing sediments are grits and gravels resting directly on steeply dipping basement gneisses. The deposits appear to be largely fluviatile, and they infill valleys up to 300 ft. deep, incised into the basement surface. Most of the fragments are derived from the underlying gneisses; but a few of volcanic origin are also present. The fossiliferous horizons in the grits have a calcareous cement.

At both localities, the sediments are overlain by olivine–basalt flows which are fresh in the centre but have rotten vesicular tops and bottoms. The total thickness of sediment exposed at Moroto I is only 12 ft. At Moroto II, up to 80 ft. of sediments were seen but they were only locally fossiliferous. At the

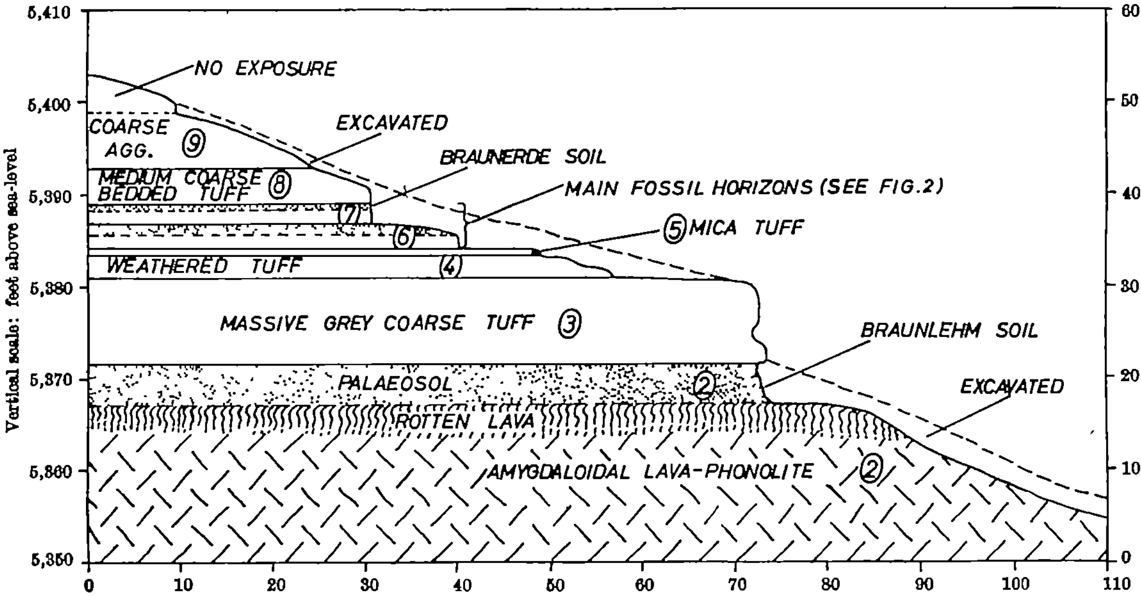

Fig. 1. Levelled section across the Fort Ternan excavation showing pyroclastics (left blank), lava (crossed ornament) and palæosol horizons (stipple)

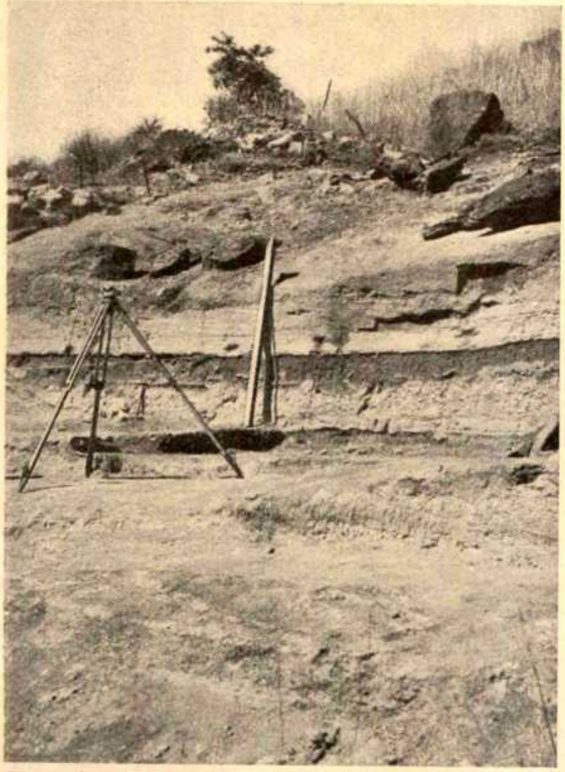

Fig. 2. Detail of the upper section of the Fort Ternan excavation. The tripod stands on grey calcareous crystal tuff (Bed 5). The weathered top of Bed 6, fossiliferous tuff, is seen below the base of the levelling staff while the geological hammer rests beside fossil mammalian bones *in situ* in the coarse tuff of Bed 7. This bed has a pronounced palæosol (dark horizon above hammer haft), developed at the top of it which contrasts sharply with the fresh bedded calcareous tuff of Bed 8. The blocks on the hill slope are derived from the upper phonolite (Bed 11)

latter site, the present-day stream is etching out a pre-existing valley cut in basement complex gneisses.

Fort Ternan. The site at Fort Ternan was referred to recently by Dr. L. S. B. Leakey[5], who published our levelled profile through the deposits as his Fig. 1 (p. 691). However, this diagram was not accompanied by details of the succession. The following is the sequence exposed immediately above and below the principal fossiliferous horizons. The numbers of the beds refer to those on the previously published section and to Fig. 1 of this article.

Bed No.			Thickness (ft.)
11	Phonolite lava (Losuguta type)	seen to	20·0+
	no exposure, about 40 ft.		
10	Hard grey tuff	seen to	3·0
	no exposure, about 10 ft.		
9	Very coarse agglomerate	seen to	5·0
8	Medium to coarse bedded tuff with agglomeratic horizons		4·5
7	Grey coarse tuff (fossiliferous) with weathered top		2·5
6	Grey medium tuff (fossiliferous) with weathered top		2·0
5	Grey medium calcareous crystal-tuff		0·5
4	Grey medium tuff, deeply weathered		2·5
3	Massive grey coarse tuff		9·0
2	Rotten amygdaloidal lava (Phonolite—? Losuguta type) with deeply weathered upper surface		50·0
1	Massively bedded very coarse agglomerate	seen to	60·0

The beds exhibit a gentle dip of 1 in 25 to the north, and have suffered faulting locally. With the exception of the lavas, all the beds are stratified tuffs of variable grade, some reaching the coarseness of agglomerate. Preliminary examination of these deposits shows that many have a matrix of finely disseminated volcanic dust while others have a calcite cement. The fragments are mainly of local volcanic rocks and include many individual crystals with Bed 5 essentially a crystal-tuff.

Although local wash-outs and the effects of water-sorting, probably the result of rain-wash on the freshly deposited ash slopes, can be seen on some of the horizons, the majority of the beds are sub-aerial accumulations of original primary volcanic ash.

Fig. 1 shows a detailed section through the beds exposed by Dr. Leakey's excavation, from the top of Bed 2 to the base of Bed 9. In this sequence weathered profiles can be seen developed on the lower lava and on several of the overlying pyroclastic horizons (Fig. 2). Dr. I. W. Cornwall has made a preliminary examination of material from two of the weathered profiles. We are indebted to him for the information that three samples spanning the weathered top of Bed 2 reveal it to be a palæosol of Braunlehm type, while two from the top of Bed 7 suggest a palæosol with Braunerde characteristics.

It seems probable that when large-scale excavations are carried out at Napak, palæosols will be found similar to those at Fort Ternan. Deep cuts are necessary before the palæosols can be seen clearly, as near the surface they merge into the zone of present-day weathering and slope creep.

The primary and sub-aerial nature of the Fort Ternan deposits is clear, and almost identical lithologies argue similar environments at Napak and Songhor. It is of interest to note the closely comparable sequence of numerous primary crystal-tuffs with soil profiles recently referred to by Curtis and Evernden as occurring in the Lower Pleistocene Bed I of the Olduvai Gorge section in Tanganyika[13].

From considerations of their petrology and altitude (5,350–5,470 ft.), the Fort Ternan lavas and pyroclastics fit into the sequence of strata outlined by Binge[11]. They represent horizons near the base of his Kericho phonolites (Tvp_1) which were derived from the south and are intercalated with the upper layers of his tuffs and agglomerates (Tmf) which had their origin from Tinderet to the north.

Although no fauna was excavated by us from Fort Ternan, a potassium-40/argon-40 date of about 14 million years has been published[5]. The tuffs and agglomerates, however, also include near their base the typical Lower Miocene assemblage of Songhor (4,420–4,490 ft.) and they immediately overlie fossiliferous beds of this same age at Koru (4,750–4,800 ft.). This suggests a prolonged 'Miocene' history for a largely ash cone on the site of Tinderet. A similar

Table 1. SUB-AERIAL PYROCLASTIC DEPOSITS

	1958	Napak 1961			Total	Songhor 1961	
	Napak I	Napak I	Napak IV	Napak V	Napak upper level 1958 and 1961	Songhor I	Songhor II
Total	1,112	365	185	372	2,034	395	78
Unidentifiable bone fragments	778	210	77	244	1,309	167	37
'Better' bones	188	100	53	72	413	131	21
Skulls, teeth and jaws	146	55	55	56	312	97	20
Details of skulls, teeth and jaws							
Rodent	33	32	46	22	133	79	17
Mastodon	84	8	—	—	92	—	—
Others	29	15	9	34	87	18	3
Details of 'Other' skulls, teeth and jaws							
Ruminant	2	2	2	16	22	11	1
Primate	5	3	2	8	18	3	—
Carnivore	7	2	2	—	11	—	1
Suid	2	3	1	1	7	1	—
Remainder (Chalicothere, Rhino, Insectivore, etc.)	13	5	2	9	29	3	1

situation with a greater amount of pene-contemporaneous erosion is suggested by the superimposition of Burdigalian and Pontian mammalian faunas on Maboko Island near Kisumu. At Tinderet, the later outwellings of phonolitic nephelinites, nephelinites, through Kenya-type phonolites to basanites and tephrites as described by Binge[11], are probably all of post-Miocene age.

Fauna

At each locality every fossil fragment seen was collected, whether identifiable or not. The results of the 1961 collections are recorded in Tables 1 and 2, and in Table 1 are compared with the figures for Napak I 1958. The following tentative conclusions are drawn concerning the nature of the assemblages:

(1) There are two distinct types of preservation and assemblage represented. In Table 1 are included only those sites where fossils were stratified beneath a mantle of calcareous sub-aerial pyroclastic material. In this group it is notable that skulls, teeth and jaws comprise 12–25 per cent of the total finds. The 'Better' bones, that is, post-cranial bones preserving recognizable facets and form, show a similar range.

The fact that far too many skulls, teeth and jaws are represented when considered in relation to the number of bones, would seem to imply selective preservation. This presumably reflects the scavenging activities of carnivores and rodents in removing a large proportion of the more edible bones from the debris which lay on the temporary land surfaces. This is supported by the fact that many of the bone fragments retain signs of gnawing and chewing. From the Table 1 localities the non-mammalian fossils collected include fossil wood, seeds, fruits and land gastropods.

(2) The sites included in Table 2 are situated at the base of the main volcanic series, and the fossils occur in calcareous sands and grits resting unconformably on the basement complex and consisting largely of quartzose fragments derived from these rocks. The very different percentage of skulls, teeth and jaws, ranging from 1–6 per cent, reflects in part a more correct proportion of cranial to post-cranial fragments, but also the much poorer preservation resulting in the fragmentation of the majority of the specimens.

The contrast in lithology between the two groups of sites is seen also in the non-mammalian fauna. These basal grits characteristically yield fossils of crocodile and chelonia. At Ombo, fish were represented in the form of jaws of *Protopterus*, and at two of the Napak sites valves of an oyster were found. Also, at Ombo and Mariwa the mammals included *Dinotherium*. These are all absent from the sub-aerial volcanic environments, and provide evidence in support of a fluviatile origin for the basal deposits.

Table 2. GRIT DEPOSITS ON BASEMENT SURFACE

	Napak 1961				Moroto 1961		1961
	Napak II	Napak VI	Napak VIII	Total Napak basal levels	Moroto I	Moroto II	Ombo and Mariwa
Total	103	696	647	1,446	818	286	82
Unidentifiable bone fragments	91	685	600	1,376	733	260	72
'Better' bones	6	2	38	46	69	17	—
Skulls, teeth and jaws	6	9	9	24	16	9	10
Details of skulls, teeth and jaws							
Mastodon	4	8	—	12	10	6	6
Rhinocerotid	1	1	4	6	—	—	—
Primate	—	—	—	—	1	2	—
Others	1	—	5	6	5	1	4

Table 3. TOTAL ASSEMBLAGES COMPARED

	Napak upper level 1958–61 (%)	Songhor I, II and III 1961 (%)	Koru 1927–32 BM collection (%)	Mfwanganu (Whitworth 1961) (%)	Napak basal levels 1961 (%)	Moroto I and II 1961 (%)
Percentages of total Mammalia						
Unidentifiable bone fragments	64·4	43·3	×	×	97·0	90·1
'Better' bones	20·3	31·7	×	×	2·0	7·7
Skulls, teeth and jaws	15·3	25·0	×	×	1·0	2·2
Total number of skulls, teeth and jaws	312	120	208	303	Only 24	Only 25
Percentages of skulls, teeth and jaws						
Rodent	42·6	82·5	86·5	68·6	—	—
Mastodon	29·5	—	—*	0·3	50·0	64·0
Ruminant	7·1	10·0	0·5	14·5	—	—
Primate	5·8	2·5	4·3	3·0	—	12·0
Carnivore	3·5	0·8	2·4	3·0	—	—
Suid	2·2	0·8	3·4	2·0	—	—
Others	9·3	3·3	2·9	8·6	50·0	24·0

* At another site near Koru several specimens of *Dinotherium* were found in sediments with a different lithology.

(3) Table 1 records the characteristic high percentage of rodents preserved in all the sub-aerial tuff environments. In Table 3, percentages of the main mammalian groups in the assemblages of skulls, teeth and jaws at Napak and Songhor are compared with similar figures for Mfwanganu[14], and Koru (based on collections made between 1927 and 1932 housed in the Department of Palæontology, British Museum (Natural History)). The proportions of the main groups reveal a remarkably constant picture at the four sites, and contrast sharply with the figures for the more sparse basal assemblages at Napak and Moroto where the small fauna is completely absent.

(4) That collection failure is also a factor to be considered, is shown in Table 1. Practically all the easily seen mastodont fragments from Napak I were collected in 1958 and only an additional eight fragments were found on return in 1961. By contrast, only one-fifth of the 1958 finds were small rodents although they represent three-fifths of the 1961 total, and were often represented by isolated incisors.

(5) The fossils from Napak I, IV and V are from identical lithologies on the same stratigraphical horizon and are virtually contemporaneous. They must represent very similar environments within one mile of each other on the slopes of the periodically active volcano. Yet there are significant differences in the mammalian assemblages which must imply local ecological changes. Rodents form 80 per cent of the fauna at Napak IV but only 30 per cent at Napak I. Ruminants account for almost 30 per cent of the total at Napak V but only 2 and 4 per cent at Napak I and IV. Primates are most numerous at Napak V where they represent almost 15 per cent of the total.

In assessing the abundance of different groups, it has been assumed that each find represents one individual. Only in the case of the primates at Moroto II and of the large primate from Napak V, does it seem probable that several finds represent only one individual.

(6) At Songhor, it is more difficult to give full weight to the percentages shown in Table 3, as a great deal of collecting has been already made. However, the broad similarity both in actual genera and species and in the relative proportions of the different family groups, between the finds from Songhor and those from Napak, Koru and Mfwanganu, is very striking.

(7) At one locality in the main Songhor I exposure, from an isolated area of fine-grained reddish

tuffs known as the 'Red Outlier', which measures only some 10 ft. in diameter, 97 mammalian fossils were collected during September 1961. These included 46 unidentifiable bone fragments, 22 better-preserved bones, and 29 skulls, teeth and jaws. This was the richest deposit which we studied, although the outcrop at Songhor II (Table 1) suggested a similar local fossiliferous patch within the tuff. At these sites again too many skulls, teeth and jaws (30 and 25 per cent of the total finds respectively) were associated with too few post-cranial bones. The Red Outlier assemblage showed the usual domination by rodents with 23 out of the 29 cranial fragments.

These fossiliferous patches within the tuffs recall on a small scale the local concentrations of fossils at Napak I, IV and V. These three areas, each with a maximum diameter of less than 50 yards, yielded the vast bulk of the fauna from the Napak upper level although there was well over a mile of good exposure in beds which appeared to be of identical lithology.

(8) The Napak sites I, IV and V have now yielded a faunal list of at least 29 genera or species as follows: primates 6, insectivores 1, rodents 6, carnivores 6, anthracotheres 1, artiodactyls 4, perissodactyls 3, proboscideans 1, hyraxes 1. The majority of the genera and species is also recorded from the other Lower Miocene (Burdigalian?) assemblages at Rusinga, Mfwanganu, Songhor and Koru, but these contrast sharply with the Fort Ternan assemblage. It therefore seemed desirable to collect where possible specimens of potassium-bearing volcanics associated with the various fossiliferous horizons, with the view of potassium-40/argon-40 dating. The specimens are being dated by Drs. J. F. Evernden and G. H. Curtis of the Department of Geology, University of California, Berkeley, and Dr. P. Damon, University of Tucson, Arizona, to whom we are indebted.

It is hoped that the association of the dates with the faunal evidence will be of assistance in assessing the reliability of these techniques when applied to Tertiary volcanic deposits. Also, as more absolute ages become available it may be possible to review the palæontological data for the various localities. It seems desirable to record differences in abundance, as in Tables 1–3, in addition to identifying lists of species. This is essential if the faunas are to yield evidence on rates of evolutionary change and on the relative importance of ecological differences between the various sites.

(9) The finds from Uganda included 13 primate specimens from Napak and 3 from Moroto. This brings the total of primate finds from the Napak upper level to 18, of which at least 9 represent the genus *Proconsul*. The two finds from the new Moroto II site included the left maxilla of a large *Proconsul*. A further expedition to this locality in December 1961 by Prof. D. B. Allbrook and other members of the Department of Anatomy, University College, Makerere, yielded several more *Proconsul* fragments, including the right maxilla of the original individual. These primate specimens, together with those from Napak, will form the subject of a further publication shortly.

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[1] For distribution of localities, see Maps (Fig. 1) in Bishop (refs. 2 and 3).

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MOLECULAR ORIENTATION OF SOME KERATINS

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Wool and related hairs (α-keratin) show no birefringence in transverse section, and it is concluded that the preferred molecular orientation must lie in the direction of the fibre axis. In the case of feather, however, which is substantially in a β-configuration, Astbury and Bell[1] observed more than twenty years ago that the molecular orientation of a thin outer layer of quill was at right angles to the feather axis. Recently, we have[2], using the polarizing microscope, found that in the case of goosewing quill, the region showing transverse orientation represented approximately the exterior third of the calamus (root-end) wall, reducing to one-seventh at the rachis. Preliminary X-ray diffraction work also indicated that the pattern of calamus is in fact composite and arises from two structures at right angles to each other.

Thick tactile whiskers, such as those of lion and tiger, exhibit Maltese-cross birefringence when a transverse section is viewed in polarized light, and it has been concluded that in these structures it is the interior region surrounding the medulla that possesses tangential orientation at right angles to the axis[2].

In the present investigation an examination has been made by X-ray diffraction and polarizing microscopy of a number of keratinous materials to determine the extent to which molecular orientation at right angles to the longitudinal axis is general in these structures. The materials examined included hairs, cattle horn and mammalian quills as these are representative of a wide range of keratinous structures. Baleen plate of whale ('whalebone') and rhinoceros horn have been studied also as these materials are interesting in that their complex structures may be regarded as intermediate between normal horn and hair. Some further observations on tactile whiskers and feather calamus are included in the present communication as they have confirmed the conclusions put forward previously regarding the molecular orientation of these structures.

The calamus was isolated from white goose feathers with scissors. Shavings of rhinoceros horn were taken near the root and both from living animals and museum specimens. Strips of commercial 'whalebone' were used with the fringe fibres attached. Although it has been suggested that hydroxyapatite may be present in the whalebone fringe fibres of the fin whale[3], it was found that the calcium phosphate content of the baleen used in this work was 1·0 per cent only. Prior to examination, all materials were degreased by Soxhlet extraction with alcohol and then with ether.

Transverse sections were obtained by hand-sectioning with a razor blade or by the use of a microtome. Specimens were mounted in xylene and viewed in ordinary and convergent polarized light. Examination at elevated temperature was made by enclosing the specimen in an electrically heated aluminium block, the apparatus being similar to that used for observing the shrinkage temperature of collagen.

Although the transverse sections of whisker were of uniform thickness, the birefringent region exhibited very marked multicolouring when viewed in white light, indicative of optical heterogeneity. In these circumstances no quantitative measurements of birefringence could be made, although the insertion of a quarter wave plate showed that in all cases the birefringence was negative.

X-ray diffraction photographs were obtained using a flat plate camera and copper $K\alpha$-radiation from a Raymax rotating anode crystallographic unit operating at 40 m.amp and 40 kV, with the beam parallel, radial or tangential to the axis. Where comparison photographs were required a quadrant type cassette was used.

(*a*) *Feather calamus*. It had been shown previously that the regions exhibiting differing birefringence were separated by a sharp boundary line[2], and there was no evidence for the two structures merging into each other. This suggested that it should be possible to separate the two phases mechanically and this was, in fact, achieved by the following method. A sample of calamus was mounted in a stretching frame in such a manner that tension could be applied tangentially in the plane perpendicular to the feather axis. The specimen was steamed for about 2 min in this state, when it split spontaneously into its two components. These components were then mounted in the X-ray camera with the direction of the quill axis vertical. X-ray diffraction photographs showed that although the molecules of the two regions possess the same configuration, in the case of the inner region the orientation is parallel to the feather axis, but in the case of the outer region it is perpendicular to this axis.

(*b*) *Tactile whiskers*. It has been reported previously[2] that the interior regions of transverse sections of whiskers of lion and tiger exhibit Maltese-cross birefringence in polarized light. Myelin sheaths however, also exhibit the same phenomenon and it has been concluded that this form of birefringence may arise from two materials within the sheath, the protein molecules which lie tangentially and incrusted lipoid materials with the molecules orientated radially[4]. Since the latter birefringence is reduced by solvent extraction[5], it was concluded that the Maltese-cross birefringence of whiskers was due solely to tangential orientation of protein molecules since the materials used had been extracted thoroughly. However, the region showing this phenomenon is always in the vicinity of the medulla where lipoid materials are very probably present, and therefore the birefringence of these materials was examined at elevated temperatures, since it is known that the birefringence due to lipoids disappears at 100° C (ref. 5). It was shown that a transverse section of lion whiskers viewed in polarized light with the specimen heated to 115° C gave a Maltese cross as sharp as that at room temperature, and it must be concluded that the original views regarding the cause of this birefringence are correct.

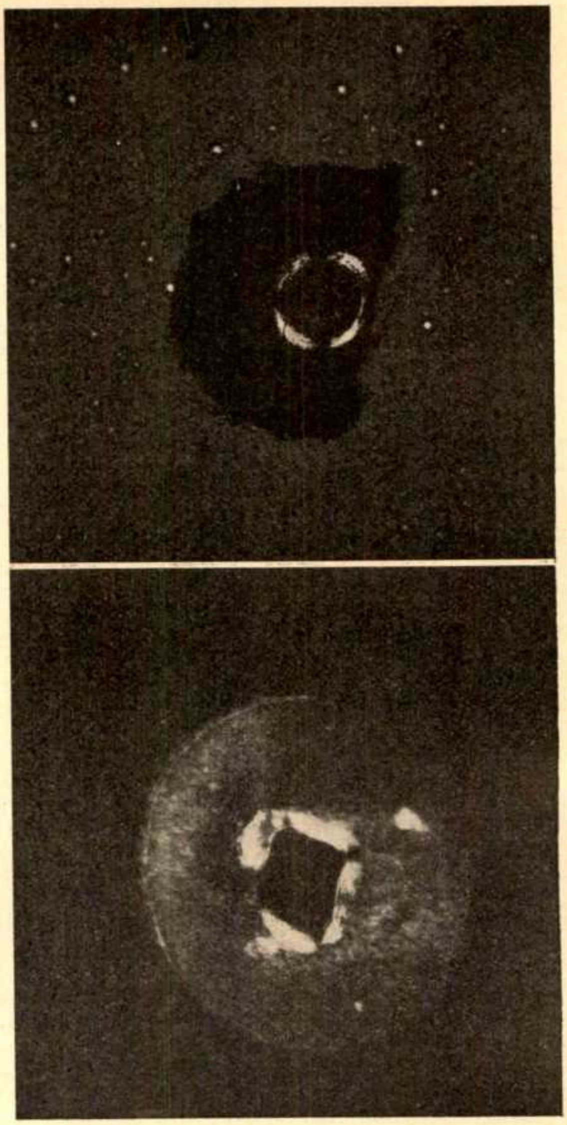

Fig. 1. Top, transverse section of whisker of lion viewed in polarized light at 115° C (× 45); bottom, transverse section of horse hair viewed in polarized light (× 400)

(*c*) *Miscellaneous animal hairs.* In view of the fact that the very extensive literature on the fine structure of wool makes no mention of Maltese-cross birefringence, it was scarcely surprising that none was found in this investigation. The literature on heavily medullated wools and kemp, which are more akin to the tactile whiskers than the finer wools where medulla is completely absent, however, is less comprehensive and these materials were, therefore, carefully examined but again no birefringence was observed.

Although it was not possible to make a complete survey, a very large number of other animal hairs was also examined, but only one example of Maltese-cross birefringence was found. This was in monster horse hair, and a transverse section taken near the root is shown viewed in polarized light in Fig. 1.

(*d*) *Cattle horn.* X-ray diffraction showed that in the case of horn, which consists of about ten concentric laminates which may be separated readily mechanically, the outer layer has very good orientation with the molecules lying perpendicular to the axis and tangentially. The inner layers, generally, did not show such good orientation, but the orientation showed a tendency to be parallel to the horn axis, although occasionally a region could be found with molecular orientation similar to that in the outer layer. No evidence could be found for a β-configuration.

A transverse section of horn, about 0·001 in. thick, obtained by using a shaping machine, was mounted in xylene and viewed in polarized light. The outer layer, which amounted to about one-third of the thickness of the horn, was highly birefringent whereas the remainder was birefringent to a lesser degree. Thus the molecular orientation as shown optically was consistent with the results obtained from X-ray diffraction.

(*e*) *Baleen plate* ('*whalebone*'). Whalebone, after extraction with alcohol and ether to remove grease, gave a normal α-keratin X-ray diffraction pattern. A transverse section of this material viewed in normal light is shown in Fig. 2, top. In the lower portion the fibres can be seen set in the matrix, and the upper portion consists solely of layers of horny embedding material. The laminated nature of the latter may be seen clearly and it is thus very similar to cattle horn. The same section viewed in polarized light (Fig. 2, bottom) shows that each embedded fibre exhibits a Maltese cross and is very similar to the whiskers of lion and tiger. The fringe fibres which emerge from baleen also show the same effect.

(*f*) *Rhinoceros horn.* There is considerable confusion in the literature regarding the structure of rhinoceros horn and an attempt to clarify the position has been made recently by Ryder[6]. There is no doubt that, whereas cattle horn is composed of sheets of keratinous material, rhinoceros horn consists of longitudinal filaments. These tend to separate, particularly at the proximal end, which, no doubt, gave rise to the earlier statements that rhinoceros horn is composed of matted hair[7,8]. Although the latter is untrue, it has been shown[6] that the interfilamentous horn is much sparser than, for example, in the case of baleen plate (Fig. 2, top), and the filaments are packed so tightly together that they are often multi-sided rather than circular. Morphologically, it is probably not incorrect to regard both baleen plate and rhinoceros horn as intermediate between hair and cattle horn.

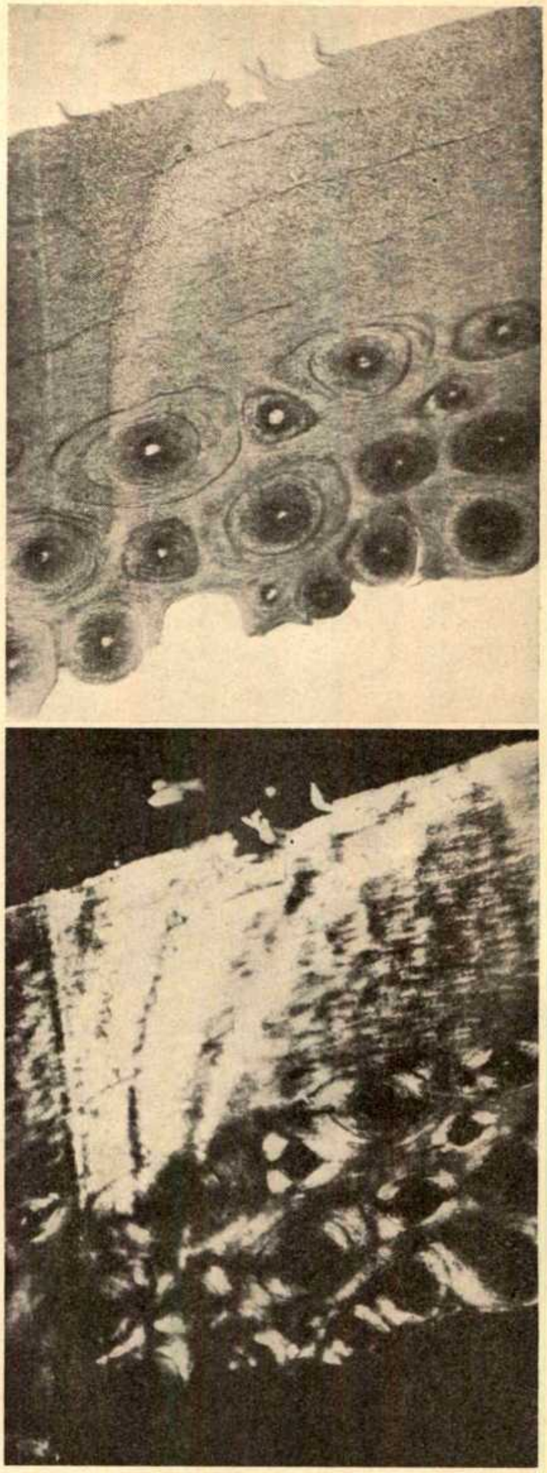

Fig. 2. Top, transverse section of baleen plate viewed in normal light (× 60); bottom, similar section viewed in polarized light (× 60)

Concurrently with the publication of the discovery of transverse orientation in whiskers of the cat family[2], Ryder[6] published photomicrographs of transverse sections of the individual filaments comprising rhinoceros horn. The latter showed clearly longitudinal cell structures orientated tangentially in the plane of the section and were, in fact, very

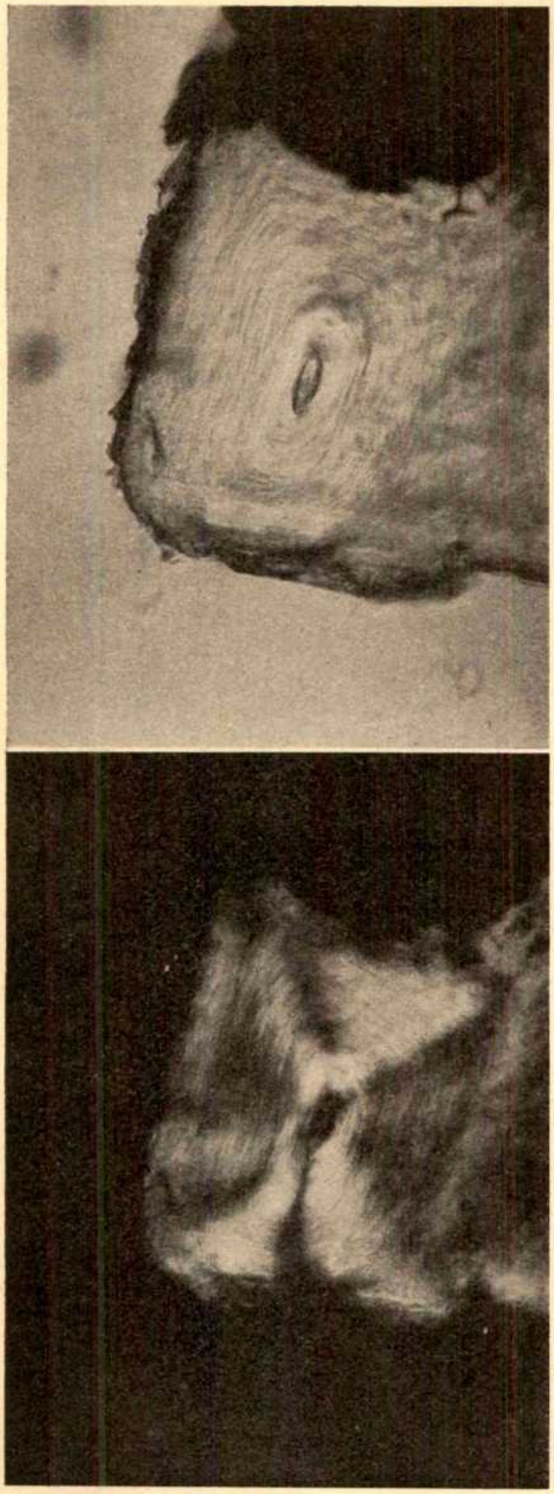

Fig. 3. Top, transverse section of a single filament of the horn of the black African rhinoceros viewed in normal light (×50); bottom, similar section viewed in polarized light (× 50)

similar to sections of whisker. It was therefore anticipated that these sections would exhibit Maltese-cross birefringence in polarized light. In Fig. 3 is shown a transverse section of a filament from the horn of the black African rhinoceros (*Diceros bicornis*) viewed in polarized light and a similar section taken from the horn of the Indian rhinoceros (*R. unicornis*) in polarized light is shown in Fig. 4.

Of the five living species of rhinoceros, the horns of four were available for examination, the exception being the Sumatran rhinoceros (*R. sumatrensis*). A comparative investigation of these four keratins was therefore made, and the results are summarized in Table 1.

Although Table 1 suggests that the Asian rhinoceroses have horns superficially more resembling

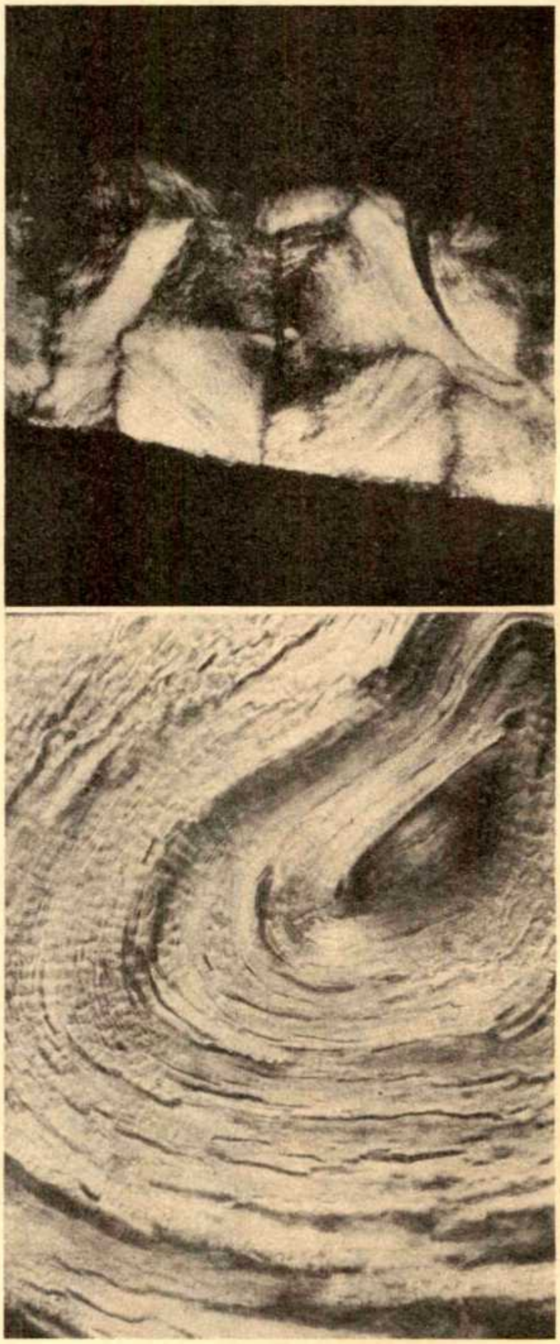

Fig. 4. Top, Transverse section of the horn of the Indian rhinoceros viewed in polarized light (× 50); bottom, transverse section of a portion of a filament of the horn of the white African rhinoceros viewed in normal light (× 400)

Table 1. STRUCTURE OF RHINOCEROS HORNS FROM DIFFERENT SPECIES

Species*	Macro structure	Appearance in polarized light of transverse section	X-ray diffraction pattern
Indian (2) (*R. unicornis*)	General appearance like cattle horn.	Maltese-cross effect (Fig. 4).	Poorly orientated α-pattern. Strong apatite reflexions.
Javan (1) (*R. sondaicus*)	Filaments very firmly embedded in matrix.	Patchy birefringence. Filaments too irregular to produce cross.	Poorly orientated α-pattern. Apatite reflexions absent.
African Black (2) (*Diceros bicornis*)	General appearance like bundle of bristles.	Maltese-cross effect (Fig. 3).	Poorly orientated α-pattern. Apatite reflexions absent.
African White (1) (*Cerathotherium simus*)	Filaments readily separated.	Patchy birefringence. Filaments too irregular to produce cross.	Poorly orientated α-pattern. Apatite reflexions absent.

* The number of specimens examined from different animals is given in parenthesis.

cattle horn that the African species, and *R. unicornis* is the only species containing apatite as a major constituent of its horn, it must be borne in mind that the observations have been made on a small number of animals.

In feather and cattle horn the keratins possessing transversal and longitudinal orientation are located in well-defined regions. In the case of rhinoceros horn, however, the poor X-ray diffraction pattern produced when the specimen was mounted longitudinally and the extensive but rather ill-defined Maltese-cross pattern shown by a transverse section in polarized light, suggest that the protein molecules showing different orientations are more intimately associated.

(*g*) *Mammalian quills.* The birefringent zones exhibited by transverse sections of porcupine and hedgehog quills in polarized light were less extensive than in the other keratinous structures examined. Nevertheless, the four extinction positions were clearly visible and there can be no doubt that a thin layer possessing transverse orientation is present. If the complex internal structure of quill be regarded as a medulla, the location of this layer is not dissimilar to that in tactile whiskers.

Thus, it has been shown conclusively by X-ray diffraction and polarizing microscopy that feather calamus and cattle horn are polyphase in structure, possessing molecular orientation of the protein molecules at right angles to the main structural axis, in addition to parallel orientation.

Strong evidence has been obtained also from polarizing microscopy that similar structures are present in rhinoceros horn, baleen plate and mammalian quills, but in hair and wool with a single exception the preferred molecular orientation lies exclusively in the direction of the fibre axis.

It is considered that the biological significance of these structures is to prevent the splitting that would occur if the structural elements were laid down exclusively in one direction.

We thank Dr. L. Harrison Matthews and Mr. E. H. Tong of the Zoological Society of London, Dr. G. B. Corbet of the British Museum (Natural History) and Dr. E. Lees of the Department of Biological Sciences, Bradford Institute of Technology, for providing specimens and advice. One of us (J. G. P. S.) is a Wool Textile Research Council Fellow.

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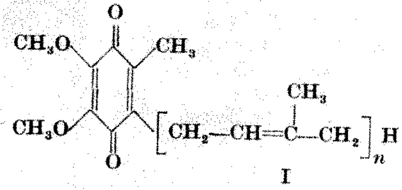
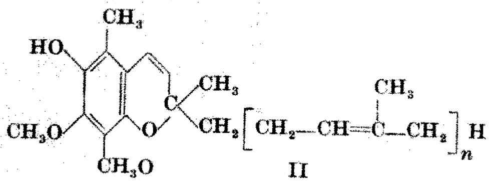
UBIQUINONE AND UBICHROMENOL

Ubiquinone and Ubichromenol in *Torula* Yeast

UBIQUINONES (I) and ubichromenols (II) are isomeric.



I



II

Ubichromenol$_{10}$ ($n=9$)[1] has been found in various animal tissues and ubichromenol$_7$ ($n=6$) occurs in a *Torula* yeast (commercially dried).

The isomerization can be effected in the laboratory by various procedures involving the use of a catalyst[2,3]. These lead to a racemic product, but the preparations from human kidney[4] and from *Torula* yeast were optically active.

The question whether ubichromenol is or is not a natural product for which it is reasonable to seek a function is not easily settled, although evidence has been reported[5] which supports a natural origin from some animal tissues. It became necessary to investigate the problem in the case of *Torula* yeast.

A commercial, dried *Torula* yeast (*Candida utilis*), kindly given by the Lake States Co., New York, and designated type *B*, was analysed for ubiquinone (UQ) and ubichromenol (UC) with the results shown in Table 1.

Simple extraction of lipid from yeast is an inefficient process[6], and the failure in experiments 1, 2 and 3 to achieve the yields of UQ and UC which occur after alkali digestion (saponification) of the yeast is not surprising. Alkali and heat tend to destroy UQ and UC, and saponification should aim at liberating these substances with minimal destruction. The fact that the ratio UC/UQ in Table 1 is nearly constant argues against conversion of UQ in the saponification

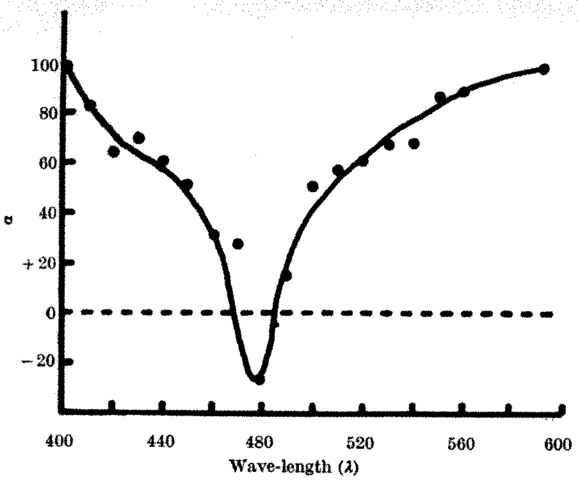


Fig. 1. Optical rotatory dispersion in petrol of ubichromenol$_7$ isolated from dried *Torula* yeast

process. The UQ and UC were separated and assayed according to the method of Green *et al.*[7] except that the second dimension of the paper chromatography was omitted.

A completely independent analysis carried out on another sample of the same yeast gave UC 485 μg/g, UQ 250 μg/g. In this experiment the unsaponifiable matter was twice chromatographed, once on alumina and once on magnesia, and by comparison with the results in Table 1 artefact formation cannot have occurred on more than a quite small scale, if at all.

Analyses in other laboratories[6] have given figures of UQ 341 μg/g and UC 420 μg/g.

A sample of yeast was digested with methanolic alkali containing pyrogallol for 1 h (Table 1). The ether-soluble unsaponifiable matter was then subjected to chromatography on acid-washed alumina (Brockmann grade 2). After further purification, involving chromatography on magnesium oxide, a sample was obtained which exhibited absorption maxima in *cyclo*hexane at 275 and 332 mμ ($E^{1\%}_{1\,\mathrm{cm}}$ of 123 and 50, respectively) and minima at 260 and 300 mμ ($E^{1\%}_{1\,\mathrm{cm}}$ of 75 and 24, respectively). The characteristic inflexion at 282 mμ was also clear. Reversed-phase partition paper chromatography indicated that the product was mainly ubichromenol$_7$ with small amounts of ubichromenol$_8$ as the contaminant. This is consistent with the $E^{1\%}_{1\,\mathrm{cm}}$ values quoted here, for at $\lambda_{\max}$ 275 mμ pure ubichromenol$_{10}$ has an $E^{1\%}_{1\,\mathrm{cm}}$ of 96·1, and so pure ubichromenol$_7$ and ubichromenol$_8$ would be expected to have $E^{1\%}_{1\,\mathrm{cm}}$ of 125·0 and 104·9, respectively. The material was therefore free of other impurities.

Table 1. Yields of Ubiquinone and Ubichromenol from Dried *Torula* Yeast using Various Methods of Extraction

Method of extraction	UQ* (μg/g)	UC† (μg/g)	UC/UQ
1. Acetone/ether, boiled, filtered	97	142	1·46
2. (*a*) Ether (Soxhlet)	96‡	148	1·54
(*b*) 1 h saponification of remaining yeast	173	268	1·55
3. Chloroform/methanol, boiled, filtered	126	190	1·51
4. ½ h saponification	270‡	416	1·54
5. 1 h sponification	266‡	419	1·58
6. 1½ h saponification	215‡	350	1·63
7. 1 h acid hydrolysis	225§	352	1·56

*UQ, ubiquinone estimated as ubiquinone$_7$.
† UC, ubichromenol estimated as ubichromenol$_7$.
‡ Refluxed *x*g with 3 *x*ml. 5 per cent (w/v) methanolic pyrogallol and 3·5 *x*ml. 25 per cent (w/v) aqueous KOH. Then diluted with water and extracted with ether.
§ Same as saponification but with 6 N HCl instead of KOH.

Table 2. Effect of Drying on the Amounts of Ergosterol, Ubiquinone and Ubichromenol in Fresh *Torula* Yeast (*Candida utilis*)

(Yeast dried at 90° C in an open beaker)*

Period of heating	Ergosterol (mg/g dry weight)	Ubiquinone (μg/g dry weight)	Ubichromenol (μg/g dry weight)
None	Not recorded	762 (±25)†	17
23 min	4·11	666	20
60 min	4·09	872	22
3 h	4·00	602	21
6 h	4·32	688	22

* 75·65 per cent of wet weight lost on freeze-drying.
† Mean of three readings.

A sample (31 mg) of this ubichromenol was then sent to Glaxo Laboratories for investigation of its optical activity. Since ubichromenol is known to be photo-labile and is probably also thermo-labile, it was kept in a sealed tube in a thermos flask containing solid carbon dioxide during transport. We are indebted to Dr. M. Barnard, Mr. A. C. Parker and others of Glaxo Laboratories for the following report:

"The solution of ubichromenol (from *Torula* yeast) in petroleum ether (b.p. 40–60°) showed a negative peak at 480 mμ with [α] of −26° (±5°). The possible error in [α] is due to the use of dilute solutions, for the sample had a high optical density."

The results are recorded graphically in Fig. 1.

Ubichromenol$_{10}$ has previously been examined[4] for us in the Glaxo Laboratories when a product isolated from human kidney was compared with a product obtained by Dr. O. Isler *et al.* (Hoffmann-La Roche and Co., Basle) by the Links[2] procedure from ubiquinone$_{10}$ (ref. 5). The synthetic compound was optically inactive. The natural compound was examined in chloroform solution and qualitative curves on the fresh solution resembled that for ubichromenol from *Torula* yeast shown in Fig. 1. On the following day there was a negative peak at 430 mμ with [α] of −50°. On the third day the solute had evidently decomposed.

The yeast sample examined for optical activity was obtained in an experiment in which saponification was conducted for 1 h and contained a minimal proportion of racemic ubichromenol artefact (Table 1).

Inasmuch as ubichromenol is not always found in extracts from tissues fairly rich in ubiquinone, the case in favour of the view that it may be an artefact of laboratory manipulation is far from strong. On the other hand, the erratic distribution might be taken as indicative of formation of UC as a result of biological differences in the sense that UQ is converted to UC, but only under special conditions. This possibility warrants investigation.

Accordingly, live yeast was obtained by courtesy of the Lake States Co. It came in two forms, one as an agar slope and the other (through Dr. J. Green, of Vitamins, Ltd.) as a frozen pressed cake (not dried). The smaller sample was for inoculating cultures and the larger sample for analyses.

Rather surprisingly, the UC content of the fresh yeast was almost negligibly low, but the UQ corresponded closely with the sum of the UQ and UC contents of the dried yeast (Tables 1 and 2).

Qualitative results obtained by Dr. J. Green[8] on the same sample of fresh yeast agree with this finding.

It is clear from Table 2 that there has been no significant destruction of ergosterol or ubiquinone, and only extremely slight formation (if any) of ubichromenol during laboratory drying of the fresh yeast.

Ten experiments have been carried out on the effect of various treatments of yeast on the minor lipid constituents. Freeze-drying alone had no detectable effect, nor did storage for one week at

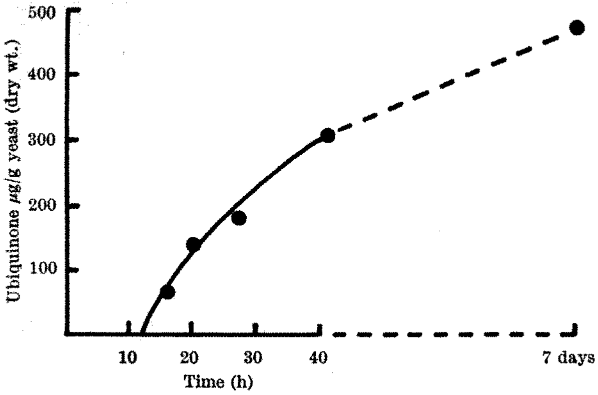


Fig. 2. Variation of the ubiquinone content with the growth period of *Torula* yeast

room temperature. Freeze-drying, followed by heating at 90° for periods up to 6 h, effected no appreciable loss of ergosterol or ubiquinone and no significant increase in ubichromenol. The most damaging treatment was to heat the yeast for 3 h at 80°–90° after spreading it out in a thin layer. This was harmful to ergosterol and to ubiquinone, but it did not raise the ubichromenol content. In fact, no treatment applied brought about more than a minimal increase in ubichromenol.

As a result of numerous trial experiments, it was found that the yeast could be grown satisfactorily in 250-ml. Erlenmeyer flasks on a shaker at 37°. The medium contained 5 per cent glucose, 1 per cent mycological peptone and the usual inorganic salts. The *p*H was maintained at 5·2 by potassium citrate buffer. By growing in 100 ml. medium for 3 days, a yield of 1·56 g (dry wt.) of yeast containing 300 μg/g (dry wt.) of ubiquinone was obtained. When ammonium sulphate was used as nitrogen source the yield of ubiquinone dropped to 112 μg/g dry wt., but this was boosted to 188 μg/g dry wt. by putting the glucose concentration up to 10 per cent although only 25 per cent of the sugar had been metabolized. When ammonium sulphate was replaced by 1 per cent leucine the yield of ubiquinone was restored to 300 μg/g dry wt., but less yeast (1·06 g) was produced.

Larger amounts of yeast could be grown in 1-litre medium in 3-litre flasks. These were aerated by attaching to a filter pump and sucking air through sintered glass distributors in the medium. As shown in Fig. 2, the yield of ubiquinone increased rapidly with time during the first 2 days, but then the rate of increase slowed down. Increase in aeration also increased the yield, but excessive frothing occurred.

Despite the great variations in yield of ubiquinone produced by these changes in conditions, on no occasion was ubichromenol found to be present.

The simplest explanation of the analytical data presented is that ubichromenol isolated from commercially dried *Torula* yeast is formed from ubiquinone during factory drying or storage of the yeast. It was not possible to reproduce the critical conditions for this conversion in the laboratory. Such an explanation presents two problems. In the first place, the ubichromenol so produced was optically active, and secondly, the conversion was extremely efficient. The optical activity could be explained by assuming that in the yeast cell ubiquinone is adsorbed on, or complexed with, protein or other material in some stereospecific manner such that ring closure during subsequent treatment to form ubichromenol can occur in only one of the two possible arrangements. An alternative hypothesis is that some optically active compound in the yeast cells can give rise to either ubiquinone or ubichromenol, depending on the treatment to which the cell is subjected. An attempt is being made to resolve the problem.

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Natural Occurrence of Ubichromenol

All procedures previously used for the isolation or analytical determination of ubichromenol have involved saponification and/or chromatographic separation on alumina. Since both these steps have been shown to cause, under certain conditions, isomerization of ubiquinone to ubichromenol[1,2], doubts have been raised about the natural occurrence of ubichromenol. However, using a method of assay that had been shown to minimize the possibility of such isomerization, Diplock *et al.*[3] found a high concentration of ubichromenol$_7$ and ubichromenol$_9$ in dried *Torula* yeast. The presence of large amounts of ubichromenol and the fairly low level of fat in this yeast make it an attractive starting material for the isolation of ubichromenol by unequivocal procedures.

Dried *Torula* yeast (American type *B*; 1 kg) was treated with iso-octane (1 litre) with occasional shaking and left to settle. The supernatant liquid was removed and fresh iso-octane added. The procedure was repeated until about 2·5 litres of extract was obtained. After filtration, evaporation under reduced pressure yielded a brown oil (9 g) which gave a positive Emmerie and Engel reaction. The oil was extracted with boiling acetone (5 × 50 ml.) and the combined extract was reduced in volume (100 ml.), cooled to – 60° C and filtered. The volume of the filtrate was reduced to 50 ml. and the process repeated. Evaporation of the filtrate gave an oil (0·85 g) that was examined by paper chromatography without further purification. In the reversed-phase system (paraffin–95 per cent ethanol) four bands were observed, two of which (R_F 0·52 and 0·30) corresponded to ubiquinone$_7$ and ubiquinone$_9$. The other two substances (R_F 0·66 and R_F 0·42) were chromatographically identical with ubichromenol$_7$ and ubichromenol$_9$, gave a positive Emmerie and Engel reaction, and possessed a typical chromenol ultraviolet spectrum ($\lambda\lambda_{max}$ 273, 280 and 330 mμ).

An attempt was made to purify the extract further by removal of volatile constituents at 180° under molecular distillation conditions, but this led to a decrease in ubiquinone and an increase in ubichromenol. A model experiment with pure ubiquinone$_6$ confirmed that conversion into ubichromenol occurred at 180° at 0·1 mm. After being heated for 5 h, the product was examined by paper chromatography under nitrogen (paraffin–85 per cent ethanol system) and found to contain ubiquinol$_6$ (R_F 0·79), ubichromenol$_6$ (R_F 0·56), and unchanged ubiquinone$_6$ (R_F 0·38). The structure of the ubichromenol was confirmed by its ultra-violet spectrum. Ubichromanol was not detected. Although, in subsequent experiments, it was shown that isomerization did not occur at an appreciable rate at temperatures below 150° (such as are used for the commercial drying of *Torula* yeast), it was considered possible that the ubichromenols in the dried yeast might have been formed at some stage after collecting. To examine this, a sample of compressed *Torula* yeast cake was obtained from Wisconsin and, after saponification by the method of Laidman *et al.*[4], the non-saponifiable extract was examined in the paraffin–95 per cent ethanol system. Ubichromenol was not detected, but the ubiquinone concentration (on a dry wt. basis) was higher than that normally found in the commercially dried *Torula* yeast[3]. When samples of the compressed yeast were dried at 150° for several minutes in the laboratory, ubichromenol was not formed. These experiments support the suggestion that ubiquinone is converted to ubichromenol at some stage after gathering of the fresh yeast but that some factor other than heat is necessary. However, this suggestion must be reconciled with the observation of Morton and Hemming[5] that the ubichromenol$_7$ isolated from dried *Torula* yeast is optically active.

In order to clarify further the situation with regard to ubichromenol in animal tissues, the procedure described here was applied to rat liver, taken from animals deficient in vitamin E. The liver (50 g) was extracted as previously described by Edwin *et al.*[6]. The dry extract (2·7 g) was re-extracted with boiling acetone (4 × 25 ml.) and the combined extract was reduced in volume (50 ml.), cooled to $-60°$ C and filtered. Evaporation of the yellow filtrate under reduced pressure gave an oil which was extracted into light petroleum. The soluble portion (0·3 g) was strongly reducing, even at this stage, but could not be chromatographed directly in the reversed-phase system without being purified further by thin layer chromatography on 'Kieselgel *G*' plates, using benzene for development. Under these conditions, a partial separation of ubichromenol (R_F 0·3) and ubiquinone (R_F 0·25) was achieved. The ubichromenol fraction was extracted with ether and, when examined in the reversed-phase system (paraffin–95 per cent ethanol), migrated to the same position as authentic ubichromenol$_9$ (R_F 0·33) and gave a positive Emmerie and Engel reaction and a typical chromenol ultra-violet spectrum. Although chromatography of pure ubiquinones on 'Kieselgel *G*' plates leads to slight, just detectable, isomerization to ubichromenols, we regard it as unlikely that this could account for the quantity of ubichromenol$_9$ (30 μg/g) observed in this rat liver.

We thank the Lake States Yeast Corp., Rhinelander, Wisconsin, for generosity in providing samples of *Torula* yeast.

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DISTRIBUTION OF RADIOACTIVE SELENIUM IN MICE

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In the course of experimental studies of the nutritional importance of traces of selenium, the distribution of radioactive selenium within the tissues of mice was investigated.

Selenium, tagged with selenium-75, was introduced into lucerne (*Medicago sativa* L.) by growing the plants in a nutrient solution containing $Na_2{}^{75}SeO_3$. Examination of protein extracted from the leaves indicated that most of the selenium became concentrated in the leaf-protein fraction. These lucerne leaves were incorporated in the rations fed to mice, for periods up to 7 days; the daily intake of selenium from this source was 2 μg selenium containing 0·1 μc. selenium-75. Thus, the concentration of selenium was within the range usually present in natural foodstuffs.

After the feeding period the mice were anæsthetized, perfused via the left ventricle with buffered formalin; the organs were then removed, weighed and their radioactivity estimated in a well-type scintillation counter. Autoradiographs (Figs. 1–5) were made with Kodak *AR* 10 stripping film closely superimposed on histological sections.

Table 1. Localization of Radioactivity in the Tissues of Mice Fed Lucerne labelled with Selenium-75

	Score*					
Tissue	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Animal 6
Pancreas	+ + + +	+ + ‡	+ + + +	+ + + +	+ + +	+ + +
Kidney	+ + +	+ + +	+ + +	+ +	–	+ + +
Liver	+ + +	+ + +	+ +	+ +	–	+ +
Adrenal	+ +	+ +	+ +	+ +	–	–
Pituitary	+ +	+ +	+ +	–	–	–
Thyroid	+ +	+ +	+ +	+ +	–	–
Skin	+ +	+ +	+ +	+ +	+	+
Gut	+ +	+ +	+ +	+ +	+	+ +
Stomach	+ +	+ +	+	+ +	+	+ +
Brain	+ +	+ +	+ +	+ +	+	+ +
Lung	– †	–	+ +	+ +	–	–
Spleen	+	+	+ +	+	–	–
Heart	+	+	+	+	+ +	–
Bone	+	+	+	+	–	–
Muscle	+	+	+	+	+	+

* Scoring system: intensity of localization of radioactivity. +, faintly present, no apparent concentration; + +, detectable localization; + + +, obvious localization; + + + +, very intense localization.

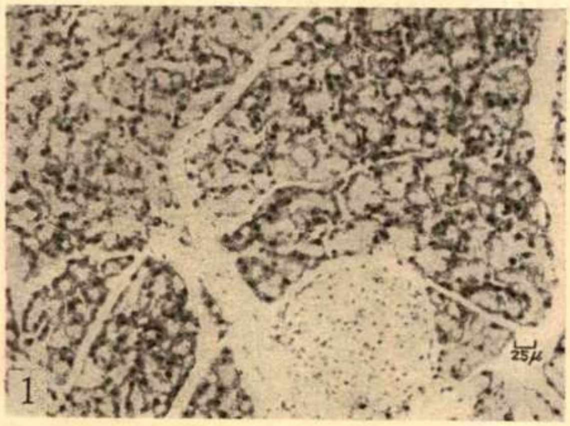
† Tissue not examined.

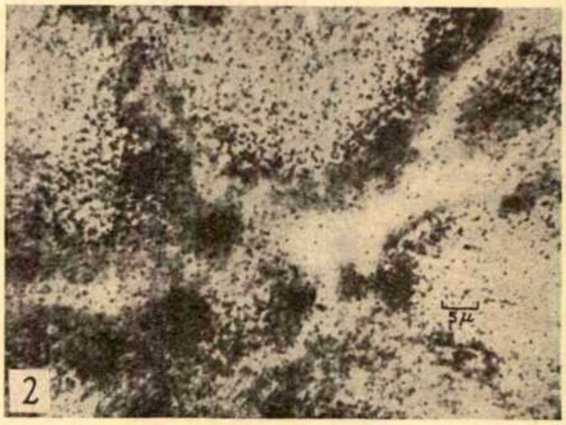
‡ Diseased pancreas.

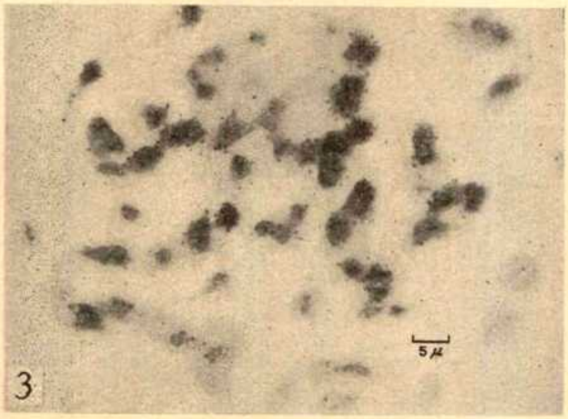
In terms of activity per unit wet weight of the organs removed at autopsy, the concentrations of selenium-75 ranged in the following order : kidney > liver > endocrine glands > pancreas ≫ lung > heart > spleen > brain. The concentrations of selenium-75 accumulated within these organs fell into two distinct groups, being appreciably higher in the first four than in the second four.

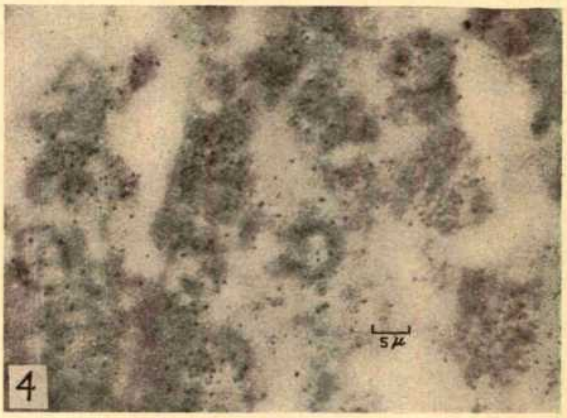
The autoradiographs (Figs. 1–5) indicated a discrete localization of selenium-75 within the cell nuclei. This was a consistent finding in all tissues examined except cardiac muscle, skeletal muscle and bone. In Table 1 a score has been recorded for each tissue, based on the degree of localization of activity as revealed by the autoradiographs. Judged from autoradiography, the greatest accumulation of selenium-75 occurred in the acinar tissue of the pancreas, and this was followed closely by the extent of the accumulation in the kidney, liver, adrenal, pituitary and thyroid. This would suggest a differential loss of selenium-75 complexes during histological processing.

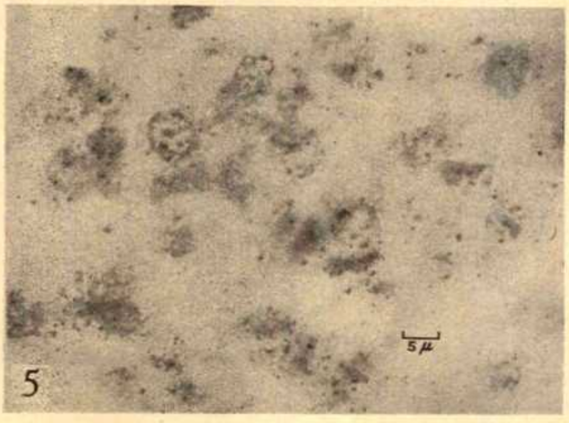
In another experiment, individuals from the litter of a lactating mouse, fed in the way described here, were examined at intervals for selenium-75. Radioactivity was detected in the baby mice 4 h after their dam had ingested the ration containing the lucerne labelled with selenium-75, and was continuously transferred in the milk at a steady level during the subsequent 6 h. The rapid transfer of selenium, when in organic combination of the sort present in the lucerne that was fed, suggests that selenium is











Figs. 1–5. Autoradiographs of sections of organs from mice that had ingested lucerne labelled with selenium-75

(1) Pancreas; unstained; low power (× 10 objective). The architecture of the organ is clearly defined by localization of selenium-75. The concentration in the islet of Langerhans (lower right) is markedly less than in the acinar cells

(2) Pancreas; unstained; high power (1/12 in. oil immersion objective). Acini in same section as Fig. 1. Radioactivity is preferentially associated with the nuclei of the acinar cells

(3) Kidney; high power (1/12 in. oil immersion objective), showing a glomerulus where radioactivity is characteristically concentrated over the nuclei

(4) Liver; high power (1/12 in. oil immersion objective). Note. The radioactivity is more generalized but relatively more highly concentrated in the nuclei

(5) Adrenal, zona glomerulosa; high power (1/12 in. oil immersion objective). Radioactivity occurs in zona fasciculata and zona reticulosa to about the same degree. There was little activity in the medulla

All sections were exposed for identical time on stripping film, Kodak *AR*.10. All illustrations have been reduced to three-quarters of original photograph readily metabolized, and that selenium-containing compounds are normal constituents of milk.

The considerable attention now being given to the function of selenium in animal tissues reveals that the liver and kidney are sites of comparatively intense selenium metabolism. Evidence from the autoradiographic studies reported here, and from the distribution of radioactivity on a weight basis, shows that the pancreas may also be of considerable importance in selenium metabolism, and that the endocrine glands are sites of relatively intense activity.

Localization of the isotope within the nuclei of the cells of most tissues suggests that selenium plays a functional part in the nucleus. Necrotic changes in the liver resulting from selenium deficiency in the rat[1] might thus originate in functional disorder of the nucleus, leading to its degeneration, thence to complete cell necrosis.

The findings recorded here are part of an investigation that will be published in detail elsewhere.

We thank Dr. H. R. Marston for his advice.

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TWO-DIMENSIONAL ELECTROPHORESIS OF PSEUDOCHOLINESTERASE COMPONENTS IN NORMAL HUMAN SERUM

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AUGUSTINSSON[1,2] showed, by electrophoresis in cellulose columns, that the cholinesterase activity of human serum could be separated from the arylesterase activity. After electrophoresis at *p*H 8·4 serum cholinesterase activity was found in the α_2-β-globulin area while the arylesterase activity was found in a position similar to that of serum albumin. Similar separations had been obtained using filter paper electrophoresis[3,4]. With starch-gel electrophoresis, however, a more complex pattern of esterase components may be observed[5-10]. In particular, the findings suggest that serum cholinesterase activity is probably made up of several electrophoretically distinguishable components. In the present investigation we have attempted to define the electrophoretic behaviour of these components more precisely by using the technique of two-dimensional electrophoresis[11] which combines both kinds of separation referred to here.

Electrophoresis was carried out first in filter paper using barbitone buffer (*p*H 8·6) and then in starch gel using the discontinuous buffer system of Poulik[12]. The substrates used to detect and differentiate between the various zones of esterase activity were α-naphthylacetate, β-naphthylacetate, β-carbonaphthoxycholine, and *o*-nitrophenylbutyrate. α-naphthylacetate, β-carbonaphthoxycholine and *o*-nitrophenylbutyrate have been shown to be hydrolysed very much more readily by serum cholinesterase than by arylesterase[13-15]. β-Naphthylacetate[13], however, is hydrolysed by both enzymes at significant rates. These enzymes may also be differentiated in terms of their response to various inhibitors and activators. In the present investigations we used physostigmine (10^{-5} M), tetraethyl pyrophosphate (TEPP) (10^{-6} M), ethylenediamine tetraacetic acid (EDTA) (10^{-3} M) and Ca^{++} (10^{-3} M) for this purpose. Both physostigmine and TEPP in low concentrations are known to inhibit serum cholinesterase activity but not to affect arylesterase while EDTA inhibits and Ca^{++} activates arylesterase[4,16-19].

Fig. 1 illustrates the main esterase zones distinguishable after electrophoresis of 15 µl. of fresh adult serum using α-naphthylacetate as substrate. Four of the zones (C_1, C_2, C_3 and C_4) showed properties characteristic of serum cholinesterase activity. They were also detected with β-naphthylacetate, β-carbonaphthoxycholine, and *o*-nitrophenylbutyrate, and they were completely inhibited by physostigmine and TEPP. The activity in these zones was, however, unaffected by EDTA or Ca^{++} in the concentrations used here. A fifth zone (*A*), obtained with α-naphthylacetate, was also found with β-naphthylacetate and *o*-nitrophenylbutyrate, but not with β-carbonaphthoxycholine. It was not affected by physostigmine, TEPP, EDTA or Ca^{++}. It does not, therefore, appear to represent either serum cholinesterase activity or arylesterase activity. This zone (*A*) was found to be co-extensive with the area occupied by serum albumin, and it may represent the thermostable albumin esterase reported by Wilde and Kekwick[20]. Its close association with albumin was confirmed by the demonstration of two distinct zones of esterase activity corresponding with the two albumin zones found in cases of bisalbuminæmia.

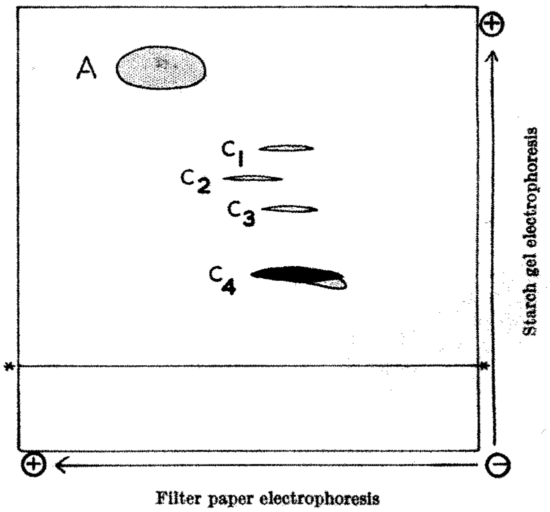

Fig. 1. Diagram of the main esterase zones after two-dimensional filter paper/starch-gel electrophoretic separation of 15 µl. of normal human serum

After 15 min incubation in 50 ml. 0·2 M phosphate buffer, *p*H 7·1, the following mixture was added to the sliced gel: 25 ml. phosphate buffer, 0·5 ml. 1 per cent α-naphthyl acetate in 50 per cent aqueous acetone, 10 mg Fast Red *TR* salt (Edward Gurr) *——*, line of insertion of filter paper in starch gel

Of the four zones, C_1, C_2, C_3 and C_4, which appear to be components of serum cholinesterase, one, C_4, was always considerably more intense than the others, and presumably accounts for most of the cholinesterase activity in normal serum. Although all four components migrated in the paper electrophoresis dimension in the α_2-β-globulin region, it is of interest to note that one of these components (C_2) had a significantly greater mobility in this dimension than the other three. Svensmark[21,22] has demonstrated, using paper electrophoresis, that the treatment of serum with neuraminidase results in a marked reduction in the mobility of the zone of serum cholinesterase activity, presumably because of the removal of sialic acid residues from the molecule. The four components, C_1, C_2, C_3 and C_4, have been observed to show this effect. The reduction in mobility in the paper electrophoresis dimension was paralleled by a similar reduction in mobility in the starch-gel dimension. Neuraminidase appeared to affect all four components in a similar manner and, using varying concentrations of neuraminidase and different periods of incubation, it was shown that the change of mobility was gradual. Zone *A* was not affected by neuraminidase.

A further zone of esterase activity with properties of serum cholinesterase could often be detected in trace amounts in adult serum. In the new-born (cord serum), however, this zone was very much more prominent (Fig. 2*a*). In cord serum it occurred together with components C_1, C_2, C_3 and C_4 described here, and appeared to account for about 15–30 per cent of the total serum cholinesterase activity as judged by the intensity of staining. This zone was also observed in seven sera obtained from human fœtuses (8–10 cm long), and in each case formed a substantial fraction of the serum cholinesterase activity present. A characteristic feature of this 'fœtal' serum cholinesterase zone was that it was obliquely orientated in the two-dimensional zymogram, in marked contrast to the zones C_1, C_2, C_3 and C_4 which were always horizontal. This finding suggests that the 'fœtal' zone is heterogeneous, the upper end of the zone consisting of enzyme molecules with a relatively greater mobility both in filter paper and in starch gel than those at the lower end. It was observed that a zone very similar in appearance to this 'fœtal' zone occurred in the very early stages of the modification of zone C_4 of adult serum by neuraminidase. It is possible, therefore, that the 'fœtal' zone may be due to the synthesis of serum cholinesterase molecules lacking their full complement of sialic acid residues, or to the partial removal of sialic acid residues after complete synthesis. Its occurrence is probably not due to the presence of neuraminidase activity in cord serum because incubation of the serum failed to produce further changes. Furthermore, there was always a clear zone of unchanged C_4 in cord or fœtal serum which was not present when adult C_4 was treated with neuraminidase to a sufficient degree to result in the formation of a zone resembling the 'fœtal' zone.

In a survey of sera from 300 randomly selected adult individuals 14 were observed to have an extra zone with the properties of serum cholinesterase (Fig. 2*b*). This zone had a slightly lower mobility than the main zone C_4 both in filter paper and in starch-gel electrophoresis. Among the individuals in whom it was found it was repeatedly detected in further serum samples. Thus it appears to be a constant individual characteristic. Furthermore, family studies showed that there was a much higher incidence of this peculiarity among the relatives of individuals showing it than in the general population. This suggests that it is genetically determined. Two typical pedigrees showing the segregation of this character are shown in Fig. 3. These and other family studies indicate that individuals possessing the extra zone are probably heterozygous for a relatively uncommon gene which determines the presence of this component. As it was not found possible, however, to detect the zone in all presumptive heterozygotes, the genetics of the peculiarity may be more complex. The occurrence of this variant was found to be independent of sex and age. So far it has not been found in association with other types of serum cholinesterase variants which may be detected by inhibition studies[23,24]. A more detailed account of the genetics of this peculiarity will be given elsewhere.

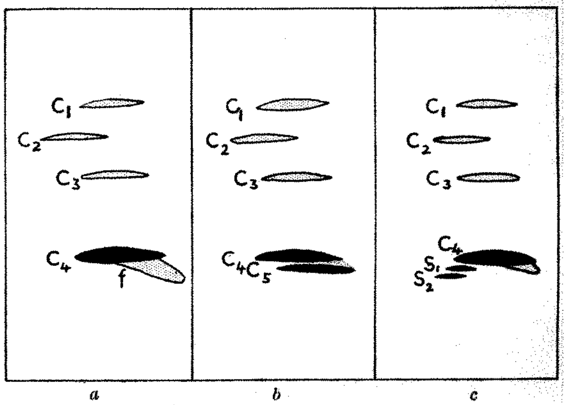

Fig. 2. Diagram showing three variations on the normal esterase pattern. Only that section of the gel containing material from the α–β area on paper electrophoresis is included
a, Cord blood—component *f*; *b*, genetically determined variant C_5; *c*, storage bands S_1 and S_2

In serum or plasma two further serum cholinesterase zones, S_1 and S_2, were observed (Fig. 2*c*). S_1 could be detected in all the samples after about ten days storage either at + 4° C or − 7° C. It migrated slightly faster than C_4 in the filter paper dimension, but slower in the starch-gel dimension. S_2 took longer to appear. Its mobility in filter paper was slightly greater than that of S_1, and its mobility in starch gel slightly less. On prolonged storage the intensity of both these zones increased. As it was accompanied by some decrease in the intensity of C_4 this suggests that it was from the C_4 zone that the two new zones appearing on storage were derived. S_1 and S_2 may be confused with the variant C_5 if the serum is only examined in starch gel in one dimension. However, in the two-dimensional system they are readily distinguished by their relative positions in relation to the main zone C_4.

Using β-naphthylacetate as substrate, and tetrazotized *o*-dianisidine as coupling reagent, two further zones of esterase activity may be detected and can be distinguished from serum cholinesterase by their properties. One of these zones migrates in paper electrophoresis with about the same mobility as serum albumin, but in starch gel it has a mobility similar to that of zones C_1, C_2 and C_3. It is a diffuse zone probably consisting of two or three separate components which, however, could not be sharply distinguished by the methods used here. It was not detected with α-naphthylacetate or inhibited by physostigmine or TEPP. The activity was, however,

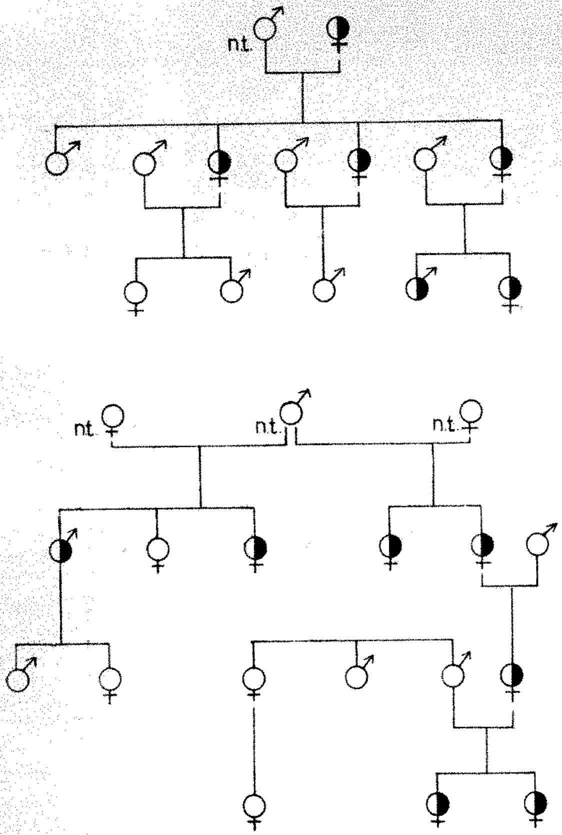

Fig. 3. Pedigrees of two families in which the esterase variant C_5 is segregating
◑, C_5 present; ○, C_5 absent; *n.t.*, not tested

increased by the addition of Ca^{++}, and inhibited by EDTA. This zone presumably represents arylesterase.

The second zone detected with β-naphthylacetate had different properties. It moved more slowly both in paper and in starch-gel electrophoresis than any of the other esterase components, and it appeared to be in the position occupied by β-lipoprotein, as detected either with lipid or with protein stains. Minor variations from serum to serum in the mobility of β-lipoprotein have been observed in starch gel electrophoresis, and these are exactly paralleled by variations in mobility of this zone. Furthermore, in certain rare sera containing two β-lipoprotein components, two corresponding zones were observed. The zone was not affected by EDTA, TEPP, physostigmine or Ca^{++}. Esterase activity associated with β-lipoprotein has been previously reported[25]. However, we have found that a similar zone can be obtained if β-naphthol itself is applied to the gel instead of β-naphthylacetate. It is possible, therefore, that in the experimental system used here any free β-naphthol arising from non-enzymatic hydrolysis may be specifically adsorbed on to β-lipoprotein and so contribute to the zone observed when β-naphthylacetate is used as substrate.

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INDUCTION OF TOLERANCE OF SKIN HOMOGRAFTS IN IMMUNOLOGICALLY COMPETENT MICE

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THAT unresponsiveness to skin homografts can be brought about across a 'strong' histocompatibility barrier (the *H*-2 locus in mice) was demonstrated by Shapiro *et al.*[1], who repeatedly injected the skin graft recipients with large doses of viable cells from the skin donor strain. The experiments reported here are also concerned with the immunological consequences arising from the injection of mice with allogeneic (= homologous) cells either on a single occasion or given over a prolonged period of time. They confirm the observation of Shapiro *et al.*, and they provide both qualitative and quantitative evidence that this kind of unresponsiveness is closely similar to, if not identical with, tolerance induced in fœtal or neonatal life.

In most of our experiments (*CBA* × *A*) hybrid (*H*-2*k*) spleen cells were injected into *A* strain (*H*-2*a*) mice; but in certain experiments the strain combinations were (*CBA* × *A*) hybrid → *CBA* or *CBA* → *C3H*. Whereas in the first two of these combinations donor and recipient strains differ at the *H*-2 histocompatibility locus, this is not true for the *CBA* and *C3H* strains, and for this reason the use of F_1 hybrid cells to avoid graft-versus-host reactions was not necessary in this strain combination. Unless otherwise stated, all cells were administered intravenously in the tail, and skin-grafting was carried out according to the technique of Billingham and Medawar[2]. The method of intravenous injection of 'adolescent' mice, the preparation of cell suspensions and other experimental techniques have been described elsewhere[3].

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Although some of the recipients were fully adult, most of the mice began their pre-treatment as soon as 13 days after birth; at this time they are immunologically competent but not far removed from that post-natal period in which it is possible to induce tolerance by conventional means, that is, by a single injection of allogeneic cells[4]. The experiments involving (*CBA* × *A*) hybrid and *A* strain mice were based on a weight-adjusted dose of 5×10^6 spleen cells/g because this is the dose which, in new-born *A* strain mice, is sufficient and necessary to establish tolerance in all recipients[4] (Fig. 1). On the other hand, when using *CBA* mice as donors and *C3H* mice as recipients the corresponding figure was found to be as low as $0{\cdot}15 \times 10^6$ cells/g, and this was therefore adopted as the weight-adjusted dose for this strain combination. The 30-fold difference in weight-adjusted dose is in some sense a measure of the strength of the *H*-2 locus compared with other histocompatibility loci.

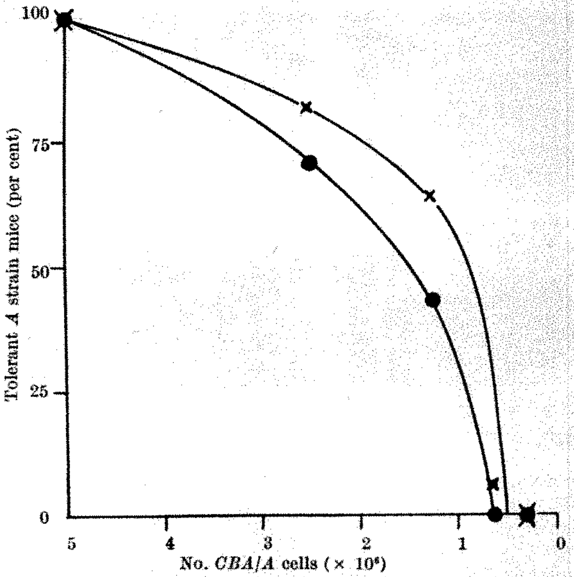

Fig. 1. Antigen dose (in the form of *CBA* × *A* hybrid spleen cells) and induction of tolerance in new-born *A* strain mice ×—×, (*CBA* × *A*) hybrid skin grafts surviving at 2 × median survival times of controls; ●—●, (*CBA* × *A*) hybrid skin grafts surviving at 5 × median survival time of controls

Our previous finding that, following a single intravenous injection of multiples of the weight-adjusted dose, 13-day-old mice do not become unresponsive to *H*-2 incompatible skin grafts[4] is supported by the results given in Table 1, lines 1–5. Far from developing unresponsiveness the mice were again weakly sensitized. (Owing to the fact that the recipients were skin-grafted almost at once it can be safely assumed that our previous results on these lines were not distorted by the interval of several weeks between injection of cells and skin-grafting.) Very similar results were obtained when *C3H* mice were injected with up to 20 times the weight-adjusted dose of *CBA* spleen cells (Table 1, lines 6–9). Regardless of whether donor and recipient strains differed at the *H*-2 locus the lower cell doses were again consistently more efficacious in sensitizing than were the higher doses, which failed to produce any significant deviation from the median survival time of the control grafts. So far as they go, the results obtained with *CBA* and *C3H* strains are in general agreement with the work of McKhann[5], who has shown that in *H*-2 compatible coisogenic *C57Bl* strains 1 or 5×10^6 cells injected intraperitoneally into adult mice sensitize, 50×10^6 cells fail to sensitize, and 200×10^6 cells induce tolerance in at least half the recipients.

Quite different results were obtained when, beginning on the thirteenth day after birth, *A* strain mice were repeatedly injected with (*CBA* × *A*) hybrid cells. The cells were administered intravenously every third or fourth day, and the recipients were grafted with hybrid skin on the day of the last injection. Following 7 injections the median survival time of the grafts was 30 days (Table 2, lines 1 and

Table 1. INTRAVENOUS INJECTION OF SINGLE DOSE OF ALLOGENEIC SPLEEN CELLS INTO FULLY ADULT RECIPIENTS

Line No.	Exp. No.	No. mice	Donor strain	Recipient strain	Cell dose	Time of skin-grafting	Approx. median survival time (days)
1	26,29*C*	12	(*CBA* × *A*)	*A*	Nil	—	13
2	49,53*B*	15	(*CBA* × *A*)	*A*	1×10^6/g	Day after injection	8½
3	48,53*A*	15	(*CBA* × *A*)	*A*	10×10^6/g	Day after injection	11
4	68	10	(*CBA* × *A*)	*CBA*	Nil	—	11
5	50, 54	11	(*CBA* × *A*)	*CBA*	30×10^6/g*	Day of last injection	10
6	75	20	*CBA*	*C3H*	Nil	—	12½
7	78	19	*CBA*	*C3H*	$0{\cdot}15 \times 10^6$/g†	Day of injection	11
8	79	21	*CBA*	*C3H*	$1{\cdot}5 \times 10^6$/g	Day of injection	12½
9	80	19	*CBA*	*C3H*	$3{\cdot}0 \times 10^6$/g	Day of injection	12

* Given as three equal injections of 10×10^6 spleen cells/g at intervals of 2 days.
† $0{\cdot}15 \times 10^6$ spleen cells/g = weight-adjusted dose for this strain combination.

Table 2. REPEATED INJECTION OF WEIGHT-ADJUSTED DOSES (5×10^6 CELLS/G) OF ALLOGENEIC SPLEEN CELLS INTO *A* STRAIN MICE, COMMENCING ON THE THIRTEENTH DAY AFTER BIRTH

Line No.	Exp. No.	No. recipients	Donor strain	No. weight-adjusted dose	Approx. total dose (× 10^6)	Route of injection	Median survival time (days)	No. mice highly tolerant (65 days)
1	26,29*C*	12	(*CBA* × *A*)	—	—	—	13	—
2	6,29*D*	22	(*CBA* × *A*)	1	20	i/v	10	0
3	37*A*	10	(*CBA* × *A*)	2	50	i/v	11	0
4	37*B*	11	(*CBA* × *A*)	4	150	i/v	15	1/11
5	26,29*A*	20	(*CBA* × *A*)	7	300	i/v	30	5/20
6	29*B*,61	28	(*CBA* × *A*)	12	650	i/v	$\geqslant 70$	17/28
7	64*	9	(*CBA* × *A*)	12	650	i/v	50	5/9
8	34,39†	9	(*CBA* × *A*)	7	300	i/v	$> 20 < 30$	3/9
9	38,62	23	(*CBA* × *A*)	7	300	i/p	$\leqslant 8$	0
10	63	11	(*CBA* × *A*)	12	650	i/p and i/v‡	11	0
11	72*A*	8	*A*	7	300	i/v	13	0
12	72*B*	8	*A*	10	500	i/v	12	0

* Skin-grafted 3½ weeks after last injection.
† Presensitized by intraperitoneal injection of 5×10^6 (*CBA* × *A*) hybrid cells when 7 days old.
‡ Injected alternately by these two routes; first injection intraperitoneal (i/p).

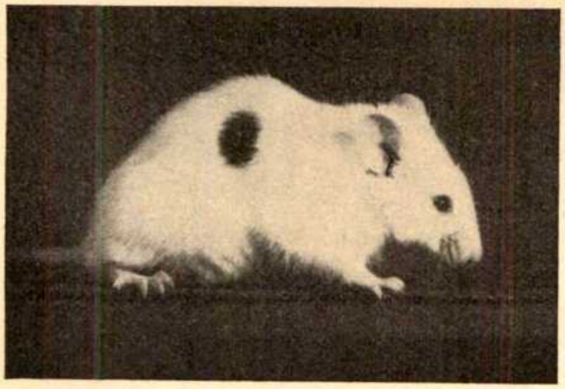

Fig. 2. Illustrating *A* strain mouse carrying a (*CBA* × *A*) hybrid skin graft 3 months after transplantation. Unresponsiveness was induced by twelve injections of the weight-adjusted dose, beginning on the thirteenth day of life

5) and 5/20 grafts survived in very good condition, with more or less normal hair growth, for at least 65 days (= 5 × median survival time of the control grafts). When the number of injections was 12 the median survival time well exceeded 70 days (Table 2, line 6) and 17/28 grafts survived in good condition (Fig. 2) until unresponsiveness was broken experimentally by the transfer of normal or pre-sensitized *A* strain lymph node cells or until the animals were killed so that their spleens could be used for chimera tests. That this unresponsiveness was not due to non-specific factors such as physiological stress was clearly shown by the normal survival times of grafts in control experiments in which 13-day-old *A* strain mice were subjected to exactly the same regimen of injections using syngeneic *A* strain cells (Table 2, lines 11 and 12).

Several other points arise from the results summarized in Table 2. (*a*) Pre-sensitization of the recipients by an intraperitoneal dose of 5×10^6 cells on the seventh day after birth did not appreciably prejudice the induction of unresponsiveness (line 8). This experiment could bear repetition with a more powerful sensitizing stimulus in the form of skin grafts. (*b*) An interval of 3½ weeks between the last injection and skin-grafting reduced the proportion of mice exhibiting some degree of unresponsiveness without significantly lowering the proportion of highly unresponsive mice (line 7). (*c*) When 13-day-old mice were injected 7 times intraperitoneally they were strongly sensitized (line 9). (*d*) Alternation between intraperitoneal and intravenous routes, commencing with the former, led to a slight shortening of the median survival time (line 10) compared with that of the control grafts; but the degree of sensitization to be read into this was nowhere near as great as that produced by intraperitoneal injections alone. (*e*) There was evidence that the development of unresponsiveness was progressive and that before unresponsiveness was finally established the mice went through a transient period of sensitization: 1 or 2 injections of the weight-adjusted dose weakly sensitized (lines 2 and 3), 4 injections raised the median survival time of the grafts just beyond that of the controls (line 4), and 7 injections resulted in a very substantial increase in the median survival time and the induction of a high degree of unresponsiveness in 25 per cent of the mice (line 5) (Fig. 3). There is, however, one qualification which must be made about these results, namely, that in the experiments summarized in lines 2–4 of Table 2 the mice were all skin-grafted 5 weeks after birth irrespective of the number of injections given, so that the interval between the last injection and grafting varied from 3 weeks (1 injection) to one week (4 injections), whereas in the long-term experiments (7 or 12 injections) the mice were grafted one day after the last injection. This variable was forced on us by the difficulty of skin-grafting very young mice, and we intend to eliminate it in future experiments with fully grown mice. The same shortcoming in experimental design prevents us from being absolutely certain that, just because antigen was continued to be administered in the long-term experiments, the development of sensitivity in the earlier stages was not blocked by excess antigen.

Experiments on the induction of tolerance by the repeated injection into adult animals of certain proteins also show that, prior to the development of tolerance, there is a phase in which antibodies are produced[6,7].

The properties of unresponsiveness are still being examined, but several conclusions can be stated here in bald terms (see also ref. 3). (1) As already indicated (Table 2, lines 11 and 12), unresponsiveness is not due to non-specific factors. Furthermore, like classical tolerance it is strain specific: five highly unresponsive mice promptly rejected *C*57 or *AU* skin grafts without deterioration of their long-established (*CBA* × *A*) hybrid grafts. It must, however, be borne in mind that, as in all specificity tests performed on mice which have become cellular chimeras (see later) as a result of the injection of allogeneic lymphoid cells, the rejection of third party grafts is likely to be influenced by the immunological reactivity of the donor cells. (2) Unresponsiveness can be broken experimentally by the transfer of normal or pre-sensitized lymph node cells syngeneic with the host. (3) There is no evidence from our experiments that *A* strain mice which accept (*CBA* × *A*) hybrid skin grafts form hæmagglutinating or cytotoxic antibodies. In view of the fact that this particular strain combination is a poor one so far as the production (or detection) of hæmagglutinins is concerned we are at present investigating the effect of injecting (*CBA* × *A*) hybrid cells into *CBA* hosts. (4) The unresponsive mice become cellular chimeras: by quantitative *in vivo* tests[3] it has been established that their spleens contain a proportion of donor cells and that this proportion is unlikely to exceed 5–10 per cent. These somewhat indirect tests are now being followed up by experiments utilizing *CBA* (*T*-6) chromosomally 'labelled' cells.

The foregoing properties are exactly those of immunological tolerance conferred on mice in fœtal or neonatal life[8,9], and it may be concluded that we are dealing here with the same phenomenon. Quantitatively the induction of tolerance in neonatal and immunologically competent mice is very different indeed: one requires one single weight-adjusted dose whereas the other cannot be brought about by as many as 10 or even 20 times the weight-adjusted dose if it is given on a single occasion. This difference is almost certainly due to the absence, or virtual absence, in the neonatal *A* strain mouse of immunologically competent cells—a conclusion which is amply supported by our investigations on the induction of tolerance in the first two weeks of life[4]. To overcome the barrier created by the presence of mature antibody-forming cells the cellular antigen must evidently be administered over a prolonged period, at least when donors and recipients differ at the *H*-2 locus. But our experiments do not, at

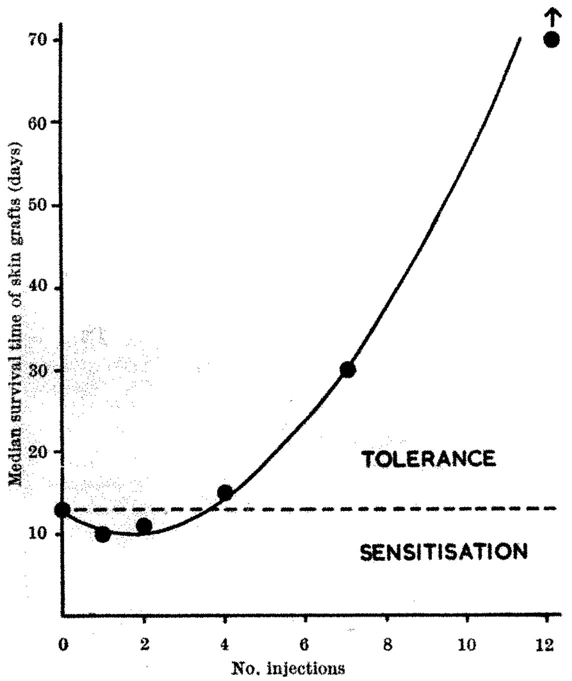

Fig. 3. The effect on the median survival times of (*CBA* × *A*) hybrid skin grafts transplanted to groups of mice injected from the thirteenth day of life, twice weekly, with weight-adjusted doses of hybrid spleen cells (Table 2, lines 1–6, and text)

present, permit us to make a statement on the nature of tolerance at a purely cellular level, that is, as to what happens when a cell becomes tolerant: our results could be equally well accommodated by the hypothesis of progressive paralysis of mature antibody-forming cells by excess antigen as by the hypothesis postulating the death of these cells by prolonged contact with antigen and the induction of tolerance in immature stem cells.

From our knowledge of the relationship between antigen dose and regimen of administration we predicted that it should be possible to induce tolerance by the repeated injection of cell doses which, when given just once to new-born mice, are inadequate for tolerance induction—provided that the course of injections is begun in neonatal life. $0{\cdot}5 \times 10^6$ cells/g (that is, 1/10 of the normal weight-adjusted dose) seemed a suitable dose (Fig. 1) and accordingly 18 *A* strain mice were injected with this dose of (*CBA* × *A*) hybrid spleen cells starting within 12 h of birth and continuing every third or fourth day. When the mice were grafted with hybrid skin 3 weeks after the last injection, 72 per cent showed some degree of tolerance and 50 per cent were fully tolerant in the sense that they grew normal crops of hair and survived for a minimum of 65 days (= 5 × median survival time of the control grafts). In mimicking the results of experiments with 13-day-old mice (Table 2, line 6) these results fulfilled our prediction, and they lend further weight to the contention that the difference between tolerance induction in new-born and immunologically competent mice is essentially a quantitative one. Since the degree of tolerance conferred in the first 5 weeks of life by 10 injections of 1/10 of the weight-adjusted dose is considerably inferior compared with the degree of tolerance induced by one weight-adjusted dose in new-born mice (Fig. 1) it is clear that, with increasing age, it is more difficult to establish tolerance even with a dose of cells based on the weight of the recipients. This would be explained if the increase in weight of young mice does not wholly reflect the increase in the number of immunologically competent cells—a point on which we have, at present, no precise information.

The results given in Table 1 (see also Brent and Gowland[4]) are different from those reported by Guttmann and Aust[10] and by Wigzell[11] who, respectively, found that the injection of a single large dose of *C3H*/*A* spleen cells into *C3H* adults, and of *CBA*/*A* cells into *A* strain adults, led to a striking prolongation of skin graft survival. Some of the cell doses used by us unsuccessfully were of the same order of magnitude. Guttmann and Aust tested their mice with very large skin grafts compared with those used in our experiments, and this might conceivably have influenced the outcome of their experiments. Genetic differences between sub-strains of *A*, *CBA* and *C3H* could also be an important contributory factor, especially in view of Wigzell's finding that skin transplanted from *A* strain donors maintained in the Karolinska Institute to *A* strain mice kept in our department were not accepted (personal communication). Unlike Wigzell[11] we have so far failed to demonstrate the presence of humoral antibodies in our tolerant mice.

The striking difference in results obtained with intravenous and intraperitoneal routes requires comment. We have yet to convince ourselves that this is due to a more efficient distribution of the cells rather than to a feature unique to the intravenous administration of antigen. The observation that alternation between the two routes does not lead to the induction of tolerance suggests that the latter explanation is the more plausible, and this question is now being investigated more fully.

The main conclusions to be drawn from our experiments are that unresponsiveness of skin homografts can be established in immunologically competent mice by the repeated injection of viable lymphoid cells; that the intravenous injection route is obligatory when attempts are made to overcome the barrier of the *H*-2 locus; that this unresponsiveness is almost certainly the same as immunological tolerance induced in fœtal or neonatal life with a single stimulus; and that the difficulties encountered when attempts are made to induce tolerance in post-natal life are intimately bound up with the increase in immunologically competent cells from birth onward.

We thank Dr. P. B. Medawar and Dr. H. A. Mitchison for their advice.

Note added in proof. We have now satisfied ourselves that immunologically mature (*CBA* × *A*) hybrid mice made tolerant, or partially tolerant, of *CBA* skin grafts do not possess hæmagglutinating antibodies, and that this cannot be due to *in vivo* absorption by donor strain cells.

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INDUCTION, REVERSION AND EPITROPHISM OF FLAX GENOTROPHS

By DR. ALAN DURRANT

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EXPERIMENTS with many thousands of plants carried out over a period of nine years have shown that heritable changes are induced in flax plants of the variety Stormont Cirrus when they are supplied with different combinations of nitrogen, phosphorus and potassium, and when they are grown for at least the first five weeks in a heated greenhouse[1]. The original variety is a plastic genotroph which is changed into a large stable genotroph, or a small stable genotroph, depending on the nutrients supplied. The large stable genotroph is three or more times the weight of the small stable genotroph and both have remained unchanged in inheritance for seven generations irrespective of the fertilizers that have since been applied. Both behave as two distinct genetic types, giving equilinear inheritance in their reciprocal crosses and no transmission through their reciprocal grafts. The plastic genotroph is intermediate in weight, but taller than the other two, and all plants are highly uniform within each genotroph.

Studies have been made on the variation in the F_1 and F_2 of the reciprocal crosses between the large (L) and small (S) stable genotrophs. Table 1 gives the results of separate crossing experiments with different groups of parent plants carried out in different years at Aberystwyth. The F_2 was examined first and the coefficients of variation, which compensate for differences in the means, show that the variation in both reciprocal crosses is higher than within the individual parent crosses, suggesting genic segregation. On the other hand, there is a marked difference between the two reciprocal crosses, the $S \times L$ variation being higher than any of the other three types of cross. In addition, the same pattern of variation is obtained in the F_1 of these crosses, and in the F_1 of other crosses, in plant weight and plant height. The correlation between the F_1 and F_2 coefficients of variation of the four types of crosses is extremely high ($r = 0{\cdot}87$ for F_1 weight/F_2 weight; $r = 0{\cdot}97$ for F_1 height/F_2 weight). Genic segregation therefore is probably not the sole explanation for the increase in variation in the F_2 of the reciprocal crosses.

To check whether the F_2 variation was at least in part due to the variation in the F_1, five F_1 plants were selected from each of the four types of crosses, and F_2 families of five plants were grown from each in winter in a heated greenhouse. Table 2 shows that the positive correlation between F_1 plant weights and F_2 family means is virtually complete in the $S \times L$ cross, and high in the $L \times S$ cross, while the small amount of variation among the F_2 family means of each of the parent crosses is negatively correlated with the F_1 plant weights, a maternal relationship commonly occurring under winter conditions. There is, therefore, an immediate breakdown, or instability, in the F_1 of crosses between the two stable genotrophs producing to all appearances random, heritable variation, which presumably arises from the opposing nature of the factors or processes determining the two genotrophs, or the destruction of their delicate balance by chance metabolic variation. Since factors in addition to nutrition appear to be involved, the various forms may be referred to as 'epitrophs'. There is evidence from exploratory experiments at the Earhart Laboratories that the F_1 instability may be arrested if the F_1 plants are grown under certain conditions but it is not clear at present which are the relevant environmental factors.

The large genotroph used for these crosses was induced at Aberystwyth by applying nitrogen, phosphorus and potassium to the plastic genotroph, and the small by applying nitrogen and potassium, but taking into account soil fertility and soil inter-

Table 1. MEAN PLANT WEIGHTS (G), HEIGHTS (CM) AND VARIANCES OF F_1 AND F_2 OF CROSSES WITH LARGE (L) AND SMALL (S) STABLE GENOTROPHS

Coefficient of variation (C of V, standard deviation divided by mean) is averaged over each set of experiments

		$L \times L$		$L \times S$		$S \times L$		$S \times S$	
		Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
F_2 plant weight	Exp. 1	33	167	27	172	28	353	7	11
	Exp. 2	35	135	20	81	22	103	10	7
	Exp. 4	36	84	22	53	28	209	11	18
	Total	104	386	69	306	78	705	28	36
	C of V	0·33		0·42		0·55		0·37	
F_1 plant weight	Exp. 4	64	293	50	203	53	240	17	11
	Exp. 5	65	233	49	153	47	228	21	31
	Exp. 6	103	1,130	81	1,166	75	2,503	26	382
	Total	232	1,656	180	1,522	175	2,961	64	424
	C of V	0·28		0·32		0·43		0·20	
F_1 plant height	Exp. 1	85	15	81	19	77	30	71	8
	Exp. 2	61	15	61	19	60	41	52	18
	Total	146	30	142	38	137	71	123	26
	C of V	0·05		0·06		0·09		0·06	

Table 2. Variances and Correlation Coefficients (r) of F_1 Plant Weights (g) and F_2 Family Means of Crosses with Large (L) and Small (S) Stable Genotrophs

	$L \times L$	$L \times S$	$S \times L$	$S \times S$
Variance of F_1 plants	4,196	7,626	3,587	865
Variance of F_2 family means	1	203	83	11
r_{F_1/F_2}	−0·19	0·62	0·94	−0·67

actions, it was suspected that the balance of nitrogen and phosphorus was particularly important, nitrogen inducing the large genotroph and phosphorus the small. At the Earhart Laboratories nitrogen and phosphorus were added separately to different groups of plants of the plastic genotroph grown in a gravel and vermiculite mixture with dilute Hoagland's solution in different day/night temperature (°C) combinations. The progeny of the nitrogen and phosphorus treated plants were approximately equal in weight to the large and small genotrophs respectively when the treated parent plants were grown in 27/15 and 23/23 temperatures, progressively smaller induced changes occurring in 27/11, 27/7 and 31/7, and none in 19/11. This confirms the importance of the balance of nitrogen and phosphorus, in the absence of soil interactions, for the induction of changes transmitted to the first generation and also shows that high day and night temperatures are necessary. On the other hand, these changes were not inherited by the second generation grown at Aberystwyth so that there remain other unknown environmental factors. These impermanent changes are nevertheless of additional interest and are of some significance in view of the temperature requirements for partial reversion mentioned here.

At Aberystwyth the large and small genotrophs were grown under conditions giving a greater day/night temperature difference, maximum natural day length and maximum light intensity during the first five weeks of their growth, and the two following generations were grown under approximately similar conditions. By the third generation the difference in weight between the two genotrophs had practically vanished although other characteristic differences such as branching habit, leaf size and colour remained distinct. At the Earhart Laboratories the original large and small stable genotrophs were grown in day/night temperatures of 27/15 and 27/7 in 50 per cent Hoagland's solution, and the two following generations in all combinations of these temperatures as shown in Table 3, where the differences between the large and small genotrophs in the second generation are entered. The differences between the two sets of differences, one set for the second generation plants grown in 27/15, and the other for those grown in 27/7, in addition to assessing the responses in this generation, remove a large amount of extraneous maternal effects of the previous generation's treatments, and show that the difference between the large and small genotrophs grown consistently in 27/7 is greatly reduced compared with those grown consistently in 27/15. Those grown previously for one generation in each of the two temperature combinations show intermediate differences. This partial reversion is probably not permanent and appears from these and other observations to occur when parents and their progeny are grown consistently in the same environment of strongly contrasting day/night temperatures (27/7). In view of these results, the induction and transmission to the first generation of only small changes at 31/7 and 27/7 temperature combinations mentioned here are not unexpected.

Altogether there are here three classes of heritable change. Class I: changes which are maintained largely in those environments inducing them, that is, the partial, temporary reversion of the large and small genotrophs. Class II: the induction of the large and small stable genotrophs from the plastic genotroph. Class III: the heritable variation arising immediately in crosses between the large and small genotrophs. Classes II and III are almost certainly nuclear with the cytoplasm having a subsidiary role. It is not clear whether intermediate genotrophic forms induced by certain fertilizer and temperature treatments, or some of the epitrophs, are stable or transitional to the three genotrophs mentioned earlier, but it is notable that all the variation originates from the influence of the environment on a hitherto uniform variety of flax.

These results are more profitably discussed when other crossing experiments are completed. Numerous comparisons can be made with other forms of heritable change, cellular differentiation, paramutation and similar phenomena, but it is particularly interesting to note that the variation in Class III is strikingly similar to the variation reported by Sonneborn[2], Kimball[3], Nanney[4], and other workers, among caryonides of the unicellular organisms *Paramecium aurelia* and *Tetrahymena piriformis*, where exconjugants give rise to daughter cells of different mating types during successive mitotic divisions. Variation of this type may therefore be common and perhaps responsible for part of the variation normally ascribed to classical segregation and recombination.

Varieties of other crop plants, unlike flax varieties, have been bred for characters such as plant weight and seed yield[1], and they may be equivalent to the stable, rather than to the plastic, flax genotrophs, so that the probability of Class I changes occurring would be higher than those of Class II. One example is known ; Highkin[5] has reported that peas of two varieties deteriorate in size and vigour when grown for several generations in constant day/night temperatures, but are restored after two or three generations in a fluctuating day/night temperature. Speculatively, assuming similar metabolic changes to those in flax, the pea varieties could have arisen initially from plastic pea genotrophs grown at a reasonably high temperature with excess phosphorus—not improbable conditions. On the other hand, it may be simply a question of breeding.

It is probable that individuals in natural populations vary in their possession of heritable factors or processes which can be altered or stabilized by the environment. Mutation anywhere in the heredity material, or crossing between unlike individuals, may

Table 3. Differences (L–S) in Weight (g) between Large (L) and Small (S) Stable Genotrophs of the Second Generation descended from Parents and Grandparents grown in Different Combinations of Day/Night Temperatures (°C)

Parental generation temperature	27/15	27/15	27/7	27/7
First generation temperature	27/15	27/7	27/15	27/7
Second generation grown in 27/7 (a)	18	18	10	10
Second generation grown in 27/15 (b)	10	16	9	15
Difference (a–b)	8	2	1	−5

stabilize some factors to give stable genotrophs, and break down others to give plastic genotrophs, the environment thereby directly altering the characteristics of a population, as distinct from its indirect action in natural selection. Although it is possible that heterozygous individuals in natural populations may be buffered to a greater extent against all three classes of heritable change, such changes may be widespread, and potent factors in biological evolution.

I thank Prof. George W. Beadle and Prof. Anton Lang for the opportunity to carry out some of these experiments in the Earhart Laboratories, California Institute of Technology.

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BIOASSAY OF TRADE WASTES

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DURING recent years river boards, with the co-operation of local authorities and industry, have done much to improve the condition of many rivers which are heavily polluted by sewage and industrial wastes. Standard methods of analysis have been developed, but there is a lack of information concerning the toxic effects of discharges of complex wastes on the plants and animals in natural waters. In particular, there appears to be a need of a quantitative method for the assessment of the toxicity to fish of trade wastes. Much work has been done on the subject; but so far all the methods have been prolonged and the interpretation of results leaves much to be desired.

A relatively simple and rapid method applicable both to field and laboratory investigations has been developed in this laboratory. A series of dilutions of a representative sample of effluent is prepared with water of similar composition to the natural water receiving the discharge. A glass bottle with a capacity of 2 l. is filled with each test solution, two fingerling brown trout are introduced and the containers are stoppered, avoiding air bubbles. The dissolved oxygen concentration in each solution is measured at the beginning of the experiment and on the death of the fish, using a portable dissolved oxygen meter which incorporates a dropping mercury electrode. It has been found that the residual oxygen concentration is moderately constant for a given batch of fish in clean water, but when harmful substances are present the residual oxygen concentrations are higher. Since the toxicity to fish of many compounds increases as the dissolved oxygen of the water falls toxicity is enhanced as the fish respires and consequently the survival period of the fish decreases.

Experimental results are presented in Fig. 1 which show that concentrations of cyanide down to 0·025 p.p.m. and copper down to 0·1 p.p.m. inhibit the uptake of oxygen by trout in the sealed containers during experiments of 300 min duration. Thus, compared with conventional bioassay, this method constitutes a more rapid and sensitive means of detecting these substances in low concentrations. For example, Herbert and Merken[1] found that the median survival period of rainbow trout was at least 10,000 min in water saturated with oxygen containing 0·05 p.p.m. of cyanide, and it is recorded in the annual report of the Water Pollution Laboratory for 1959[2] that the median survival period of rainbow trout is of the order of 1,000 min in aerated water containing 0·1 p.p.m. of copper.

The new technique has also been used for the bioassay of complex chemical wastes, and the results compare favourably with those obtained from a more conventional method of bioassay where fish were immersed in aerated solutions of effluent and their survival time noted. Concentrations of effluent which killed fish within 96 h in aerated waters also impaired the reduction of the oxygen concentration by trout in sealed containers within 300 minutes.

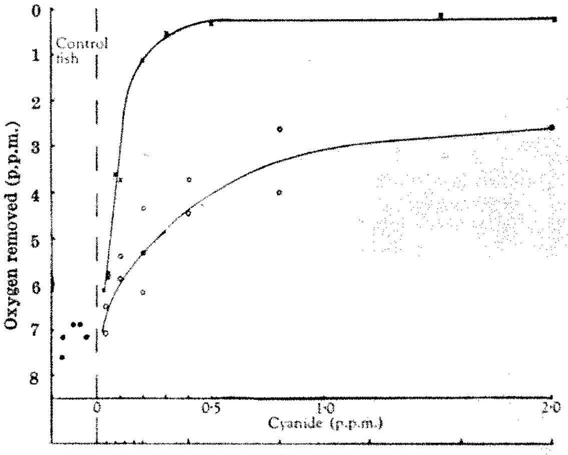

Fig. 1. Effect of cyanide (×) and copper (○) on the uptake of oxygen by brown trout

It is concluded that the technique permits the rapid detection of solutions containing low concentrations of copper and cyanide which are toxic to fish. It seems likely that the method might also be used to make rapid assessments of the toxicity to fish of complex trade wastes.

Where many effluents are discharged to a watercourse it would be advantageous to pinpoint the more toxic discharges at the site of their discharge. The new technique is particularly suitable for this type of investigation, as it is a rapid method and the apparatus is simple and portable. Investigations at the site of discharge would also avoid transporting samples to a central laboratory during which time the quality of the effluent might be altered by chemical reactions or biological activity.

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LETTERS TO THE EDITOR

ASTRONOMY

A Corrugated Model for the Lunar Surface

We would like to suggest that a number of previously uncorrelated visual and infra-red lunar observations may be explained by assuming that the surface of the Moon has indentations the scale of which is too small to be resolved optically by terrestrial observations. For simplicity of argument, we consider the shape of the indentations to be as shown in Fig. 1. In addition, for the arguments given here to be rigorously true, the linear dimensions of the indentations must be appreciably greater than the parametric distance for thermal conduction during a lunar cycle (~ 3 cm).

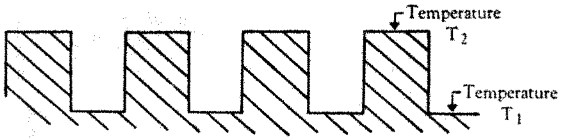

Fig. 1

It is clear from radiation considerations that during an eclipse the temperature T_1 of the bases of the indentations of our model vary very much less than will the temperature T_2 of the upper surfaces. Eclipse measurements[1,2] show a greater variation of effective temperature for regions close to the lunar limb than for regions at the centre of the lunar disk. This result is in agreement with the proposed model since radiation received from the lunar limb would come only from the top of the indentations, whereas that from the centre of the disk would consist of equal contributions from the base and top.

A similar argument may be applied to the results of observations of the effective temperature of the centre of the disk measured as a function of time throughout a complete lunar period[3,4]. With the model considered, the variation of temperature of the base of the indentations would be very much less than for a simple flat surface, for in addition to radiation considerations the bases of the indentations would receive solar radiation for only a small fraction of the Moon's period. The observed variation of effective temperature during lunation is less than that predicted from the application of the results of eclipse measurements to a simple plane surface, a result which would be expected from the model here proposed.

Infra-red measurements[1] show that when the sub-solar point is near the lunar limb (first and last quarters) its effective temperature is about 50° C lower than when it coincides with the centre of the lunar disk at full Moon. Previously, this effect has been attributed to the large-scale mountainous nature of the lunar surface, but an examination of lunar topography shows that the average lunar slope (~ 0·01 radian) is insufficient to account for the large effective temperature change, although such a change could well be accounted for by an indented model.

Finally, photometric measurements of radiation of wave-length 3000 Å–9000 Å show deviations of the Moon's surface from that of a simple diffuse reflector. In particular, at full Moon the radiation received from the lunar limbs is relatively greater than that expected from a diffusely reflecting sphere, while the sub-solar point appears considerably brighter than would be expected from observations of this point at other times during the lunar period. Models similar to that suggested here have, in fact, already been investigated both experimentally[5] and theoretically[6,7] and they have been found to give good agreement with lunar photometric measurements.

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PHYSICS

Slicks on Lake Huron

While examining the movement and dispersion of groups of drift-bottles released simultaneously at Douglas Point, on Lake Huron, we came upon some pronounced 'slicks', well known to oceanographers. These can be recognized by eye as streaks of smooth water from which the capillary waves are absent and in which quantities of organic matter and other floating debris collect. There are apparently a number of possible mechanisms for the production of slicks, all leading to accumulation of surface material through confluence of the surface water layers, which sink at the slick, leaving any floating material to collect on the surface.

In our experiments a group of small cylindrical jars, 5 cm in diameter, 8 cm high, lids painted fluorescent yellow and weighted so that the lid floats level with the surface, were released from a boat, with the intention of following their dispersion by turbulent diffusion. In the initial phase the growth of the 'cloud' exhibited the typical features of 'relative' turbulent diffusion in that the rate of growth increased with the size of the cloud. This is in accordance with the observations of Richardson and Stommel[1] and the theory of Batchelor[2]. At some stage of this regular turbulent diffusion then the group would typically arrive at a slick and the bottles would re-collect along a line oriented at some angle against the original direction of mean drift. When this angle was not too far from 90°, the whole group became quite concentrated again, because the diffusion in a direction perpendicular to the direction of the mean drift was typically much less than along it. Afterwards, for a prolonged period, the bottles would remain in the slick spreading out along it but remaining in a narrow line (a slick is typically 1–2 m wide) and travelling along the slick with the local drift velocity. On some occasions the bottles remained in the slick for 5–6 h and apparently only left it when the entire slick disappeared in a change of wind. The slicks seemed to be most pronounced and permanent around a wind speed of 5 m.p.h., in May, before the development of the thermocline.

Surface temperature measurements in and near slicks invariably showed a temperature difference of 0·5°–2·0° C from one side to the other, often over a very short distance. On one occasion, when strong solar heating was present, the temperature at the centre of the slick was 1·0° C higher than on the warmer side. The surface temperatures measured in these observations were between 8·0° and 14·0° C. All the observations were carried out within 500 m from the shore in water 8–10 m deep.

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A Superconducting Solenoid

Tests have been made recently on a superconducting solenoid constructed from what is believed to be the longest continuous length of niobium–zirconium alloy wire so far produced in the United Kingdom. An ingot of the alloy (75 at. per cent niobium, 25 at. per cent zirconium) was swaged and cold drawn to give approximately 320 feet of 0·010-in. diameter wire. 100 ft. of this was used to construct the coil, which is shown in the accompanying photograph. The insulation was provided by a single layer of silk wrapping round the wire and by sheets of 'Melinex' between the layers of the coil.

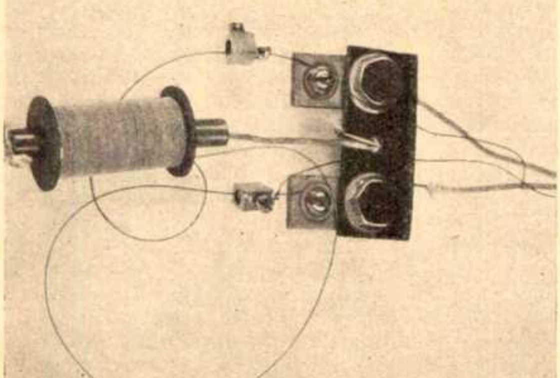

Fig. 1

The coil had the following characteristics: length of winding, 3 cm; internal diameter, 1 cm; external diameter, 1·6 cm; No. of turns, 992; maximum magnetic field, 382 gauss/amp.

A copper rod was inserted in the centre of the solenoid to absorb the energy released when the coil was driven normal (that is, became resistive). During operation the coil was immersed in liquid helium at 4·2° K.

The maximum current required to drive the wire normal was 23 amp; corresponding to a critical current density in the superconductor of $4 \cdot 5 \times 10^4$ amp/cm^2 and a maximum magnetic field at the centre of the solenoid of 8·8 kilogauss. No 'training' effect[1] was noted during repeated runs. This current density agrees with the values of $5–7 \times 10^4$ amp/cm^2 in a field of 10 kilogauss obtained by extrapolating data obtained by Berlincourt[2] for straight wire specimens of the same composition.

The work described here forms part of an extensive programme, initiated at the Nuclear Research Centre of C. A. Parsons and Co., Ltd., at the beginning of this year, to investigate the fabrication, properties and practical applications of superconductors. Pieces of the wire have been passed to Government and university laboratories for additional tests.

We thank the Directors of C. A. Parsons and Co., Ltd., for permission to publish this communication.

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CRYSTALLOGRAPHY

Anomalous Extinction Effects in X-ray Reflexion by Crystals

It is commonly supposed that the effect of the presence of perfect blocks produces a decrease in the intensity of X-ray reflexion from a mosaic crystal. This phenomenon is therefore known by the name of extinction (primary or secondary extinction, according to the conditions of the experiment). However, it appears that this is not necessarily so, and that, under certain conditions, a perfect crystal can give an integrated reflexion which is larger than that of a mosaic crystal. In this case, one would not have an extinction, but an enhancement in the reflected intensity due to the deviation of the crystal from the ideally mosaic state. These results follow from the dynamical theory, in particular from the formulæ developed by Ramachandran[1], and have actually been verified in this laboratory using perfect and milky crystals of calcite as representatives of the two extreme limits of perfection.

As is well known[2], if the reflexion occurs from the surface of the crystal, then it can be shown that the integrated reflexion of a mosaic crystal is larger than that of a perfect crystal and is, in fact, the upper limit under different conditions for the latter. However, for an internal reflexion ('Laue case') the integrated reflexion for a perfect crystal can be much larger than that for a mosaic crystal, due to the occurrence of the Borrmann effect. Using the notation of Ramachandran[1] and considering a thickness for which $(\mu t/\gamma)$ is of the order of 5, the integrated reflexion of a symmetric internal reflexion from a perfect crystal in y-units can be represented by the approximate formula:

$$R_y^P = \frac{\pi}{2}\,\frac{1}{(2\pi\mu t\varepsilon K/\gamma)^{1/2}}\,e^{-\mu(1-K\varepsilon)t/\gamma}$$
$$= \text{constant} \times \frac{e^{-\mu(1-K\varepsilon)t/\gamma}}{t^{1/2}}$$

where ε is equal to (ψ''_H/ψ''_0), the other symbols having the same significance as in ref. 1. The corresponding expression for a mosaic crystal is:

$$R_y^M = \frac{\pi}{2}\cdot K\,.\,(\mu t\varepsilon/\gamma k)e^{-\mu t/\gamma} = \text{constant} \times e^{-\mu t/\gamma} \times t$$

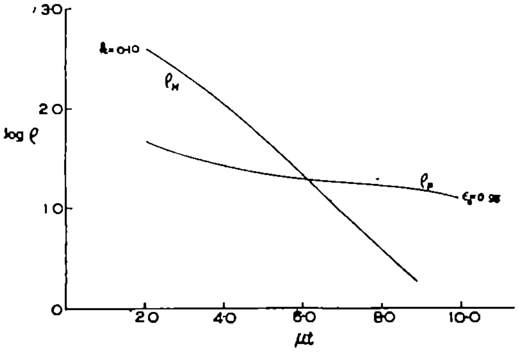

Fig. 1. Variation of the integrated reflexion of a perfect crystal (ρ_P) and of a mosaic crystal (ρ_M) with thickness for a typical X-ray reflexion (expressed in arbitrary units)

Fig. 1 shows the variation with thickness of the integrated reflexions ρ_P and ρ_M which are proportional to R_y^P and R_y^M, respectively, for a typical crystal with $\varepsilon = 0{\cdot}95$, $k = 0{\cdot}10$. In Fig. 1, ρ_P and ρ_M are expressed in arbitrary units, and the exact formulæ for R_y^P and R_y^M have been used to compute these quantities. It will be seen from this that while the mosaic crystal has a higher integrated reflexion than a perfect crystal when the crystal is thin, the situation is reversed when it is thick. The reversal occurs at a thickness of the order of 0·2 mm and greater for typical crystals of reasonable absorption, and the thickness at which the reversal occurs for a particular reflexion can be even smaller than 0·2 mm, if the reflexion is exceptionally strong. It will be seen from the foregoing formulæ that the essential reason for this strange result is the occurrence of the factor $(1 - K\varepsilon)$ in the exponent in the expression for a perfect crystal.

The fact that ρ_P is greater than ρ_M under certain conditions has actually been verified by experiment using calcite crystals of thickness 0·4 mm. The internal reflexion for a perfect crystal has an integrated intensity about six times more than the mosaic crystal. The extension of this observation to the case of the ordinary small crystal bathed in the X-ray beam is not straightforward. Yet, it will be clear that in these cases a reflexion will be partly from the surface and partly through the crystal, and consequently it does not follow that the occurrence of a small degree of perfection in a crystal would necessarily lead to a reduction in intensity.

It has been suggested from a study of polarization effects in perfect and mosaic crystals[2-7] that studies using polarized X-rays can be used to correct for extinction effects. However, a study of the present theory shows that the very basis of the polarization method of correcting for extinction effects requires a re-examination. The results are being worked out and will be reported elsewhere.

Finally, these studies have indicated a very elegant method for studying the variation of the anomalous dispersion part of the scattering factor ψ''_H with the angle of scattering. It is possible to obtain this quantity from the ratio for the different states of polarization from a single perfect crystal.

We thank Sri M. Mallikarjunan for help with the experiments. One of us (R. P.) thanks the Council of Scientific and Industrial Research for the award of a senior fellowship.

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CHEMISTRY

Bond Alternation in Heterocyclic Ring Systems

IN a recent communication Davies[1] has made an interesting extension of Longuet-Higgins and Salem's treatment[2] of bond alternation to the case of monocyclic ring compounds of the type $(AB)_n$ such as the phosphonitrilic halides. The total energy is separated into σ- and π-parts: $E = E^\sigma + E^\pi$. He shows that:

$$E^{p\pi} = -\sum_l [\delta^2 + 4(\beta_1^2 + \beta_2^2 + 2\beta_1\beta_2\cos 2\pi l/n)]^{\frac{1}{2}} \quad (1)$$

for 'homomorphic' systems[3], where the combining orbitals on A and B have the same site symmetry, and that:

$$E^{d\pi} = -\sum_l [\delta^2 + 4(\beta_1^2 + \beta_2^2 - 2\beta_1\beta_2\cos 2\pi l/n)]^{\frac{1}{2}} \quad (2)$$

for 'heteromorphic' systems, where the site symmetries are different[4].

Hence $E_{xx}^{p\pi} \equiv (\partial^2 E^{p\pi}/\partial x^2)_{x=0} =$
$$-16\beta_0^2 a^2 \sum_l [\delta^2 + 16\beta_0^2 \cos^2 (l\pi/n)]^{-\frac{1}{2}} \quad (3)$$

and $E_{xx}^{d\pi} \equiv (\partial^2 E^{d\pi}/\partial x^2)_{x=0} =$
$$-16\beta_0^2 a^2 \sum_l [\delta^2 + 16\beta_0^2 \sin^2 (l\pi/n)]^{-\frac{1}{2}} \quad (4)$$

The following symbols have been used in equations (1) to (4): alternate bonds are of length $r_1 = r_0 + x$, $r_2 = r_0 - x$; the resonance integral is[5]:

$$\beta(r) = \beta_0 \exp[-a(r - r_0)]$$

(note that we write a where ref. 2 has $1/a$); δ is the difference between the Coulomb integrals for atoms A and B; and l takes the values

$0, \pm 1, \ldots \pm (m-1), m$, where $m = \frac{1}{2}n$ for n even, and $m = \pm \frac{1}{2}(n-1)$ for n odd. Furthermore[6]:

$$E^{\sigma}_{xx} \equiv (\partial^2 E^{\sigma}/\partial x^2)_{x=0} = 2n(f'')_{x=0} = 2nf''_0, \text{ say} \quad (5)$$

where $f(r)$ is the energy of one σ-bond. Davies stated[1] that E^{π}_{xx} is finite whatever n, and he concluded that: (*a*) for the infinite heterocyclic polyene, the stable configuration always has equal bonds, whatever the value of δ (other than zero); (*b*) for the finite polyene, and for sufficiently small δ, there may be a critical value of n below which bond alternation should occur.

The analysis given here shows that (*a*) is incorrect, and that (*b*) is valid only for certain classes of heterocyclic ring systems.

Let us consider first the infinite ring, whether homomorphic or heteromorphic. It is clear that as $n \rightarrow \infty$ the number of terms in equations (3) or (4), all finite and larger than $16\beta_0^2 a^2(\delta^2 + 16\beta_0^2)^{-\frac{1}{2}}$, also tends to infinity, so that E^{π}_{xx} cannot remain finite. For large n we re-write E^{π}_{xx} as an integral:

$$\begin{aligned} E^{\pi}_{xx} &= -(16\beta_0^2 a^2 n/\pi) \int_{-\frac{1}{2}\pi}^{+\frac{1}{2}\pi} (\delta^2 + 16\beta_0^2 \cos^2 \theta)^{-\frac{1}{2}} d\theta \\ &= (8\beta_0 a^2 n/\pi)\, kK(k) \end{aligned} \quad (6)$$

where $$k = [1 + (\delta/4\beta_0)^2]^{-\frac{1}{2}} \quad (6a)$$

and $K(k)$ is the complete elliptic integral of the first kind. The mathematical analysis is similar to that of the polyacenes[8]. Clearly E^{π}_{xx} is proportional to n, and the same is true for E^{σ}_{xx}. We conclude that whether or not bond alternation occurs in the infinite heterocyclic ring cannot be settled by algebraic reasoning alone. Bond alternation will exist if:

$$E_{xx} \equiv E^{\sigma}_{xx} + E^{\pi}_{xx} = n[2f''_0 + (8\beta_0 a^2/\pi)\, kK(k)] < 0 \quad (7)$$

For $\delta = 0$, $K(k)$ is infinite, and the conclusions of ref. 2 follow. As δ increases, the second term in equation (7) becomes less negative, so that there is a critical value of δ, denoted by Δ, for which E_{xx} changes sign, and above which the infinite ring has equal *A–B* bonds. For all $\delta < \Delta$ the infinite heterocyclic polyene will show bond alternation.

Let us consider next the case of finite n. It is necessary to distinguish two cases.

(1) *Homomorphic systems with n odd* $(=2n'+1)$. These correspond, in the limit $\delta = 0$, to the cyclic 'Hückel' hydrocarbons $C_{4n'+2}H_{4n'+2}$ with a closed shell of π-electrons. When $\delta = 0$,

$$(1/n)|E^{p\pi}_{xx}| = (4|\beta_0|a^2/n) \sum_l \sec\, l\pi/n$$

which is a monotonically increasing function of n. Hence, Longuet-Higgins and Salem found that for fixed a and β_0 there is a critical value of n, which we denote by N_0, above which bond alternation should occur. In the present case $(1/n)|E^{p\pi}_{xx}|$ as given by equation (3) is by inspection also a monotonically increasing function of n. Thus the same conclusions hold good. For all $\delta < \Delta$ there is a critical value of n, denoted by N, above which bond alternation should occur. As δ increases from 0, so will the value of N from N_0. For $\delta > \Delta$ bond alternation never occurs whatever the value of n. As δ increases, less mixing of the π-orbitals of A and B occurs; and for sufficiently large δ, the σ-electrons dictate a symmetrical structure for all values of n.

(2) *Homomorphic systems with even n, and heteromorphic systems.* In our one-electron Hückel approximation, all these systems possess, in the limit $\delta = 0$, a pair of degenerate non-bonding orbitals[3] (for the symmetrical configuration with $r_1 = r_2$) which are to be occupied by two electrons; it is known[9,10] for hydrocarbons that the configuration with bond alternation (and the degeneracy thus removed) is more stable for all values of n. If we now include electron repulsion, this will result in considerable mixing with low-lying states[10,11]. We may describe all this as a 'pseudo' Jahn–Teller effect. For small non-zero δ, the situation will clearly be similar. In both cases, no quantitative significance should be attached to the results of applying Huckel theory; but the interesting conclusions from (4) are worth recording. For small δ, we do find bond alternation predicted for all n; for intermediate δ, there is a critical value of n, *below* which bond alternation occurs; and for larger δ, there is no bond alternation, as in case (1). We can explain this by saying that the separation between the near-degenerate orbitals is independent of n, but the total binding energy increases with n; thus the ratio, and hence the 'pseudo' Jahn–Teller effect, will decrease as n increases.

Examination of the equations shows a close parallelism between the energy $|E^{\pi}|$ and the quantity E_{xx}; so much so, in fact, that we may take over Craig's figure (Fig. 5 in ref. 3*a*, Fig. 4 in ref. 3*b*) as a schematic representation of the Hückel results. The ordinate is now to be read as $(1/n)E_{xx}$; the thick line represents homomorphic, the dashed line heteromorphic systems. When $\delta < \Delta$, $\lim_{n \rightarrow \infty} (1/n)E_{xx}$ is negative; when $\delta > \Delta$ it is positive. For larger δ, $(1/n)E_{xx} > 0$ for all points in the figure. Of course, $(1/n)E_{xx} < 0$ implies bond alternation.

Experimentally, the lowest cyclic phosphazenes do not exhibit bond alternation[12].

It is of interest to estimate the values of the critical parameters Δ and $N(\delta)$. Assuming a bond-order versus bond-length relationship[2] $P(r)$ we find

$$f''_0 = -2\beta_0 a^2 (P_0 - P'_0/a) \quad (8)$$

where P_0 is the bond-order in the symmetrical configuration, given for the infinite polyene by:

$$P_0 = (1/2n)(\partial E^{\pi}/\partial \beta_1)_{r_1 = r_2 = r_0} = (2/\pi)k[K(k) - D(k)] \quad (9)$$

which is very nearly $2/\pi$. Here $D(k) = k^{-2}[K(k) - E(k)]$, where $E(k)$ is the complete elliptic integral of the second kind. P'_0 is the gradient at $r = r_0$ of the curve $P(r)$. From equations (7), (8) and (9) Δ is given after simplification by the solution of:

$$kD(k) = -\pi P'_0/2a \quad (10)$$

where $$k = [1 + (\Delta/4\beta_0)^2]^{-\frac{1}{2}}$$

Davies considered the particular case of the heterocyclic phosphazenes. The spectroscopic assignments[13] for $(PNX_2)_3$ are not sufficiently unambiguous to warrant a separate calculation of P'_0/a but the discussion in ref. 13 suggests that it should be at least comparable with the value for hydrocarbons. As a preliminary estimate we choose $P'_0/a = -2{\cdot}07$ derived from the two benzene force-constants[2,14]. It follows from equation (10) that $\Delta = 0{\cdot}23|\beta_0|$

To determine $N(\delta)$ for case (1) when $\delta < \Delta$, we find from equations (1), (3), (5), (6*a*) and (8) that N is the lowest value of n for which:

$$(k/n) \sum_l \sin^2 l\pi/n \,.\, (1 - k^2 \sin^2 l\pi/n)^{-\frac{1}{2}} > -P'_0/a$$

Of course $N > N_0$, and $N_0 = 17$ (ref. 14) from our chosen value of P_0'/a (but see ref. 15). N_0 and N are, however, sensitive to changes in P_0'/a and might be substantially different for borazoles or cyclic azines.

It must be recognized that a more refined treatment of this question would need also to include: mixing in of d_{yz} character (in Craig's notation); the effect of non-planarity; and also the participation of lone-pair electrons ('π'-bonding')[16].

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I AM indebted to Mr. Haigh and Dr. Salem for pointing out the mistake in my communication. They correctly state that $E^{\pi}_{xx} \to \infty$ as $n \to \infty$, and that the infinite heterocyclic ring can have, respectively, equal or unequal bond-lengths depending on whether δ is greater or smaller than a critical value.

The other conclusions of my communication are not affected by this mistake, and they are confirmed by Mr. Haigh and Dr. Salem. I confined myself to the cyclic phosphazenes, and treated them as heteromorphic systems. This gave the interesting result that bond alternation might occur below a critical value of n. Mr. Haigh and Dr. Salem consider other possibilities implicit in my equation (9), and show that, for suitable values of δ, odd homomorphic heterocyclic rings can behave like homocyclic rings, with bond alternation occurring above a critical value of n. In particular, they show that treating the known cyclic phosphazenes as odd homomorphic systems does not affect my result that bond alternation should not occur.

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Role of Hydroperoxyl in Hydrogen–Oxygen Flames

THE hydroperoxyl radical has been postulated as an intermediate in numerous oxidation reaction mechanisms. In particular, mechanisms involving it are capable of giving detailed explanations of the explosion limits and reaction-rates in hydrogen–oxygen systems in static reaction vessels[1,2]. In discussing hydrogen–oxygen and hydrogen–air flames, however, reactions of HO_2 are usually omitted, on the assumption that most of the flame reaction occurs at too high a temperature for the HO_2 forming reaction (2) to compete with the chain-branching reaction (1).

$$H + O_2 = OH + O \qquad (1)$$

$$H + O_2 + M = HO_2 + M \qquad (2)$$

We have recently obtained evidence, arising from a detailed investigation of a slow-burning hydrogen–oxygen–nitrogen flame and from calculations involving two faster (higher temperature) flames, that this assumption may be invalid in many cases.

The atmospheric pressure flame selected for detailed examination had a final temperature of 1,072° K and a burning velocity of 9·2 cm sec^{-1} (gas flows measured at 18° C and 760 mm). The initial gas temperature was 336° K. Analysis of the measured temperature profile gave a maximum heat release-rate of 20 (± 2) cal cm^{-3} sec^{-1} at 900° K, while the maximum hydrogen atom concentration, measured by studying the rate of the $H + D_2$ exchange reaction in the flame, was about $2{\cdot}5 \times 10^{-8}$ mole cm^{-3} at this temperature. Further, at 900° K $(O_2) \sim 1$, $(H_2) \sim 11{\cdot}3$ and $(H_2O) \sim 7{\cdot}2$ mole per cent.

Now the second limits of hydrogen–oxygen mixtures in potassium chloride coated reaction vessels at temperatures up to about 850° K are normally assumed to be ultimately controlled by a competition between reactions (1) and (2). At the limit the partial pressures of the reactants and inert gases obey the relationship $2k_1/k_{2\mathrm{H}_2} = (\mathrm{H}_2) + k_{\mathrm{O}_2}(\mathrm{O}_2) + k_M(M)$, where k_M denotes the efficiency of molecule M (relative to hydrogen) as the third body in reaction (2). Clearly, the ratio $2k_1/k_{2\mathrm{H}_2}$ may be found from a study of the variation of the second limit with mixture composition. A short extrapolation of the Arrhenius plot for this ratio from second limit data leads to $k_1/k_{2\mathrm{H}_2} = 150$ mm at 900° K. Second limit data[3–5] also show that $k_{2\mathrm{N}_2} = 0{\cdot}45 k_{2\mathrm{H}_2}$ and $k_{2\mathrm{H}_2\mathrm{O}} \sim 6{\cdot}5 k_{2\mathrm{H}_2}$. For the flame composition at atmospheric pressure it might therefore be expected that reaction (2) is some four to four and a half times as fast as reaction (1), that is, that HO_2 reactions are important at the position of maximum reaction-rate. This conclusion is supported by a simple calculation of the heat release-rate in the flame using the experimental concentration values. In the absence of HO_2 forming reactions, and using $k_1 = 2{\cdot}1 \times 10^{10}$ cm^3 mole^{-1} sec^{-1}, at 900° K (a value which is consistent with the recent literature[5]), together with recent experimental values[6] for the hydrogen atom recombination rate constant, a maximum heat release-rate of about 3 cal cm^{-3} sec^{-1} is obtained (cf. experimental 20 cal cm^{-3} sec^{-1}). Similarly, the measured rate of oxygen consumption in the flame is some four to five times larger than would be predicted from the foregoing value of k_1. It seems highly likely, therefore, that much of the reaction path in this flame is by way of HO_2.

By making the assumption that the HO_2 concentration assumes a kinetic steady state value, it is possible to make an estimate of the HO_2 concentration in the part of the flame near the maximum reaction-rate and also to suggest its main reaction path. Work[1,2] on the hydrogen–oxygen slow reaction and explosion limits suggests that reactions (3)–(6) may have to be considered as steps consuming HO_2.

$$HO_2 + H_2 = H_2O_2 + H \qquad (3)$$

$$HO_2 + H_2O = H_2O_2 + OH \qquad (4)$$

$$H + HO_2 = OH + OH \qquad (5)$$

$$HO_2 + HO_2 = H_2O_2 + O_2 \qquad (6)$$

The ratios of rate constants $k_3^2/k_6 = 0{\cdot}96$ cm^3 mole^{-1} sec^{-1} and $k_5^2/k_6k_2^2 = 1{\cdot}2 \times 10^{-4}$ mole cm^{-3} sec at 500° C have been derived by Baldwin and Mayor[1] on the basis of reaction mechanisms which satisfactorily interpret in detail the features of the hydrogen–oxygen reaction in aged boric acid coated vessels at that temperature. Taking $k_2 = 4{\cdot}7 \times 10^9$ at 500° C, $k_5^2/k_6 = 2{\cdot}6 \times 10^{15}$ cm^3 mole^{-1} sec^{-1}, a value only slightly less than the ratio $Z_5^2/Z_6 = 4{\cdot}8 \times 10^{15}$ quoted by Baldwin and Mayor. Foner and Hudson[7] have recently obtained $k_6 = 1{\cdot}8 \times 10^{12}$ cm^3 mole^{-1} sec^{-1} at room temperature, using mass spectrometry to measure HO_2 concentrations. Further, the very reasonable inclusion of reactions (5) and (6) in the mechanism proposed by Burgess and Robb[8] for the mercury photosensitized reaction of hydrogen and oxygen at room temperature would require the activation energy E_6 to be low, regardless of the actual value of k_6. Taking $E_6 = 0$, the values $k_3 = 1{\cdot}3 \times 10^6$ and $k_5 = 6{\cdot}9 \times 10^{13}$ cm^3 mole^{-1} sec^{-1} at 500° C their result.

An alternative value for k_6 ($= 6{\cdot}5 \times 10^{13}$ cm^3 mole^{-1} sec^{-1} at about 30° C, and implying a collision efficiency close to unity for both reactions (5) and (6)) has been derived by Burgess and Robb[8] in the work already quoted. For reaction (6) such a high collision efficiency seems to us to be somewhat surprising. Taking $E_6 = 0$, it would lead to $k_3 = 7{\cdot}9 \times 10^6$ and $k_5 = 4{\cdot}1 \times 10^{14}$ at 500° C. It should be noted also that both values of k_3 are more than 50 times greater than a third value[9-11] of $1{\cdot}2 \times 10^{11} \exp(-24{,}000/RT)$, giving $k_3 = 2{\cdot}2 \times 10^4$ at 500° C, due to Voevodsky and Tal'Rose[10], and derived from slow reaction and third limit studies of the hydrogen–oxygen system in vessels coated with potassium chloride. In view of the more recent developments in connexion with the hydrogen–oxygen system, values derived from older third limit mechanisms may be open to question.

In the flame at 900° K, $(H)/(H_2) \approx 10^{-3}$ and, for $E_3 - E_5 \approx 25$ kcal mole^{-1}, $k_5(H)/k_3(H_2)$ has a minimum value of the order of 10^4. Reaction (3) is therefore unimportant in this region of the flame. Reaction (4), which is endothermic to the extent of about 29 kcal mole^{-1}, is also unlikely appreciably to affect the kinetics. The main reaction path for the HO_2 is therefore most likely to be via reactions (5) and (6), which together with the HO_2 forming reaction (2) would lead to a kinetic steady state concentration $(HO_2) \sim 8 \times 10^{-10}$ mole cm^{-3} (or about one-thirtieth of the hydrogen atom concentration), if Foner and Hudson's value for k_5 is used, or $(HO_2) \sim 1{\cdot}3 \times 10^{-10}$ mole cm^{-3} using Burgess and Robb's value. Further, the rates of reactions (5) and (6) at 900° K in the flame may now be compared approximately. The ratio, using either value of k_6, is:

$$\frac{R_5}{R_6} = \frac{k_5(H)}{k_6(HO_2)} \approx 1{,}200$$

It therefore seems that reaction (5) provides the main reaction path, and that HO_2 concentration in the flame is considerably smaller than the hydrogen atom concentration. The figures quoted depend, of course, on the kinetic steady state assumption, which is not strictly true in the flame due to the diffusion and gas flow processes. A more accurate numerical calculation will be published in due course.

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Determination of the Adsorption of Ethylene on Platinum Electrodes using a Radiotracer Method

THERE has been a recent surge of interest in the mechanism of the electrochemical oxidation of hydrocarbons. Important to any determination of mechanism is a knowledge of the fractional surface coverage of reaction species. This communication deals with the adsorption of ethylene labelled with carbon-14 at the platinum-electrolyte interface as a function of potential and time.

The method for measuring the electrode surface concentration of carbon-14 containing species under electrochemically controlled conditions consists basically in an adaptation of the Joliot method[1,2]. In this work the electrode under study is a thin metal foil (10^4 Å thick), which is placed over the window of a proportional counter (suitable for detecting weak β-rays). The electrode is brought into contact with the solution, and from the count rate, after suitable calibration, it is possible to determine the surface concentration of carbon-14 and from that the surface

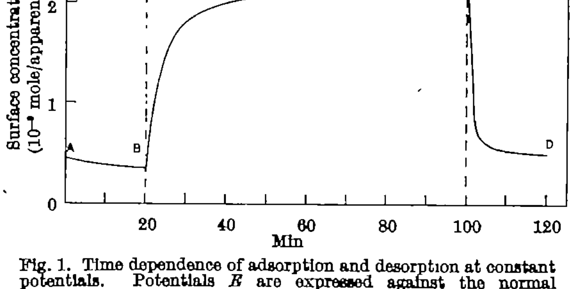

Fig. 1. Time dependence of adsorption and desorption at constant potentials. Potentials E are expressed against the normal hydrogen electrode

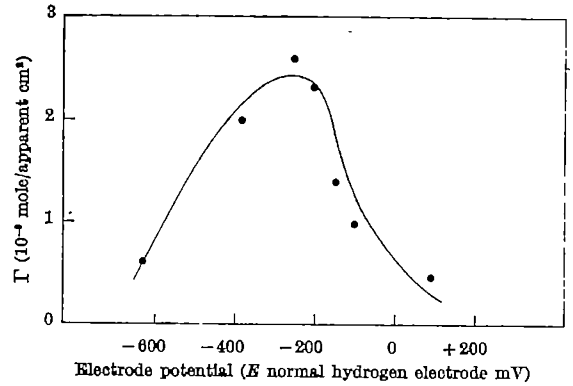

Fig. 2. Dependence of adsorption on potential

concentration of carbon-containing species. Details of the method will be published elsewhere[3].

The electrolysis cell consisted of the usual three-electrode arrangement: a working electrode (metal foil) with reference electrode separated from an auxiliary electrode. The electrode potential of the working electrode was controlled using an electronic potentiostat.

Our results refer to the electrode platinum/1 M sodium hydroxide, ethylene (diss.). The platinum electrode was prepared by electrodepositing a thin layer of platinum black on a gold foil. The electrode was brought into contact with sodium hydroxide solution with an inert (nitrogen) atmosphere above the electrolyte. The working electrode was cleaned by alternate anodic-cathodic pulsing (going from oxygen evolution to hydrogen evolution) and terminating always on a cathodic pulse. The evolved oxygen and hydrogen were removed from solution by sweeping with nitrogen. The electrode was then brought to a potential of +250 mV (normal hydrogen scale). At this stage an aqueous solution of ^{14}C-labelled ethylene was added to the electrolyte of the working electrode compartment so that the concentration of dissolved ethylene was 4×10^{-5} mole/l. The ethylene solution had been prepared in a separate vacuum line. Carbon-14 adsorption measurements, as a function of potential and time, were then started. The solution was kept stirred throughout and the temperature of the system air-thermostatted to 60° C.

The dependence on time of the adsorption at constant potentials is given in Fig. 1. It can be seen that most of the adsorption occurs within 10 min (*BC*). The rate of desorption is somewhat faster (*CD*). The equilibrium values for different potentials are plotted in Fig. 2.

The dependence on potential of adsorption is in general agreement with the adsorption of methanol on platinum determined by an electrochemical method[4]. The potential ranges of desorption correspond to those potentials where a significant increase in coverage of the electrode with hydrogen and oxygen occurs[5].

The electrochemical oxidation of ethylene under identical experimental conditions only proceeds in that range of potential where there is significant ethylene adsorption. At more anodic potentials, where rapid desorption of ethylene occurs (Fig. 1), the electrochemical oxidation of ethylene does not take place[6].

Determination of the roughness factor of our electrode by a gas adsorption method yield surprisingly low values, typically 14. It is thought that this low value may be ascribed to oxidation of the electrode on exposure to air. However, even if we use this roughness factor we find that the maximum fractional coverage of carbon-14-containing species on our electrodes does not rise above about 0·1.

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Mass-Spectrometric Observations of Negative Ions in Flame Gases

In view of recent interest in negative ions, it seems useful to report certain results obtained here in the early stages of a programme concerned with negative ions in flames. We have described elsewhere[1] a mass-spectrometric system suitable for the abstraction of ions from hot flame gases, and their subsequent analysis. This has so far largely been used for positive ions[2,3], but it is adaptable to the study of negative ions, albeit with a somewhat reduced sensitivity. A small flame plays directly on a very small sampling hole in a platinum diaphragm, on the other side of which a low pressure is maintained by pumping. It has been shown that the positive ions produced in flames may in some cases undergo reaction to produce other ions in the course of traversing the cooler boundary layer near the walls of the sampling hole[3,4]. In the work described here, no attempt has been made to ascertain whether the negative ions were produced in the flame or in the boundary layer, the primary interest lying rather in the wide variety of ions found.

Flames of premixed hydrogen, oxygen and nitrogen, without additives, showed no detectable negative ions. This implies a concentration of less than 10^7 ions cm^{-3} in the flame gases. Even when alkali metal salts were introduced in small quantities, so as to give electron concentrations of at least 10^{10} electrons cm^{-3} in or near the reaction zone, no negative ions were found there. The species which might have been expected would be OH^- (mass 17) and possibly O^- (mass 16), the corresponding neutral species being the only ones with reasonably high electron affinities known to be present. The absence of OH^- suggests strongly that the electron affinity of the hydroxyl radical is much closer to Branscomb's value[5] of 40 kcal/mole than to that of 65 kcal/mole given by Page[6].

Extension of the observations to the region of the visible tip of the burnt gases, about 3·5 cm downstream from the reaction zone, however, showed a large variety of negative ions in readily measurable amounts. This suggests attachment of the electrons in the cooler regions of the burnt gases, and is in broad agreement with the results of Kinbara and Ikegami[7] on the mobility of negative charge carriers at different

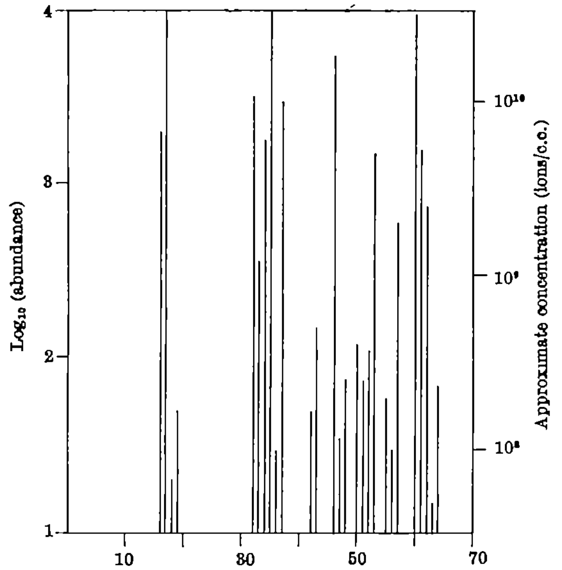

Fig. 1. Relative abundance of negative ions in the burnt gases of a pre-mixed hydrogen–oxygen–nitrogen flame ($H_2/O_2/N_2$ = 3·3/1·0/4·0) at atmospheric pressure with approximately 1 part in 10^6 of added sodium chloride. Measurements relate to a position 3·5 cm downstream from the reaction zone, near the inside tip of the gases

points in the flame. The negative ion count-rates observed at 3·5 cm from the reaction zone are shown in Fig. 1.

Approximate concentrations in the flame gases, to be taken from the right-hand ordinate axis, were obtained by calibration with positive potassium ions at the same sensitivity of detection.

Attention may first be directed to the major peaks. Mass 17 is undoubtedly OH^-, an assignment strengthened by the sizes of the isotopic satellites at masses 18 and 19. Masses 35 and 37 are largely Cl^-, from chlorine derived from the sodium chloride spray, although 35 may be partially a monohydrate of OH^-. Masses 46 and 60 are difficult to assign otherwise than to NO_2^- and CO_3^- respectively, the carbon arising from either flame or atmospheric impurities.

Masses 16 and 32 suggest O^- and O_2^- respectively, while 34 may be assigned to a monohydrate of O^- (not $H_2O_2^-$). Hydrated positive ions have already been found with this apparatus[1], and have been shown to be formed in the cool boundary layer[3,4]. Hydrated negative ions are expected to be less stable than positive ones, but will be most stable for small parent ions. The relative sizes of the peaks at masses 53 and 55 imply that 53 is rather a dihydrate of OH^- than a monohydrate of the larger Cl^- ion.

The other relatively abundant negative ions are at masses 33, 57, 61 and 62. In assigning possible formulæ to these, there seem to be few alternatives to the respective suggestions of HO_2^- (or HS^-), $C_2O_2H^-$, HCO_3^- and NO_3^-. So far as the remainder are concerned, the group 50–52 could be hydrated forms of the group 32–34, 47 a satellite of 46, 48 O_3^- (or possibly SO^-), 63 a satellite of 61 or 62, and 64 SO_2^-. Masses 42 and 43 are here unassigned, and it is hoped that future work will reveal the nature of these, and confirm and strengthen the other suggestions made.

No attempt is made to give mechanisms of formation, although it is clear that, while certain negative ions may form by direct attachment of electrons to the small amounts of the parent species present (for example, OH, O, NO_2), in some cases (for example, CO_3^-) this cannot be the case.

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Surface Tension of Aqueous Solutions of Potassium Hydroxide

MANY inorganic substances behave as volume active agents when they are dissolved in water. One such substance is potassium hydroxide. Surface tension measurements were made on a series of aqueous solutions of potassium hydroxide ranging in concentration from 6·5 to 41·2 weight per cent potassium hydroxide.

The potassium hydroxide solutions were prepared with A.C.S. reagent grade potassium hydroxide pellets. The water used in making the solutions was distilled twice in Barnstead tin-lined stills and then put through a two-stage fused quartz still. The specific electrical conductivity of this water at 25° C ranged from $1{\cdot}0 \times 10^{-6}$ to $1{\cdot}2 \times 10^{-6}$ Ω^{-1} cm^{-1}. In preparing these solutions no precautions were taken to eliminate the absorption of carbon dioxide from the atmosphere. The density values of each solution were measured at 20° and at 40° C using vacuum-jacketed pycnometers. Density values at 25° C were calculated assuming linear variation of density with temperature between 20° and 40° C.

The surface tensions were determined by the sessile drop method as prescribed by Bashforth and Adams[1]. The sessile drop was supported by a 'Teflon' block in an air atmosphere. The drops were 20–25 mm in diameter. The 'Teflon' block was cut from a large cylinder obtained from the A–1 Plastics Company, Dallas. The surface of the block was carefully prepared on a lathe; the surface profile is illustrated in Fig. 1.

The system was housed in a special cell made of optical glass, with a loose-fitting cover.

Measurements were made quickly to minimize the effect of atmospheric carbon dioxide. The drop dimensions did not seem to be affected by carbon dioxide during the first hour that a drop stood in the

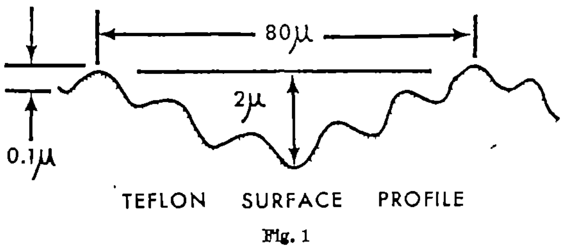

Fig. 1

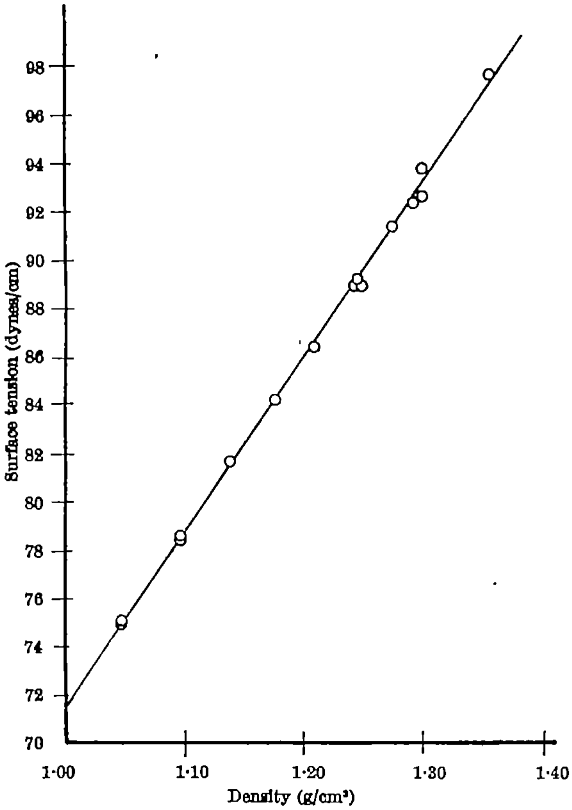

Fig. 2

cell. However, all measurements were completed within 20 min after the drop was formed.

Table 1. SURFACE TENSIONS OF AQUEOUS POTASSIUM HYDROXIDE SOLUTIONS, 25° C

Solution density (g/cm³)	Surface tension (dynes/cm)	Solution density (g/cm³)	Surface tension (dynes/cm)
1·0479	75·1	1·2433	88·9
1·0480	75·1	1·2438	89·2
1·0484	74·9	1·2480	88·9
1·0943	78·5	1·2785	91·4
1·0947	78·5	1·2980	93·4
1·0953	78·7	1·3012	92·6
1·1368	81·7	1·3015	93·8
1·1763	84·2	1·3023	93·9
1·2092	86·4	1·3515	97·6

The surface tension results at 25° are listed in Table 1. Their agreement with a least-squares relationship is shown in Fig. 2. The least-squares line passes through a surface tension of 71·32 at a density of 0·997, while a value of 71·97 dynes/cm is given in the literature[2] for the surface tension of pure water. The surface tension values were collected over a temperature range of 24·7°–25·5° C. During any one measurement the temperature range was never broader than 0·2° C.

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BIOCHEMISTRY

Calcium Hydroxide in Sucrose Solution as a Reagent for the Preparation of Water-dispersible Calcium Proteinates

CALCIUM caseinate is extensively applied in nutritional work and in therapeutic practice. Precise details regarding its preparation are not available in the literature. Mechanical drying of mixtures of ammoniacal solution of casein and aqueous calcium hydroxide in our experience offered several practical difficulties. The operations became comparatively easy when a solution of calcium hydroxide in sucrose was used as the reagent. The final drum-dried product so prepared is comparable with pharmaceutical calcium caseinate in all its properties and has met our requirements for nutritional studies. It is a light, white powder, highly acceptable organoleptically, with a good water dispersibility and has a *p*H of 5·8 in solution. The preparation contains 87 per cent protein, and about 6 per cent sucrose. Details of the method (Indian patent application pending) as developed by us with the use of calcium hydroxide in sucrose will be published elsewhere later. We have been regularly producing in this manner small batches of the material for our requirements from easily available and comparatively cheap commercial casein.

Inherent defects in the use as such of aqueous solutions of calcium hydroxide for incorporating calcium into proteins are: (*a*) its low solubility, necessitating the addition of large volumes of the solution for every 1 per cent calcium to be added to the protein; (*b*) the increased heat requirement to remove such large excess of water in the subsequent process of drying; (*c*) the difficulty of adding aqueous calcium hydroxide as a pure solution without its containing some amounts of calcium carbonate. Calcium hydroxide in sucrose solution, wherein a concentration as high as 5 per cent Ca^{++} can be easily built in, overcomes these disadvantages to a large extent. Besides, calcium hydroxide (in sucrose solution) and protein (dispersion in ammonia) would provide an ideal condition, both the reactants being in a homogeneous phase, for the calcium proteinate formation to proceed smoothly and fast. Experiments have shown that such indeed is the case.

Besides casein, the use of calcium hydroxide in sucrose for the preparation of water-dispersible powders from ammoniacal dispersions of proteins or protein-rich preparations of peanut, fresh-water fish and mutton have been tried. Preliminary observations have revealed some remarkable features. Though the calcium-treated proteins from these sources, unlike calcium caseinate, are not fully water dispersible, even so treatment with calcium endows highly acceptable organoleptic and physical properties to the proteins; for example, protein from fresh-water fish acquires a pleasing birefringence. The calcium-treated and drum-dried product obtained in each case, including fish, has remained remarkably free from odour for months. These improved properties are not shared by the products obtained from the same sources (peanut, mutton and fish), but without the use of calcium. Thus this method of processing meat, fish and vegetable proteins would appear to offer scope for extended practical application especially in the preparation of protein-rich foods from those natural sources which by themselves are not readily acceptable. This would depend on proving whether the destruction of odours by treatment with calcium hydroxide is a general phenomenon.

It cannot be stated definitely whether the concepts with regard to binding of calcium as calcium caseinate in milk would hold good for drum-dried preparation of calcium caseinate. In this product, the calcium-protein bond, whatever its nature, has withstood drastic heat treatment (drum drying at 50 lb./in.^{2}) and has preserved easy water dispersibility. We could not obtain evidence for even a trace of Ca^{++} as seen with calcium precipitants or with ethylenediamine tetraacetic acid (EDTA). Indeed, we could add EDTA to the reaction mixture and this made no difference to the water dispersibility and *p*H of the final drum-dried product. More interesting is the observation that even after Ca^{++} in sucrose solution was all chelated with excess EDTA, addition of this chelate to casein in ammonia gave, after drum-drying, a product having the normal water-dispersibility and *p*H characteristics, suggesting that the protein can combine even with EDTA-bound calcium under such conditions. Further experiments to understand this phenomenon are in progress.

These observations give rise to the suggestion that calcium hydroxide in sucrose can possibly find general application as a calcium reagent for reactions of chemical or industrial importance where residual sucrose in the final preparation would not matter.

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Influence of Molecular Weight of Deoxyribonucleic Acid on its Incorporation into Neoplastic Cells

In previous publications some kinetic investigations concerning the optimal physico-chemical conditions of incorporation of heterologous and homologous DNA into Novikoff hepatoma cells and Ehrlich ascites carcinoma cells have been given[1,2]. The first phase of incorporation of DNA process into somatic cells is an adsorption of donor DNA molecules on to the cell surface. This adsorption process is not dependent on time and temperature in the Novikoff hepatoma cells. The amount of radioactive DNA is the same at 0° C and 37° C, but is significantly dependent on the kind and composition of the incubation medium (optimum: Robinson's medium and 1/15 M phosphate buffer, *p*H 6·5, v/v).

Preliminary investigations of interaction between tracer DNA and Novikoff hepatoma cells showed that the amount of donor DNA which we found to be resistant to DNase increased during 3–5 h at 0° C, and after reaching a maximum value, did not change at prolonged exposition time. Recent publications[3,4] showed that donor DNA molecules are able to penetrate into the cell nucleus.

The experiment described by Amos[5] suggested that incorporation of donor DNA into chick embryo cells is strongly influenced by basic proteins. We have found that as a result of basic protein interaction with DNA molecules an insoluble complex was formed and precipitated. The enhancing effect of basic proteins could be ascribed rather to the extra-cellular contamination than to incorporation of donor DNA. This phenomenon leads to the conclusion that perhaps the incorporation of donor DNA depends on its molecular weight. Recently published results have shown that only DNA molecules with high molecular weight are able to demonstrate their transforming activity in the bacterial cells.

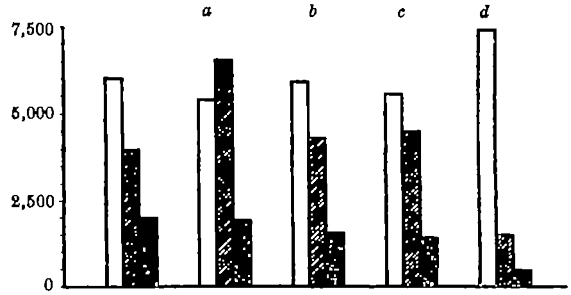

Fig. 1. Incorporation of ^{14}C-DNA into Ehrlich ascites carcinoma cells. DNA-^{14}C (thymidine-^{14}C, biosynthesis *in vitro*). Incubation mixture: 0·8 ml. Ehrlich ascites carcinoma cells, 1 : 1 in Davidson's medium with ascites fluid, 1·5 ml. Davidson's medium, 150 μg DNA-^{14}C, 13,700 c.p.m./150 μg DNA, 120 min at 37° C. The cells were separated from the medium, three times washed with 0·14 M sodium chloride solution, incubated with excess of DNase, five times washed with 0·14 M sodium chloride containing sodium fluoride, and the radioactivity was measured in a Tracerlab flow counter. The DNA was determined at 260 mμ in a Beckman *DU* spectrophotometer. Similar experiments were performed with ^{32}P-DNA samples. Left, control DNA; denatured DNA, *a*, ultra-violet light; *b*, heating; *c*, X-radiation; *d*, homogenization. White, medium; shaded, DNase; black, DNA

The general experimental procedure in our investigations was as follows:

(1) Preparation of radioactive DNA (carbon-14, phosphorus-32), by extraction with 1 M sodium chloride solution and deproteinization with chloroform-*n*-amyl alcohol. Our samples of DNA contained 0·8 per cent protein (Lowry), 1·1 per cent RNA and had a molecular weight of $5·4 - 6·1 \times 10^6$ as measured by viscosity determination. The differentiation of native and denatured DNA was examined as described by Hotchkiss[6]. (2) Exposure of Ehrlich ascites tumour cells in optimal conditions[1] to donor DNA. (3) Separation of the cells from the medium. (4) Determination of the radioactivity in both medium and various cell fractions, for example, free nucleotide fraction, phospholipid fraction, phosphoprotein fraction, free phosphate fraction and in DNA and RNA fractions by various methods. Immediately before exposure of recipient cells to donor DNA, the samples of DNA were degraded and denatured by: (*a*) exposure to 2,000-r. X-radiation; (*b*) exposure to ultra-violet light, λ_{max} 256 mμ, distance 15 cm, for 15 min; (*c*) heating at 100° C for 20 min, followed by rapid cooling; (*d*) homogenization in a glass Potter–Elvehjem homogenizer at 0° C for 5 min at 1,000 r.p.m.

The viscosity decreased significantly only after homogenization. In some experiments the exposed recipient cells were treated with excess of DNase in optimal conditions. The detailed description of materials and experimental methods were published previously[1,4]. It was clearly demonstrated by our experiments that after exposition at 0° C a small amount of donor DNA changes into a form resistant to DNase treatment. Perhaps this form represents the amount of donor DNA which is incorporated and adapted in the cells.

Fig. 1 shows the effect of adsorption and incorporation of various degraded donor DNA samples. Lerman and Tolmach[7] point out that only highly polymerized DNA molecules possess a transforming activity. Fig. 1 also shows that DNA molecules after depolymerization procedure lose their adsorption and incorporation ability. The recipient cells fixed only a very small part of donor DNA added. It is possible that the transforming activity of small DNA molecules is not demonstrated because the incorporation of small DNA molecules is highly decreased. The mechanism of the transforming process is not sufficiently proved as yet. It is not known what amounts of nucleotides are present in a DNA molecule to enable it to demonstrate its transforming activity after incorporation into the genetic apparatus of recipient cells. Neither is it certain whether an oligonucleotide can be incorporated into the terminal position of DNA molecules, which are localized in the genetic apparatus of recipient cells. If it is so, the problem remains whether the process is a mutation or one dependent on transformation.

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Isolation from Cottonseed Oil of a Halphen-negative Fraction which causes Pink-'White' Discoloration in Stored Eggs

Sterculic acid, isolated from *Sterculia fœtida* seed oil by Nunn[1] and from cottonseed oil by Shenstone and Vickery[2], and malvalic acid, isolated from cottonseed oil by Shenstone and Vickery[3,4], contain the *cyclo*-propene ring[5], give positive Halphen colour reactions[4,6], and cause pink-'white' discoloration in stored eggs from hens fed these compounds[2,7]. That cottonseed oil may contain a substance, other than malvalic and sterculic acids, which causes pink-'white' discoloration in stored eggs was indicated by our observation that crude cottonseed oil that had been heated at 150° for 4 h or at 200° for 1 h and no longer gave a positive Halphen colour reaction still produced discoloration[8].

We saponified crude cottonseed oil by mixing it with alcoholic potassium hydroxide and allowing the reaction to proceed overnight at room temperature. The fatty acids obtained after acidification were dissolved in acetone, and a fraction soluble at – 60° was obtained. Further fractionations were made by formation of urea inclusion compounds and by crystallization at – 60° from petroleum ether (b.p. 30°–60°) and from acetone. A fraction consisting of 45 g of fatty acids obtained from 4,000 g of crude cottonseed oil gave a strong Halphen reaction. Eggs produced by hens fed this fraction in the ration to supply 89 mg/hen/day developed very pink 'whites' after storage.

Urea inclusion compounds were prepared from 45 g of the active fatty acids and 45 g of urea. A fatty acid fraction (25 g) isolated from the insoluble urea inclusion compounds gave a strong Halphen colour reaction. When this fraction (as urea inclusion compounds) was mixed in the ration to give 50 mg of fatty acids/hen/day varying degrees of pink-'white' discoloration of stored eggs occurred in two experiments, but none occurred in a third. Gas-liquid chromatographic separation of the fatty acid methyl esters indicated that this fraction contained 2·8 per cent methyl sterculate and 56·6 per cent of fatty acid methyl esters with gas-liquid chromatographic retention times similar to methyl linoleate (Table 1). Malvalic acid methyl ester has a retention time similar to methyl linoleate. This fatty acid fraction probably contained 8·2 per cent or more malvalic acid, depending on whether sterculic and malvalic acids were in the same proportion as in cottonseed oil[9] or whether sterculic acid was selectively precipitated in certain steps of the isolation procedure. The variable degrees of pink-'white' egg discoloration obtained were probably caused by losses of the *cyclo*-propenoid fatty acids during the 6 weeks the fractions were mixed with the rations, even though urea inclusion compounds are more stable than the free acids[10].

Table 1. Fatty Acid Composition of Cottonseed Oil Fractions
(Percentage of total fatty acids)

Fatty acid	Urea-insoluble	Soluble
Myristic	1·0	0
Palmitoleic	18·6	5·8
Oleic	19·1	2·8
'Linoleic'	56·5	90·5
Sterculic	2·8	0
Halphen reaction	+	–
Egg 'white' discoloration	slightly pink	very pink

Fatty acids not precipitated by an equal weight of urea (25 g) gave a negative Halphen colour reaction but still produced pink-'white' discoloration consistently when included in the hens' diet at a level to supply 40 mg/hen/day. More than 90 per cent of the methyl esters of these fatty acids had gas-liquid chromatographic retention times similar to methyl linoleate (Table 1). The small amount of fatty acid fraction required to produce pink-'white' discoloration (40 mg/hen/day compared with 25 mg of *cyclo*-propenoid fatty acids given by Shenstone and Vickery[2]) indicates that the active material is present in quite high concentration and must be a constituent of the 'linoleic' peak. The active material in the soluble fraction did not contain a *cyclo*-propenoid ring (negative Halphen reaction), formed a urea inclusion compound less readily than the *cyclo*-propenoid fatty acids, was readily soluble in acetone or in petroleum ether (b.p. 30–60°) at – 60°, had an equivalent chain-length similar to linoleic acid, and was more heat-stable than the *cyclo*-propenoid fatty acids but was still labile on prolonged heating at high temperatures.

Although we believe that crude cottonseed oil contains a fatty acid, other than malvalic and sterculic acids, which causes pink-'white' discoloration of stored eggs, the possibility has not been ruled out that this fatty acid was formed from malvalic or sterculic acid in the isolation procedure.

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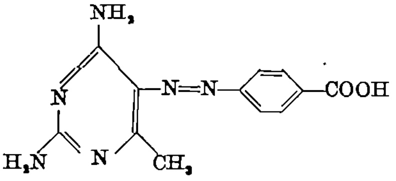
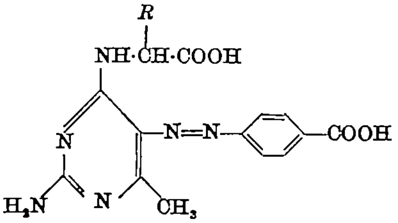
N-(5-Arylazo-4-Pyrimidyl)-Amino-Acids as Growth Inhibitors of *Streptococcus faecalis*

It was shown by Tanaka *et al.*[1,2] that 5-arylazo pyrimidines having different groups (for example, hydroxyl, methyl or amino) at the 2, 4 and 6 positions are strong folic acid antagonists and that some of these possess marked anti-neoplastic properties. They also found that the nature of substituents in the benzene ring of the phenylazo radical was the determining factor for this activity.

We considered it to be of interest to investigate the biological properties of pyrimidine systems containing a 5-arylazo as well as an amino-acid moiety. It is expected that in the proposed compounds the amino-acid side-chain would increase penetration and help maintain a higher blood-level and thus render the molecule as a whole more effective. In continuation of our synthesis of *N*-pyrimidyl amino-acids[3,4], we have, therefore, synthesized several *N*-(2-amino-5-*p*-carboxybenzeneazo-6-methyl-4-pyrimidyl)-amino-acids (compounds II–VII) and have examined their action on *S. faecalis*. The parent compound (I) was also synthesized for comparison. (So far as we know, these compounds (I–VII) are being reported for the first time.)



(I)



(II–VII) (Table 1)

We chose the *p*-carboxybenzeneazo moiety because pyrimidines having this group in the 5-position show marked anti-tumour activity although their inhibitory action on certain micro-organisms are somewhat less pronounced than that of some other compounds of this type[1,2].

All the compounds synthesized were found to inhibit the growth of *S. faecalis*. However, the amino-acid moiety was found to decrease the inhibitory effect to some extent. Thus compounds (II–VII) are less inhibitory than the parent compound (I). It has also been found that among the compounds II–VII the inhibitory activity decreases with the increase of the bulk of the amino-acid moiety (Table 1).

Inhibition studies have also been carried out using other concentrations of pteroylglutamic acid (PGA) and leucovorin and it has been observed that all these compounds can be regarded as competitive antagonists of both PGA and leucovorin in regard to the growth of *S. faecalis*. These compounds also inhibit the growth of *S. faecalis* when PGA in medium (†) is replaced by a combination of adenine and thymine, but to a much lesser extent. The inhibition can be reversed by certain metabolites.

Table 1. INHIBITION OF *S. faecalis* BY *N*-(5-ARYLAZO-4-PYRIMIDYL)-AMINO-ACIDS

Compound	Concentration (μg/ml.) required for 50 per cent inhibition of growth* PGA†	Leucovorin‡
I	0·105	2·17
II : $R = -\mathrm{H}$	1·07	50 00
III§ : $R = -\mathrm{CH_3}$	2·25	178·00
IV§ : $R = -\mathrm{CH_2.CH_3}$	2·70	380 00
V§ : $R = -\mathrm{CH(CH_3)_2}$	7·19	¶
VI§ : $R = -\mathrm{CH(CH_3)CH_2CH_3}$	7·80	¶
VII§ : $R = -\mathrm{CH_2.C_6H_5}$	22·80	¶

* Growth measured turbidimetrically after 18 h at 37° C (micro-organism used was *Streptococcus faecalis* A.T.C.C. 8043).

† Folic acid assay medium (ref. 5) excluding the purine and pyrimidine metabolites was used. Medium contained pteroylglutamic acid (PGA) (0 001 μg/ml.).

‡ Medium same as in †. No PGA used. Concentration of leucovorin (0 001 μg/ml.) refers to 50 per cent of the weight of 'leucovorin' used, since synthetic leucovorin is half as active as naturally occurring citrovorum factor due to the presence of an inactive isomer.

§ All these compounds are of DL-variety.

¶ Half-inhibition concentration could not be reached because the compound was not sufficiently soluble in the basal medium to permit the attainment of the required high concentration.

The metabolites used in the reversal experiments were adenine, guanine, xanthine, uracil, thymine, orotic acid, thymidine, cytidine, PGA, leucovorin, serine and methionine. The inhibition caused by the compounds could, however, be reversed only by thymine, thymidine, PGA and leucovorin. Although PGA and leucovorin are both equally effective for the growth of *S. faecalis*, these *N*-pyrimidyl amino-acid derivatives are much more inhibitory in presence of PGA than in presence of leucovorin (Table 1). These facts suggest that the compounds exert their inhibitory activity mainly by interfering with the enzymatic conversion of PGA to citrovorum factor in the metabolic pathway of *S. faecalis* cell.

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Glucose-6-Phosphate and 6-Phosphogluconate Dehydrogenase Activities in Erythrocytes of Monkeys infected with *Plasmodium knowlesi*

CERTAIN drugs, when administered to individuals deficient in erythrocyte glucose-6-phosphate (G-6-P) dehydrogenase, can cause hæmolytic crises. Episodes of acute hæmolytic anæmia caused by a variety of infections have been reported in enzyme-deficient individuals[1]. To investigate if the presence of a similar deficiency in monkeys could explain the severe hæmolysis which is a frequent feature of acute *Plasmodium knowlesi* infections in monkeys, quantitative estimations of erythrocyte G-6-P dehydrogenase were carried out. Fifty-eight rhesus monkeys, *Macaca mulatta*, of both sexes, were examined, and no evidence of dehydrogenase deficiency was found. This finding is in agreement with the recent report of Lie-Injo Luan Eng[2].

Blood was obtained from the saphenous veins of monkeys under general anæsthesia and was stored at 4° C in acid–citrate–dextrose solution. The test was normally performed within 3 h. Glucose-6-

Table 1. LEVELS OF G-6-P DEHYDROGENASE AND 6-PG DEHYDROGENASE IN PARASITIZED AND NON-PARASITIZED ERYTHROCYTES FROM MONKEYS INFECTED WITH *P. knowlesi**

Monkey No.	Percentage of parasitæmia	Pre-infection G-6-PD	Pre-infection 6-PGD	Infected blood G-6-PD	Infected blood 6-PGD	Separated non-parasitized cells G-6-PD	Separated non-parasitized cells 6-PGD	Separated parasitized cells G-6-PD	Separated parasitized cells 6-PGD
1	7	228	57	294	87	262	80	1,150	276
2	9	237	58	330	148	278	109	732	207
3	10	258	27	280	48	230	84	566	184
4	15	250	69	338	103	218	63	958	208
5	16	180	54	328	—	194	—	520	200 (55)†
6	19	212	–	330	130	245	—	404	200 (40)
7	31	260	113	365	156	256	102	644	297
8	32	185	58	310	132	220	—	540	227 (56)
9	44	174	—	286	—	180	—	406	— (44)
10	61	351	—	736	—	320	—	1,060	—

* All erythrocyte preparations for assay were suspended in Krebs–Ringer-phosphate solution (*p*H 7·4) following removal of the plasma and leucocytes.

† Figures in parentheses refer to the concentration of parasites achieved expressed as a percentage of the total number of erythrocytes in the preparation used for assay of both enzymes, otherwise this was more than 90 per cent.

phosphate dehydrogenase was assayed by following the rate of reduction of added nicotinamide–adenine dinucleotide phosphate (NADP) spectrophotometrically at 340 mμ in the presence of substrate (Motulsky, private communication, 1959). All experiments were carried out at 25° C. The individual results gave a mean value of 265 units/min/100 ml. erythrocytes (*S.D.* ± 70), which is very similar to the mean level found in 30 British human subjects of both sexes (275 units/min/100 ml. erythrocytes, *S.D.* ± 62).

The dye test of Motulsky and Campbell[3] was also performed on some of the same animals, but it was found that complete decolorization was not obtained in many of the tests. The difficulty in assessing complete dye reduction was overcome by increasing the amount of NADP in the reagent mixture from 0·1 mg to 0·3 mg, after which a range of incubation times necessary for complete decolorization agreed well with those reported by Lie-Injo Luan Eng[2] for *M. mulatta*. It was thought that the incomplete decolorization might have been due to inefficient hydrogen acceptance by the final acceptor, brilliant cresyl blue, since parallel tests with samples of this dye from different sources gave very variable times of decolorization, a finding in accord with the observations of Knight[4]. Recently, we have performed ageing experiments at 37° C on lysates of monkey erythrocytes and have found that the glucose-6-phosphate dehydrogenase is inactivated much more rapidly than that of similar preparations of human blood. The coenzyme NADP has been shown, in our laboratory and elsewhere[5], to protect the enzyme against inactivation by heat and on dilution.

No linear relationship was found between the levels of parasitæmia in monkeys infected with *P. knowlesi* and the time of decolorization in the Motulsky dye test, although there were indications that parasitæmias of 50 per cent or more shortened the necessary time. This was further investigated by quantitative measurements of erythrocyte G-6-P and 6-phosphogluconate (6-PG) dehydrogenases throughout the course of the infection. Increases in both enzymes were found on parasitization. Moreover, the increases were restricted to the parasitized cells, which were separated from the unparasitized cells by differential centrifugation or sedimentation on standing at 4° C. The uninfected cell enzyme contents remained at the pre-infection levels. Results from 10 experiments are shown in Table 1. There was no reticulocytosis during the course of this acute infection to account for the increased enzyme levels found in infected blood by virtue of the higher levels present in young erythrocytes. Extracts of liberated parasites prepared by a variety of methods revealed virtually no enzyme activity.

Such increases in erythrocyte dehydrogenases were normally detectable within 2 h of parasitization of the cell. Opportunities of observing these changes were facilitated by the synchronous nature of the 24-h asexual life-cycle of *P. knowlesi*.

These findings indicate either that activation of inactive forms of the enzymes, as suggested for G-6-PD (ref. 5), occurs as a result of parasitization, or that the parasite is able to produce the enzymes and to secrete them into the cytoplasm of the host erythrocyte. The second possibility is the less likely, since no residual enzyme activity was found in the parasite extracts. Whether activation is initially an erythrocyte response to the presence of a parasite or is brought about by the parasite for its own requirements, the same material benefits will in either case be derived by both parasite and erythrocyte. Hence, any changes which attempt to stabilize erythrocyte integrity and function are likely to help in maintaining a suitable environment for the parasite.

Such changes in erythrocyte dehydrogenases can regenerate increased amounts of NADPH, which, in addition to parasite requirements, may be required for reduction of any oxidized glutathione or methæmoglobin formed in the host erythrocyte. The results may also suggest that *P. knowlesi* utilizes the pentose phosphate pathway not only for regeneration of NADPH but also for production of the pentose sugars which are required for nucleotide synthesis. That the malaria parasite can synthesize large amounts of nucleic acids has been demonstrated in the studies by Ball *et al.*[6] of *P. knowlesi* and by Whitfeld[7] of *P. berghei*. An increased turn-over of NADPH is required for the large amount of lipid synthesis carried out by *P. knowlesi*[8], for enhancing synthesis of proteins[9] (such synthesis has been shown to occur in *P. knowlesi*[8]), and for regeneration of reduced glutathione which contributes to the sulphur requirements of *P. knowlesi*[10]. An increased rate of NADPH regeneration resulting from an increased utilization of the pentose phosphate pathway is also likely to be required for many other essential syntheses in a rapidly growing parasite.

These observations offer further explanations of why the malaria parasite is an obligate intracellular parasite within the erythrocyte. Furthermore, if deficiency of erythrocyte G-6-PD in young African children is shown convincingly to afford some degree of protection against *P. falciparum* malaria, as suggested by Allison and Clyde[11] on the basis of their investigations, our findings would possibly explain this phenomenon. If *P. falciparum* has a vital requirement of the pentose phosphate pathway similar to that which *P. knowlesi* appears to have, the parasite is unlikely to thrive in enzyme-deficient erythrocytes.

Further experiments with erythrocytes from *P. berghei*-infected mice and from *P. vinckei*-infected hamsters have revealed no significant increases in the levels of these enzymes on infection, but the erythrocytes of both animals before infection have much higher enzyme levels than those of uninfected monkeys. There was no evidence of any reticulocytosis in either of these infections.

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Isolation of an Anabolic, Uterotrophic Compound from Corn infected with *Gibberella zeae*

A METABOLITE with possible anabolic and marked uterotrophic activity has been isolated from extracts of the fungus *Gibberella zeae* (*Fusarium graminearum*).

Numerous reports of genital involvement in animals fed spoiled grain[1–4] prompted us to investigate the possible relationship of micro-organisms to this syndrome. During 1957 and 1958 we observed seven widely separated swine herds showing vulvar hypertrophy and occasional vaginal eversion in females, preputial enlargement in castrated males and prominent mammary glands in both sexes. In each case the animals were fed moulded corn. Spoiled grain was procured from each farm, treated with sodium hypochlorite and plated on potato–dextrose agar. Predominant micro-organisms isolated from the samples were *Penicillium* spp., *Cladosporium* sp., *Mucor* spp. and *Gibberella zeae*. Cultures of these moulds were grown on sterile ground corn (35 per cent moisture) in flasks stored at about 24° C for 2–3 weeks. This moulded corn was fed *ad libitum* to sexually immature female pigs. Within four days, vulvar and mammary enlargement were elicited only in those animals fed corn seeded with *G. zeae* isolates. Similarly prepared corn fed for six days to five mature, ovariectomized mice produced an average uterine weight of 94·3 mg compared with 22·6 mg for the control animals. An alcoholic extract of the *Gibberella* moulded corn incorporated in sesame oil and injected subcutaneously into 10 castrated female mice at a dose of 0·1 ml. per animal daily for 3 days produced an average uterine weight of 60·7 mg compared with 20·6 mg for the controls. Nine isolates of *G. zeae* were obtained which produced varying degrees of uterotrophic activity.

To obtain material for chemical and pharmacological tests, a study was made of the cultural requisites for the production of the active principle. Suitable substrates include wheat or barley as well as corn or a synthetic medium containing mineral salts and carbohydrates including corn starch, high test molasses or D-glucose. An easily separable, water-soluble fraction, toxic for mice, is occasionally produced concurrently with the metabolite. This confirms the earlier work of Christiansen and Kernkamp[5] and Mitchell and Beadles[6] associating toxicity with the ingestion of *G. zeae*-infected grain.

Recovery of the metabolite is effected by water extraction of the corn–mycelial mixture in a high-speed blender to remove the water-soluble fractions. The wet cake remaining after filtration is repeatedly blender-extracted with anhydrous ethanol to remove the active principle. The alcoholic extracts are combined and the solvent removed by distillation. An aqueous-organic phase partition of the resulting residue is followed by application of the concentrate to a column of synthetic magnesium-silicate. Subsequent development by gradient elution using solvents of increasing polarity separates the active component. The solvent mixture is evaporated from the active fraction resulting in a white, crystalline mass which upon recrystallization has a melting point range of 161°–163° C, $(\alpha)25^\circ/\mathrm{Hg} = -82^\circ$ ($c. = 2{\cdot}4$ ethanol). A methanolic solution of the metabolite exhibits a bright, greenish-blue fluorescence when exposed to ultra-violet light. Ultra-violet absorption peaks occur at about 237, 275 and 315 mμ (methanol). Additional chemical, physical and structural data will be forthcoming as pure material becomes available.

Preliminary experiments indicate that partially purified concentrates of the active principle are effective in improving growth-rate and feed efficiency in sheep.

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Bile Pigment in *Eucypris virens* (Jurine)

Eucypris virens (Crustacea : Ostracoda) is a common ostracod with green patches on the valves of its carapace. When treated with yellow nitric acid these green patches become purple, then red, orange and finally yellow. This sequence is typical of the later stages of the Gmelin reaction, indicating the presence of a bile pigment.

Several hundred *E. virens* were soaked overnight in acid methanol (5 per cent hydrochloric acid), to give a clear blue-green solution with an absorption maximum at 655 mμ. The pigment passed readily into chloroform after dilution of the acid methanol with water. In dry chloroform absorption maxima were found at 362 and 660 mμ. The chloroform solution was then evaporated and the pigment taken into methanol. The solution in methanol did not fluoresce in ultra-violet light, nor did it fluoresce on the addition of zinc acetate, but when a trace of iodine was added a brilliant pink fluorescence appeared. This fluorescent solution, stabilized with a trace of ammonia, showed a strong absorption maximum at 625 mμ. This indicates that the pigment was mesobiliverdin, or a pigment very similar to it.

A pigment with similar properties has been obtained by cold acid hydrolysis of C-phycocyanin[1]. This raises the possibility that the original pigment in the ostracod was not a bilatriene of the mesobiliverdin type, but a biladiene similar to those occurring in the blue-green algae on which this ostracod feeds. This possibility was tested by taking fresh *E. virens* which had been passed through several changes of clean water to allow them to empty their intestines, and homogenizing them in 2 per cent hydrochloric acid in methanol. The blue-green solution was cleared by centrifuging, then diluted with water and the pigment taken, not very easily, into chloroform. The chloroform solution was washed with dilute sodium bicarbonate, and a small volume of methanol was added. This solution showed a pink fluorescence in ultraviolet light, and the fluorescence was greatly intensified by the addition of zinc acetate. Addition of iodine increased the fluorescence slightly, but then turned it to a pale green. The pink fluorescence was completely quenched by the addition of mineral acids, thus distinguishing it from the fluorescence of porphyrins, and indicating that the pigment was a biladiene of the a, b type.

It has previously been shown[2] that another ostracod, *Heterocypris incongruens* (Ramdohr), accumulates biladienes, derived from blue-green algae, in the form of small granules in the wall of its gut, but not outside the gut in the valves of the carapace. *Eucypris virens* also accumulates biladienes in the wall of its gut, but in addition transfers some to the valves of its carapace. The mesobiliverdin obtained from the first extract was due to the hydrolysis of the biladiene in the strong acid; when the pigment was extracted in weaker acid and examined immediately to minimize hydrolysis the indications were that in the ostracod the pigment was present as a biladiene.

It is probable that the green colour in the carapace valves of other ostracods is due to bile pigments: the patches on the shell of *Cypridopsis aculeata* (O. G. Costa) give a positive Gmelin reaction.

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PHYSIOLOGY

Injection of Alkaline Cations into Cat Spinal Motoneurones

The effects of electrophoretic injection of some cations into cat spinal motoneurones have already been reported[1]. The injection of K^+ displaces only the equilibrium potential of the inhibitory post-synaptic potential because of the increased intracellular chloride concentration which is caused by the inward movement of Cl^- ions during the flow of the potassium-injecting current. On the other hand, the effects of Na^+ injections are characterized by changes in the resting-, spike-, and after-potentials and the inhibitory post-synaptic potential, which can be explained by the intracellular increase of Na^+, the reciprocal decrease of K^+, and the secondary increase of Cl^-. The recovery of these potentials subsequent to a Na^+ injection represents the time-course of the sodium extrusion from the motoneurones. According to a recent analysis, the increased intracellular sodium concentration decreases exponentially with a mean time constant of 91 sec; virtually full recovery being attained after about 6 min[2]. Several other cations have now been injected into cat motoneurones, and this article will describe the effects of injections of Li^+, Cs^+, and Rb^+ ions on the motoneurone potentials and the rates of their removal from the cells.

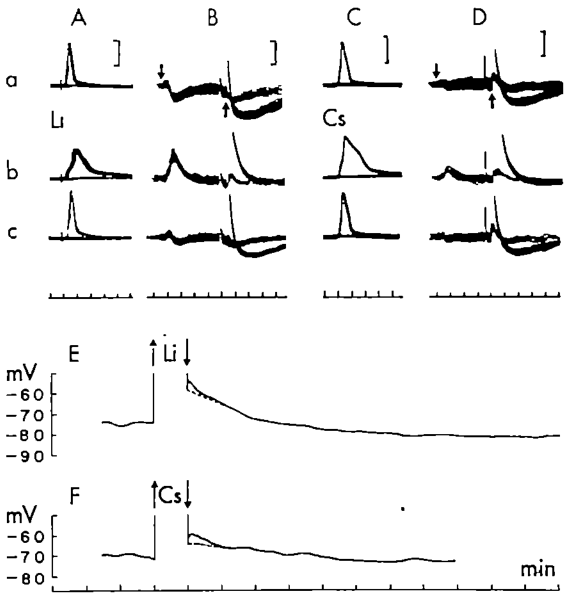

Fig. 1. *A* and *C*, antidromically evoked spike potentials in two different motoneurones which were impaled with Li^+ and Cs^+ containing micro-electrodes respectively. *B* and *D*, illustrating, with higher amplification and slower sweep velocity, the polysynaptic inhibitory post-synaptic potentials (stimulation at downward arrows) and the after-potentials following the spikes in *A* and *C*, respectively. The ventral root stimulus was at threshold intensity for the axon which belonged to the impaled motoneurone, so as to induce an antidromic invasion in about half the trials. Traces without invasion show the inhibitory post-synaptic potential evoked through the Renshaw pathway. Note that in *D* the antidromic invasion was facilitated by the monosynaptically induced excitatory post-synaptic potential. Records in upper row (*a*) of *A–D* were taken before injection, those in *b* of *A* and *B* at 41 sec after the Li^+ injection and of *C* and *D* at 35 sec after the Cs^+ injection. Records in *c* were taken at 660 sec for *A* and *B* and at 355 sec for *C* and *D*, after respective injections. Time-scales, 2 msec for *A* and *C* and 10 msec for *B* and *D*. Voltage calibrations, 50 mV for *A* and *C* and 5 mV for *B* and *D*. *E* and *F*, resting potential before and after injections of Li^+ (*E*) and Cs^+ (*F*) in trials from which records *A–D* were obtained. Solid lines were reproduced from charts of an ink-writing recorder and the interrupted lines superposed thereon indicate the correction for the artefact that was introduced by the passage of the ion-injecting current through the recording micro-electrode (ref. 3)

A laminectomy including all lumbar vertebræ was made in cats anæsthetized with pentobarbitone sodium. The cord was transected at the L_1- or L_2-level. Glass micro-electrodes were filled with solutions containing 2 M lithium sulphate, 1 M cæsium sulphate or 1·6 M rubidium sulphate, and inserted into motoneurones at the L_7- and S_1-levels. Intracellular recording from motoneurones and electrophoretic injections of cations into them were done as described previously[3]. The injections described here were performed by the passage of a current of 5×10^{-8} amp for 60 sec.

Fig. 1, *A*, *B* and *E*, shows changes in a motoneurone following an injection of Li^+ ions. Immediately after the injection, there was a depolarization of the resting potential (*E*) and a decline of the spike with a concomitant decrease of its rising and falling slopes (*A*). The active membrane potential (overshoot or reversal) at the spike peak was reduced, or there was even failure of reversal. There was also a reduction of the after-hyperpolarization and a conversion of the inhibitory post-synaptic potential from the original hyperpolarization to a depolarization (*B*). The pattern of these changes due to a Li^+ injection is the same as that obtained by a Na^+ injection[1]; the decrease of the active membrane potential and of the rising slope of the spike indicates that the enhanced permeability which causes the spike potential fails to discriminate between Li^+ and Na^+, which also occurs with other excitable membranes[4–6]. The effects of a Li^+ injection on the resting potential, after-potential and the falling slope of the spike can be explained by the displacement of intracellular K^+ ions.

The effects of Cs^+ ions are illustrated in Fig. 1, *C*, *D* and *F*. The most striking difference between Li^+ and Cs^+ was the lack of depression of the rising slope and active membrane potential of the spike subsequent to a Cs^+ injection. As was assumed already for choline ions[1], Cs^+ appears to be distinguished from Na^+ and Li^+ by the sodium-carrier system which is activated in the spike initiation[7]. Changes in the falling slope of the spike, the after-potential, and the resting potential were qualitatively identical to those produced by Li^+ or Na^+, thus indicating that Cs^+ ions are not effective substitutes for K^+ ions in the generation of these potentials. In contrast, injection of Rb^+ did not affect the motoneurone potentials except for the inhibitory post-synaptic potential that was reduced or sometimes reversed to a depolarizing potential, just as was observed on injection of K^+. It was not possible to discriminate between the effects of Rb^+ and K^+, either qualitatively or quantitatively.

The depolarizing action on the resting membrane can be related to the permeability of the motoneurone membrane to the injected cations, which determines the degree of depletion of the intracellular K^+ during their injection as well as their ability to replace the intracellular K^+ in generating the resting potential. Since, however, the depolarization produced by Na^+ injections varied according to the level of the resting potential[2], a precise comparison of this effect between different cations requires further statistical treatment. Nevertheless, it may be pointed out that the depolarizing action decreases in the order: $Li^+ > Na^+ > Cs^+$; the membrane depolarization actually obtained was up to 47 mV with Li^+, up to 35 mV with Na^+, and never more than 13 mV with Cs^+. The sequence of the order of permeability of the cat motoneurone membrane thus would be given by the series: $K^+ = Rb^+ > Cs^+ > Na^+ > Li^+$, which is in reasonable agreement with those reported in other tissues[4,8].

Changes induced by Li^+ injections completely recovered within 13 min. The recovery of the action potential and of the inhibitory post-synaptic potential was exponential with a mean time constant of 188 sec. Li^+ ions thus appear to be extruded from motoneurones at a rate about half that for Na^+. Since the inefficacy of the active extrusion of Li^+ ions has been shown in frog muscle[4], human erythrocytes[9], frog skin[10] and, though indirectly, in mammalian C fibres[11], the fast rate of Li^+ extrusion here obtained for cat motoneurones would provide a peculiar and unique feature of the membrane of mammalian nerve cells. The pharmacological action of Li^+ on the human central nervous system may not be attributable to accumulation of Li^+ ions in nerve cells[4,12]. The effects of Cs^+ injections decreased exponentially with a mean time constant of 81 sec, full recovery being attained within 6 min. Cs^+ ions are thus removed from motoneurones at a rate comparable with that for Na^+.

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Dissociation of Electrocortical Activation and Behavioural Arousal

THE fact that electrocortical 'activation' and behavioural 'arousal' are often correlated has led to interchangeable use of these terms in recent years. This identity stems from the extensive research of Magoun and Lindsley[1], who have put forward the unifying hypothesis that both wakefulness and low-voltage, fast electro-corticographic (ECG) activity depend on the 'tonic' facilitatory effect of the ascending reticular system on the cerebral cortex. Early work by Ranson[2] and Hess[3] had demonstrated that: (*a*) stimulation of the posterior hypothalamus results in behavioural arousal from sleep; (*b*) bilateral lesions of this region are accompanied by a chronic state of somnolence, or coma. Such effects were later duplicated by: (1) stimulation or (2) extensive destruction of the midbrain reticular formation[1]. In the interest of parsimony it was proposed that the posterior hypothalamus is involved in maintaining the waking state only in so far as it is a part of the ascending reticular system[1]. The present experiments were designed to test the following hypothesis: while the ascending reticular system mediates electrocortical 'tone', the posterior hypothalamic region has a functionally separable role in maintaining the waking state.

Thirty cats were used. Under pentobarbital anæsthesia, bilateral lesions were made in the posterior hypothalamic region by passing radio-frequency currents through stereotaxically placed electrodes. Bipolar stimulating electrodes were permanently implanted in the reticular formation, approximately 3 mm lateral to the midline at the level of the superior

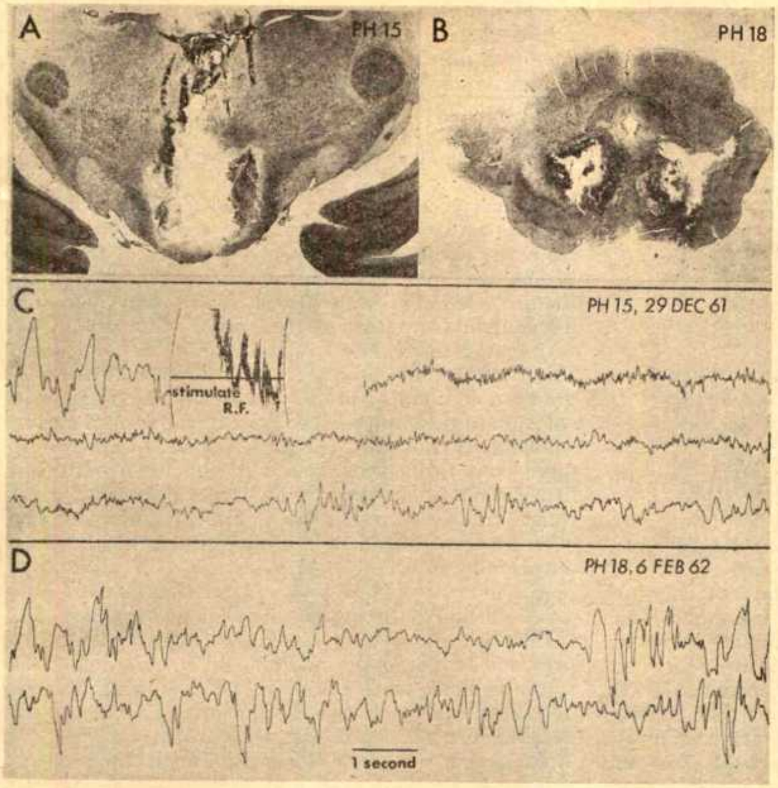

Fig. 1. ECG activity following lesions in posterior hypothalamic region and reticular formation. *A*, 40μ frozen section, stained with cresyl violet, showing hypothalamic lesion in cat *PH*-15; *B*, 40μ frozen section, stained with cresyl violet, showing tegmental lesion in cat *PH*-18; *C*, effect of reticular formation stimulation on activity of left posterior cortex of somnolent cat (*PH*-15) with lesion in posterior hypothalamic region. Line 2 starts 60 sec, line 3, 120 sec, after stimulus termination (cal. = 100 μV); *D*, activity of left posterior cortex during visual tracking, in awake cat (*PH*-18) with tegmental lesion. This ECG pattern was also present before and after the tracking session. The two tracings are continuous. (Cal. not available)

colliculus; recording electrodes were positioned epidurally on anterior and posterior cortex. After recovery from anæsthesia, the state of the surviving cats ranged from uninterrupted somnolence throughout the period of survival, to alert, attentive behaviour, depending on the locus and extent of the lesions. Animals with nearly complete lesions of the posterior hypothalamic region (sparing the ventromedial nuclei anteriorly and caudal portion of the mammillary bodies posteriorly, but including varying amounts of lateral hypothalamus, Fig. 1*a*) were apparently unresponsive to sensory stimuli. They required tube-feeding, showed no spontaneous movement, and could not be behaviourally aroused.

Beginning with the second postoperative day, ECG recordings were taken. One to two-second trains of 0·1 msec pulses were delivered to the midbrain reticular formation at a frequency of 100/sec when the cats appeared to be behaviourally asleep and when the ECG was also characterized by high-voltage, slow activity. It was found that cats with hypothalamic lesions resulting in somnolence showed a clear-cut dissociation between ECG activation and arousal. Although ECG desynchronization resulted from midbrain reticular formation stimulation, the only behavioural response of the somnolent cats was a highly stereotyped head and limb extension during the stimulus strain, followed by passive return to the sleeping posture within a few seconds. When stimulus intensity was at ECG desynchronization threshold, not even the passive return outlasted the stimulus. These movements were never accompanied by orientation to visual or auditory stimuli and clearly could not be interpreted as arousal. The ECG activation, on the other hand, outlasted the stereotyped, stimulus-bound movement by periods ranging from one or two seconds to more than four minutes, depending on the intensity of stimulation (Fig 1*c*). Peripheral stimulation also induced lasting ECG activation despite the absence of signs of behavioural arousal or orientation to the environment. This desynchronization of ECG activity can be distinguished from what has been described by Jouvet[4] as 'paradoxical sleep'. Stimulation of the midbrain reticular formation, which produced ECG activation alone in somnolent cats, produced desynchronization and behavioural arousal in normal or drowsy cats; the desynchronization pattern in the somnolent cats is therefore what has commonly been identified with behavioural arousal.

Since these data show that it is possible to dissociate behavioural arousal and ECG activation, the next problem to be considered was whether it is possible to dissociate behavioural sleep and ECG synchronization[5]. Bitateral lesions were placed in the midbrain reticular formation, sparing most of the peduncles and periventricular grey (Fig. 1*b*). Such lesions were followed not by somnolence but rather by sluggish behaviour. The animals fed themselves, attended to visual and auditory cues, and showed alternation of sleep and wakefulness. The ECG pattern, however, showed spontaneous shifts from slow, high-voltage activity to fast, low-voltage activity which correlated only approximately with behavioural arousal. In the record shown in Fig. 1*d*, for example, the animal followed a visual stimulus during the 24-sec period shown. This pattern persisted for 5 min, and was only momentarily interrupted by low-voltage, fast activity during the course of the experimental session, although the cat was awake and attentive throughout.

These experiments confirm the classical findings of Ranson[2] that lesions of the posterior hypothalamus are accompanied by behavioural somnolence. The critical findings of this study are: (*a*) that behavioural arousal is lost following posterior hypothalamic lesions despite the functional integrity of an ascending pathway from the midbrain reticular formation which can provide ECG desynchronization; (*b*) that slow ECG activity is the dominant pattern following lesions of the midbrain reticular formation, despite the integrity of a conducting pathway which can mediate arousal and alert attentive behaviour. This leads to the conclusion that although behavioural arousal requires the integrity of the posterior hypothalamic region, induced ECG activation is not critically dependent on pathways funnelling through this region.

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Independence of Transfer of Solute and Solvent across the Rat Ileum

FROM experiments on the rat ileum *in vitro* Fisher[1] and Smyth and Taylor[2], using glucose-free media and metabolic inhibitors, suggested that the movement of water across the ileum was an active, metabolic dependent process. Parsons and Wingate[3] observed that net movement of water took place against an osmotic gradient, that is, with the mucosal solution hypertonic to the serosal there was a net water movement from the mucosal to the serosal surface. These workers again suggested that the process was an active one relatively independent of osmotic forces. Also, Smyth and Taylor[4] using a glucose-free medium with isolated rat ileum found that the water movement was lowered in the absence of glucose and interpreted the process of water movement as being an active one. Hendley and Smyth[5] have reported experiments showing that the concentration of sodium in the fluid appearing on the serosal surface is higher than the concentration on the mucosal surface of the rat intestine, indicating that there was active sodium transport, but have drawn no conclusions as to its primary role in solvent movement. Curran and Solomon[6] and Curran[7], on the other hand, have shown, both *in vivo* and *in vitro*, that the movement of water across the rat ileum is secondarily dependent on active transport of solute. Removal of the glucose from the medium results in a lowering of the solute transport and hence a fall in water movement. Similarly, using metabolic inhibitors the primary action of these was demonstrated to be on the salt transport mechanism and the water movement was shown to be only a secondary effect. Furthermore, the experiments of Curran[7] show that, with isotonic conditions on both sides of the gut, a gradient of water activity is established as a result of the net solute transfer, and this is sufficient to explain the net solvent movement, thus affirming the passive nature of the water transfer.

The quantitative relation between water movement across the ileum and the sugar concentration in the medium, particularly that of glucose, which has been demonstrated by many authors (Smyth *et al.*[8]; Crane[9] and Wilson[10]) would appear to be a secondary phenomenon, the glucose being primarily required to supply the necessary energy for the active salt transport.

The active salt transport (both sodium and chloride are transported, Curran[7]) across the isolated ileum is from the mucosal to the serosal surface of the membrane, and under isotonic conditions the net movement of water is towards the serosal surface (Table 1). To demonstrate further the passive nature of the water movement, and to illustrate the dependence of this transfer on water activity gradients, an osmotic gradient could be established with the mucosal surface solution hypertonic with respect to the serosal surface solution. The net water movement and sodium fluxes determined in such experiments would confirm either of the two main hypotheses concerning water transfer across the rat ileum. If the water were actively transported then it should be moved against an activity gradient (for definition of 'active transport' see reviews by Rosenberg[11] and Ussing[12]); however, as the results indicate, this is not the case.

Female rats (Wistar strain) were starved for 18 h previous to the experiments, and after ether anaesthesia the intestines were removed following washing in cold 0·9 per cent saline solution. The ileum was divided into five equal length segments (16–17 cm); only the first three portions beginning at the duodenal end were used, however, and everted sacs made from them according to the method of Wilson and Wiseman[13], using the modification of Parsons, Smyth and Taylor[14] for longer pieces of intestine. The sacs were filled with 1 ml. of Krebs–Ringer bicarbonate solution containing 0·5 per cent glucose, and suspended in 10 ml. of this medium. After 3 min gassing with a 95 per cent oxygen–5 per cent carbon dioxide mixture, the flask was sealed and placed in a water bath at 37° C. The sacs were weighed empty and when containing the saline medium, both before and after the incubation period of 1 h. From these results the net water movement and the tissue water uptake were determined. In the same experiments influx and outflux of sodium was determined using sodium-24 (R.C.C., Amersham). The influx and outflux, however, were measured in different experiments. Counting was done, using dried 0·2 ml. samples of the solutions labelled with sodium-24, with an end-window Geiger–Müller tube; the suitable corrections were applied to the counts for decay during the time of the experiment, all counts being corrected to zero time.

Six animals were treated as already described, with identical solutions of the saline medium on either side of the membrane. A further group of six animals differed in having on the mucosal surface the saline

Table 1

Isotonic conditions:	M_{ms}^{Na}	M_{sm}^{Na}	M_{n}^{Na}	M_{n}^{W}	M_{t}^{W}
Segment 1	22·61	13·90	8·71 ± 0·33	+0·419 ± 0·0417	+0·327 ± 0·051
Segment 2	20·50	11·88	8·62 ± 0·39	+0·366 ± 0·1238	+0·282 ± 0·043
Segment 3	20·81	12·55	8·32 ± 0·35	+0·305 ± 0·0979	+0·251 ± 0·039
Hypertonic conditions:					
Segment 1	21·73	13·18	8·55 ± 0·37	−0·007 ± 0·0007*	−0·028 ± 0·019†
Segment 2	20·69	12·02	8·67 ± 0·41	+0·006 ± 0·0018	+0·018 ± 0·022
Segment 3	20·80	11·93	8·88 ± 0·29	−0·019 ± 0·0014*	+0·030 ± 0·031

M_{ms}^{Na}, Mucosal to serosal flux of sodium; M_{sm}^{Na}, serosal to mucosal flux of sodium; M_{n}^{Na}, net flux of sodium (expressed as μequiv./cm length/h); M_{n}^{W}, net water flux (ml./total length/h); M_{t}^{W}, tissue gain or loss in weight.

* Net water flux from serosa to mucosa.

† Indicates net loss in tissue weight.

containing 150 mmoles/l. of mannitol, resulting in an osmotic gradient of 150 mmoles from the serosal to the mucosal side of the gut. Mannitol has been shown to penetrate very slowly through the rat gut and thus the osmotic gradient used would not be expected to vary significantly during the course of the experimental period[7,15]. In each group of six animals three animals were utilized (that is, nine pieces of ileum) for determining sodium outflux by placing the sodium-24 on the serosal surface, that is, inside the sac, and three animals (again nine pieces of gut) were used for determining the sodium influx with the sodium-24 on the mucosal surface. In all the experiments the net flux of water was determined by changes in weight of the tissue and of the serosal fluid. There was no statistical difference between the water movement measurements irrespective of whether the sodium-24 was inside the sac or in the outside solution.

The results of this experiment are summarized in Table 1, where each of the three segments per animal are given, segment 1, the most anterior, being taken from the ileum immediately posterior to the duodenum. The results show that with isotonic conditions on each side of the membrane, in each segment there is a net transfer of solvent from mucosal to serosal fluids occurring together with a net transport of sodium (expressed as μequiv./cm length/h).

When the mucosal surface is made hypertonic to the serosal, with no change in the sodium concentration on either side of the membrane, there is still active sodium transport of quantitatively the same order as that obtained under isotonic conditions. The net water transfer, however, is almost negligible, the hypertonicity of the mucosal fluid reducing the net water flux to zero. In addition the tissue fluid is reduced when non-isotonic conditions prevail. These results clearly indicate that the water transfer across the ileum of the rat is dependent on the water activity gradients alone. That the process is not an active one is seen from the fact that the transfer is entirely accounted for by the physical properties of the two phases separated by the membrane.

Although concentration gradients of the magnitude used in these experiments are perhaps not those found in the intact animal, they do offer a method of separating the active and passive processes occurring across the membrane, and demonstrate that water is not absorbed against an osmotic gradient.

Since the net sodium flux remains unaltered with isotonic or hypertonic conditions existing across the ileum this further suggests that the sodium and water move in different channels across this membrane. Evidence has been presented by Ussing and Andersen[16] and Koefoed-Johnsen and Ussing[17] that solvent drag, as a result of osmotic water flow, may alter the ionic flow ratio ($M_{\text{in}}/M_{\text{out}}$) in the case of frog skin. It has also been shown recently by Schoeffeniels and Tercafs[18] that in isolated frog skin the passive movement of sodium (that is, outflux) and the water flux take place through spacially separate structures.

Our results indicate, since outflow of water does not cause any alteration in the sodium outflux, that also in rat ileum this spacial separation of the passive sodium movement and water movement may exist. The sodium influx, which is an active process taking place in the non-short-circuited membrane against an electrochemical gradient (serosa positive with respect to mucosa), remains unaffected by the reduction in net water movement, thus indicating that solvent drag plays only a small part in the salt transport across the rat ileum. Since our results indicate that there is no 'drag' effect on the influx of sodium, as well as none on the outflux, then it may be that the active movement of sodium (that is, influx) and the water movement take place through spacially separate structures. This appears to be unlike the conditions in amphibian skin, where evidence has been presented by Ussing and Andersen[16] that the sodium influx takes place through water-filled pores in the membrane. Curran[7], like ourselves, regards the solvent drag as playing virtually no part in the transport process across the rat ileum.

The results obtained here support the concept of a passive movement of water through the rat ileum, as in many biological membranes, and provide further information for the understanding of cellular permeability problems.

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PHARMACOLOGY

Ability of Some Antirheumatic Drugs to uncouple Oxidative Phosphorylation

Adams and Cobb[1] directed attention to the fact that certain non-hormonal antirheumatic (anti-inflammatory) drugs uncouple oxidative phosphorylation in liver mitochondria, citing phenylbutazone and salicylic acid as two examples. This correlation can now be extended to include certain analogues of these two drugs and at least three other types of anti-inflammatory agents not chemically related to either salicylate or phenylbutazone, exemplified by cinchophene, glycyrrhetinic acid and certain derivatives of anthranilic acid.

Cinchophene (2-phenylcinchoninic acid) and its 3-hydroxy derivative were formerly used as analgesic-antirheumatic agents but are now known to cause hepatic injury. Glycyrrhetinic acid (3-hydroxy-11-oxo-olean-12-en-30-oic acid), the aglycone of glycyrrhizin from liquorice, is a relatively powerful anti-inflammatory drug in animals[2-4]. Glycyrrhizin itself also exhibits some anti-inflammatory activity[5]. *N*-(α,α,α-trifluoro-*m*-tolyl)anthranilic acid (*CI*-440, flufenamic acid)[6] and *N*-(2,3,xylyl)anthranilic acid (*CI-473*, mefenamic acid)[7] are comparable with phenylbutazone in anti-inflammatory assays with laboratory animals. We have now found that these drugs also uncouple oxidative phosphorylation and stimulate the adenosine triphosphatase (ATPase) activity of rat liver mitochondria. Furthermore, we have evidence that these particular drugs also uncouple oxidative phosphorylation in at least two connective tissues, that is, tissue upon which antirheumatic drugs must exert some effect *in vivo*. All these drugs and 2 : 4-dinitrophenol, a powerful uncoupler of oxidative phosphorylation, inhibited both the incorporation of orthophosphate into organic phosphates and the ATP-dependent biosynthesis by cartilage and cornea of mucopolysaccharide sulphates (which are characteristic structural components of animal connective tissues).

Oxidative phosphorylation with succinate as substrate at 30° and mitochondrial ATPase activity at 20° were measured by described procedures[8,9]. Drugs were added either as solutions in 25 µl. *N,N*-dimethylformamide or in aqueous solutions neutralized with sodium bicarbonate. These drugs were also tested for their effect on: (1) the incorporation of sulphur-35 from sodium sulphate-^{35}S into tissue mucopolysaccharide sulphates by corneal and cartilage slices[10,11]; (2) the oxidation of octanoate-1-^{14}C to carbon dioxide-^{14}C by cartilage slices[11]; (3) the incorporation of phosphorus-32 from sodium orthophosphate-^{32}P into organic phosphates by cartilage slices incubated in a Krebs–Ringer type buffer solution constituted with *tris* hydrochloride, *p*H 7·4. The incubated tissue slices were digested with papain[11] to release non-protein constituents. Inorganic phosphate-^{32}P was precipitated together with added non-radioactive orthophosphate, *p*H 7·4, by magnesium and ammonium ions at 4° and *p*H 9·2, leaving the bulk of the organic phosphates in solution. 1,2-Diphenyl-3,5-dioxo-4(3′-hydroxybutyl)-pyrazolidine, a metabolite of phenylbutazone (metabolite II), was freshly prepared from the tautomeric lactone, δ-caprolactone-α-carboxylic-*N,N*′diphenylhydrazide (*G* 28,423) by treatment with alkali[12].

Table 1 shows that the relative activity of these drugs and some of their chemical analogues as inhibitors of phosphate and sulphate metabolism by cartilage slices *in vitro* parallels their activity as uncouplers of oxidative phosphorylation and agrees with their relative potencies as anti-inflammatory agents, where this is known[6,13,14]. More hydrophilic analogues of these antirheumatic drugs such as their principal *in vivo* metabolites (oxyphenbutazone), 'stripped down' chemical analogues (anthranilic acid, isonicotinic acid) or a glycoside (glycyrrhizin) were in all cases less potent drugs *in vitro*; the only exception being 3-hydroxycinchophene, which is also an analogue of salicylic acid, in contrast to cinchophene itself. Rendering salicylic acid more lipophilic by conversion to its anilide greatly enhanced its activity *in vitro*, not associated with the anilide group *per se* (cf. benzanilide). These relationships fully support the generalization that the activity of anti-inflammatory drugs is intimately associated with their lipophilic character[10] and are reminiscent of the Meyer–Overton hypothesis for narcotic action.

Only phenylbutazone, flufenamic acid and salicylanilide at concentrations which inhibited sulphate metabolism by more than 50 per cent inhibited octanoate oxidation by cartilage to any significant degree. The following isoprenoids, which contain one or more of the functional groups of the glycyrrhetinic acid molecule, neither uncoupled oxidative phosphorylation in liver mitochondria nor inhibited cartilage metabolism at 5×10^{-5} M: oleanolic acid, *O*-acetyl oleanolic acid, morolic acid, cholest-4-ene-3-one, deoxycorticosterone (DOC), menthol. This inactivity of DOC suggests that the mineral corticoid activity of glycyrrhetinic acid[4] is largely dissociated from its anti-inflammatory properties.

Table 1. EFFECT OF ANTI-INFLAMMATORY DRUGS AND SOME OF THEIR ANALOGUES UPON: (*a*) THE PHOSPHORYLATION QUOTIENT (P/O) WITH RAT LIVER MITOCHONDRIA AND SUCCINATE; (*b*) THE INCORPORATION OF ^{32}P$_i$ INTO ORGANIC PHOSPHATES (O.P.) BY BOVINE TRACHEAL CARTILAGE; (*c*) THE INCORPORATION OF ^{35}S$_i$ INTO MUCOPOLYSACCHARIDE SULPHATES (MPS) BY BOVINE TRACHEAL CARTILAGE

Compound	Liver Conc. $\times 10^{-4}$ M	Liver P/O	Cartilage Conc. $\times 10^{-4}$ M	Cartilage O.P.-^{32}P	Cartilage MPS-^{35}S
Phenylbutazone (PB)	0·5	36	5	35	34
Oxyphenbutazone	1	43	5	46	54
Metabolite II (γ-HydroxyPB)	1·5	89	5	85	65
Di-*p*-carboxy(phenyl)PB (HP358)	5	81	10	105	103
Salicylic acid	5	46	40	45	40
Salicylanilide	0·5	63	1	56	43
Benzanilide	5	95	1	94	95
Cinchophene	2·5	63	4	74	37
3-Hydroxycinchophene	0·5	2	4	28	15
Isonicotinic acid	5	97	10	96	93
Glycyrrhetinic acid	0·5	41	0·5	19	13
Glycyrrhizin	5	74	5	38	10
	0·5	100	0·5	75	43
Mefenamic acid	0·5	45	1	66	70
Flufenamic acid	0·5	13	1	46	54
Anthranilic acid	2	99	10	108	97
2 : 4-Dinitrophenol	0·3	30	0·5	65	53

Values for P/O ratio, O.P.-^{32}P and MPS-^{35}S after incubation with drugs are expressed as percentage of controls (incubated without drugs)

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RADIOBIOLOGY

Sensitization of Radio-resistant Bacteria to X-rays by Iodoacetamide

IODOACETATE has been shown to sensitize animals[1,2], cells in tissue culture[3] and bacteria[4] to X-rays. We have found[5] using iodoacetamide that the sensitization was particularly marked with the more radio-resistant bacteria.

Bacterial cells were suspended in 0·1 M phosphate buffer *p*H 7·0 and iodoacetamide added to give a concentration of 10^{-3} M or less. Exposure to iodoacetamide at 10^{-3} M alone for a period of 1 h caused no cell inactivation. After 20-min treatment with

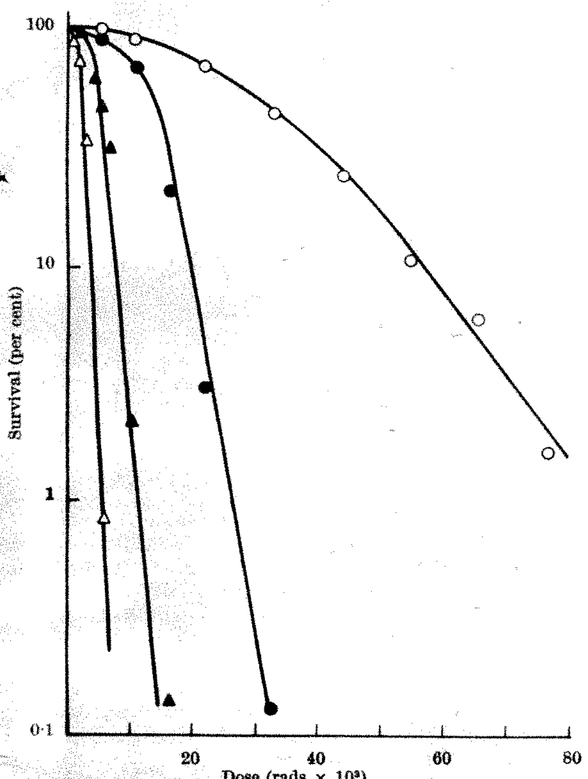

Fig. 1. Effect of iodoacetamide (added to the phosphate buffer in which the organisms were suspended) on the inactivation by X-rays of *Micrococcus sodonensis*. ○, No iodoacetamide; ●, 10^{-4} M iodoacetamide; ▲, 3×10^{-4} M iodoacetamide; Δ, 10^{-3} M iodoacetamide

iodoacetamide at room temperature samples were irradiated under controlled gas conditions with 220 kVp X-rays. The dose-rate was $1{\cdot}1 \times 10^3$ rads/min. Survivors were estimated by the surface viable count technique[6] on 'Oxoid' nutrient agar.

In aerated conditions we found no sensitization of *E. coli. B* by 10^{-4} M iodoacetamide. With *Pseudomonas fluorescens* there was a slight increase in sensitivity but the slope of the exponential survival curve was increased by not more than 10 per cent. With *E. coli B/r*, however, there was a marked increase in sensitivity. Not only was the initial shoulder of the survival curve decreased but there was also an increase in the slope of the exponential part. The lethal dose (expressed as dose required to give 37 per cent survival, D_{37}) was decreased from 13 krads to 6 krads, a dose enhancement factor of approximately 2·2. Both the initial shoulder and the final slope were equally affected in *Micrococcus sodonensis* (Fig. 1). The degree of sensitization was increased by raising the concentration of iodoacetamide; the dose enhancement factors being 2·6, 5·8 and 12·6 for concentrations of 10^{-4}, 3×10^{-4} and 10^{-3} M iodoacetamide respectively. At the highest concentration used (10^{-3} M) the lethal dose was reduced from 38 krads to 3 krads. Under anaerobic conditions *Micrococcus sodonensis* was 2·0 times as resistant to X-rays than when irradiated in aerated conditions (based on the slope of the exponential part of the survival curve). Iodoacetamide also sensitized the organism under anaerobic conditions and the magnitude of the effect was approximately the same as in air, that is, 10^{-4} M iodoacetamide reduced the anaerobic D_{37} from 89 krads to 39 krads, a factor of 2·3.

Micrococcus radiodurans, an organism exhibiting extreme radio-resistance, showed a very great increase in sensitivity when exposed to iodoacetamide during irradiation (Fig. 2). In air 3×10^{-4} M iodoacetamide reduced the lethal dose (D_{37}) from 263 krads to 37 krads and with 10^{-3} M iodoacetamide it was reduced to 3 krads. At the latter concentration the dose enhancement factor was nearly 90, and again both the initial shoulder and the slope of the final exponential curve were reduced.

Although the time and temperature of treatment with iodoacetamide before irradiation did not influence the sensitization of *Micrococcus sodonensis*, the effect was lost if the iodoacetamide was removed immediately before irradiation by dilution into buffer at 0° C. The addition of iodoacetamide immediately after irradiation produced no sensitization. Suitable experiments showed that the increased cell inactivation was not due to toxic irradiation products of the iodoacetamide produced in the external medium. Apparently the only condition necessary to obtain sensitization was the presence of iodoacetamide in the buffered suspension during irradiation.

We have no explanation to offer for the mechanism of sensitization of these resistant organisms to X-rays by iodoacetamide, but biochemical investigations indicate that the action may involve the cell membrane. In addition to sensitizing the inactivation of cells, two radiation-induced biochemical changes, namely the bleaching of the yellow pigment in *Micrococcus sodonensis* and the reduction in the oxidation of pyruvate by this organism, were considerably enhanced if the irradiation was carried out in the presence of iodoacetamide. As in the case of cell inactivation, iodoacetamide by itself had no effect on pyruvate metabolism or the pigment. From spectrophotometric data the extinction of the pigment was reduced by 50–60 per cent for a dose of 110 krads given in the presence of 10^{-3} M iodoacetamide, but only by 10 per cent in its absence.

The oxidation of pyruvate measured manometrically was reduced by less than 10 per cent with a dose of 110 krads. When 10^{-4} M iodoacetamide was present during irradiation the oxygen consumption was reduced by 58 per cent. On the other hand, the

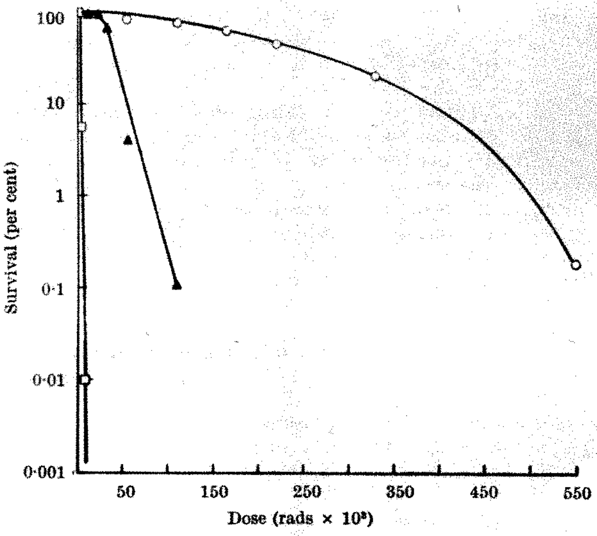

Fig. 2. Effect of iodoacetamide on the inactivation by X-rays of *Micrococcus radiodurans*. ○, No iodoacetamide; ▲, 3×10^{-4} M iodoacetamide; □, 10^{-3} M iodoacetamide

degradation of DNA, measured viscosometrically, in samples extracted from *E. coli B/r* immediately after irradiation was not influenced by the presence of iodoacetamide.

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Influence of X-rays on the Incorporation of Deoxyribonucleic Acid Precursors into some Mammalian Cells *in vitro*

Dose-response curves for the influence of ionizing radiations on *in vitro* incorporation of DNA precursors into human bone marrow[1] and experimental tumours[2,3] irradiated in the period of synthesis of DNA are well known. Recently published investigations[4] showed a striking similarity between these curves for human bone marrow and Ehrlich ascites carcinoma while both systems were oxygenated. Our experiments show the relationship between the dose of X-rays and the depression of thymidine incorporation in anoxic suspensions of rat bone marrow and Ehrlich ascites tumour.

The suspensions[5] contained an average amount of $2 \cdot 4 \times 10^7$ nucleated bone marrow cells together with $0 \cdot 7 \times 10^7$ erythrocytes or $1 \cdot 1 \times 10^7$ tumour cells in 1 ml. of medium, consisting of 50 per cent of supplemented Hanks's solution and 50 per cent of rat serum. The suspension of cells was irradiated in room temperature with X-rays, 160 kVp, dose-rate of 140 r./min. Thymidine-2-^{14}C, 0·75 μc./ml. culture, specific activity 16·0 or 18·3 mc./mM, was added immediately afterwards. 2-ml. aliquots of cell suspension were incubated at 37·5° C in a number of screw-cap bottles for 2 h without shaking. After incubation the cells were washed twice in ice-cold solution of 0·85 per cent sodium chloride and once in a 2 per cent acetic acid. Each time the cell suspension was centrifuged for 10 min at 0° C (2,000*g*). Finally, the sediment was suspended in 2 ml. of 2 per cent acetic acid and three aliquots of 0·3 ml. were placed in aluminium planchettes. A windowless gas-flow counter was used for measuring the activity. The mean values of the experimental groups were expressed as a percentage of the control group. For each dose 4–6 separate experiments were carried out. Standard deviation of individual experiments are indicated on the graph (Fig. 1). The tendency of the resulting curves to converge allowed us to draw a common curve for both tissues.

In the foregoing experiments both systems are anoxic: the suspension of Ehrlich ascites tumour cells does not contain red blood cells; the ratio of erythrocytes to nucleated bone marrow cells is about 1 : 3, while Berry *et al.*[4] added 50–100 red blood cells for each tumour cell to the suspensions so as to oxidize the cells of Ehrlich carcinoma. The same X-ray dose-response curves for the inhibition of incorporation of thymidine were obtained even though the compared suspensions contained cells that were taken from two species of animals and were of a different tissue character, one being a rather homogeneous population of tumour cells of epithelial origin, and the other normal, inhomogeneous, hæmopoietic tissue. Finally, they also differ in the rate of incorporation of thymidine that was found in other experiments[6] in which irradiation was not performed.

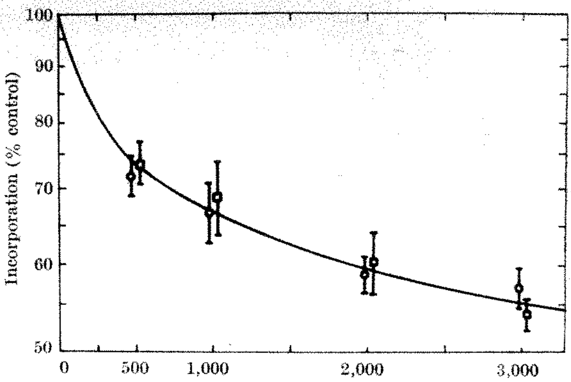

Fig. 1. Dose–response curve for X-rays on incorporation of thymidine-2-^{14}C into rat bone marrow and Ehrlich ascites tumour cells *in vitro* (S period). □, Bone marrow; ○, Ehrlich ascites tumour

This lack of specificity of X-ray action on incorporation of thymidine into different tissues depends probably on the environment of the cell. The kind of serum used seems to be an important factor. Berry, Hell, Lajtha and Ebert obtained similar results testing oxygenated suspensions of human bone marrow and Ehrlich ascites tumour cells; in both cases they used human serum. Lajtha[7], on the contrary, noticed changes in the effect of X-rays on synthesis of DNA induced by various sera. This may account for the differences between dose-response curves for Ehrlich carcinoma obtained by Berry *et al.*[2] and by us.

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BIOLOGY

Tumour Induction by an Indolyl-3-Acetic Acid–Kinetin Interaction in a *Nicotiana* Hybrid

It has been demonstrated that auxins influence the ability of bacteria to induce plant tumours[1], and that kinetin in the presence of auxin can substitute for extracts of tumours in the production of tumours on isolated tobacco pith tissue[2].

Table 1. EFFECTS OF SEVERAL GROWTH FACTORS* ON TUMOUR FORMATION AND GROWTH OF THE HYBRID, AMPHIDIPLOID *Nicotiana suaveolens-langsdorffii*. PLANTS WERE TREATED 16 DAYS AFTER SEEDING AND SCORED 15 DAYS AFTER TREATMENT

Treatment	No. of plants	Plants with tumours (per cent)	Mean length of second leaf (mm) ± *S.D.*
Control	14	0·0	40·0 ± 5·6
IAA	12	8·0	—
Kinetin	16	44·0	—
GA	14	7·0	59·0 ± 6·8
IAA + kinetin	14	86·0	40·0 ± 6·6
IAA + kinetin + GA	12	92·0	48·0 ± 5·2
*p*CiBA	12	0·0	—
IUDR	14	0·0	

* Micromoles of each growth factor applied per seedling: $4{\cdot}70 \times 10^{-4}$ μm IAA, $1{\cdot}53 \times 10^{-3}$ μm kinetin, $2{\cdot}14 \times 10^{-3}$ μm GA, $4{\cdot}7 \times 10^{-4}$ μm *p*CiBA, and 1×10^{-3} μm IUDR.

It was of interest to determine whether spontaneous genotypically determined tumour formation in *Nicotiana* hybrids[3] involves the same or similar hormonal and biochemical alterations as occur in tumours induced by the crown-gall bacterium. Earlier work with *Nicotiana* species and crosses[4] has shown that the hybrid, amphidiploid *N. glauca-langsdorffii*, has a higher free auxin-level as well as a greater capacity for the conversion of tryptophan to auxin than the parental species. This report describes the interaction of indolyl-3-acetic acid (IAA), 6-furfurylaminopurine (kinetin) and gibberellic acid (GA) on tumour induction in seedlings of the interspecific hybrid, amphidiploid *Nicotiana suaveolens-langsdorffii*.

The tobacco seedlings were grown aseptically in 125-ml. Erlenmeyer flasks containing 1 per cent agar and a modified Hildebrandt–Riker nutrient medium[5] with 'Versene'-chelated micronutrients. The plants were maintained in cotton-stoppered flasks in a constant temperature chamber at 22° C, with an 18-h photo-period at 600–650 ft.-candles. Seedlings 16 days old, including the controls, were pre-treated with 1 drop of 0·1 per cent 'Tween-20' surfactant for 1 h after which the excess was removed with sterile cotton. The seedlings were then treated separately and in several combinations with IAA, kinetin, GA, *p*-chlorophenoxyisobutyric acid (*p*CiBA) and 5-iododeoxyuridine (IUDR). The growth-factor solutions containing IAA and kinetin as well as the 'Tween-20' were prepared in White's basal inorganic medium[6] as a carrier. The solutions were adjusted to *p*H 4·5 and autoclaved for sterilization. The heat-labile compounds, GA, *p*CiBA and IUDR, were added aseptically to the mineral-growth-factor mixtures after autoclaving. Measured volumes (5 μl.) of the growth factors were applied aseptically over the leaf axils enclosing the shoot apex. Care was exercised to avoid wounding the apex and the surrounding tissues.

As shown in Table 1, kinetin in the presence of IAA exerts a strong influence on tumour induction in these *Nicotiana* seedlings. Spontaneous tumours usually form on the plants only late in development subsequent to flowering. The treatment results clearly show a synergistic effect of IAA and kinetin. IAA and GA alone exert little effect on tumour induction. The IAA–kinetin mixture partially suppresses excessive elongation caused by GA. This is illustrated in Table 1 by the measurements of the length of the second leaf. Tumour induction by kinetin alone may reflect an IAA–kinetin interaction with endogenous IAA. It may be that kinetin interacts with IAA to interfere with normal apical control since the IAA–kinetin-treated seedlings seldom produce a normal apex. The anti-auxin *p*CiBA and the nucleic acid analogue IUDR obviously had no effect on tumour induction at the concentrations used.

The foregoing results from IAA and kinetin treatments suggest that hormonal interactions may be involved in tumour formation on genetically tumour-conditioned plants. The synergistic role of IAA with kinetin in the *Nicotiana* hybrid system and the fundamental role of IAA in the bacterially induced tumour system suggests that the two systems may be basically similar. This system, however, does not require wounding, and seems uniquely suited for future investigations in various aspects of plant tumour induction and development as well as for research on metabolic inducer phenomena as they relate to differentiation and morphogenesis in plants.

Numerous possibilities exist for the function of the IAA–kinetin interaction in the formation of the hybrid tobacco tumours. One is that kinetin may act directly as a purine analogue causing the production of abnormal nucleic acids and cellular proteins. Perhaps a better argument would be that the IAA–kinetin treatments elicit the production of a variety of cell constituents in the hybrid which can induce tumours and that precursors for similar compounds are lacking or are diverted in the parental species. This concept is supported by the findings that kinetin in the presence of IAA stimulates the production of certain specific compounds which may function in governing morphogenesis and development[7]. The natural cell metabolites produced during development, which mediate morphogenesis and differentiation, are believed to be concerned with the repression of certain specific components of the total genetic potential[8,9]. By an extension of this hypothesis, the IAA–kinetin treatment can be viewed as affecting the synthesis or activation of certain cell constituents which interfere with the action of normal repressors. Metabolic antagonists to normal repressors could have a direct effect on the synthesis of messenger RNA and consequently on proteins concerned with differentiation from meristematic cells.

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Methylcholanthrene-induced Lymphosarcomas in *Xenopus laevis*

THE number of spontaneous neoplasms found in Amphibia[1] and the success of Leone[2,3] in chemically inducing tumours in newts uphold the view[4] that Amphibia, like other vertebrates, are susceptible to the occurrence of neoplastic growth.

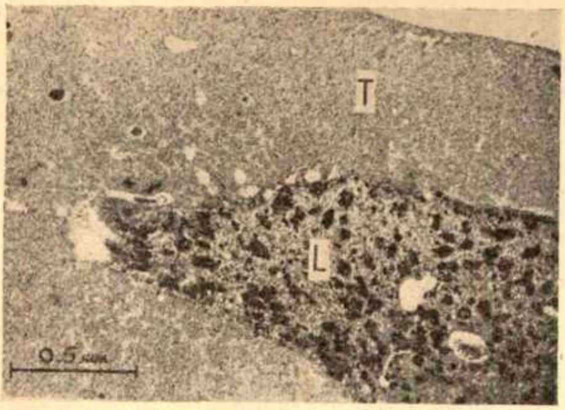

Fig. 1. Case 2, showing an unpigmented, vacuolated mass of lymphoid cells (*T*) around the pigmented liver tissue (*L*), which also contained many tumour cells. (Stained with hæmatoxylin and eosin)

Attempts at the chemical induction of tumours in Amphibia have, until recently, been unsuccessful. Thus Lucké and Schlumberger[5] were able to conclude that there was no convincing evidence that true neoplasia had been induced by the chemicals used. However, the success of Breedis[6] and Leone[3] in obtaining sarcomas in newts, using methylcholanthrene, and that of Briggs[7] with the anuran *Rana pipiens* indicate that this topic should be re-investigated.

Experiments have been performed in an attempt to induce neoplasia in adult *Xenopus laevis* using methylcholanthrene. Single doses of the chemical were given, either by injection in sterilized arachis oil into the dorsal lymph sac, abdominal cavity, thigh muscle or under the skin (14 animals), or by the implantation of the crystals alone into the dorsal lymph sac (12 animals).

The results of the experiment in which methylcholanthrene was given with oil are summarized in Table 1. The first individual (case 1) became emaciated and was killed 263 days after the injection. The kidneys were enlarged and yellow, while the enlarged liver was covered with white nodules. Microscopical analysis showed that the kidney, liver and spleen were being actively infiltrated and destroyed by masses of small, spheroidal cells the nuclei of which stain deeply and in which the amount of cytoplasm is very small. Fig. 1 shows the appearance of the liver in another animal (case 2), which was killed 384 days after injection and showed infiltration of the kidney, liver, oviduct, spleen and bladder by the same type of cells as observed in case 1. Four other animals, including 1 control, showed lymphoid tumours of varying degrees of development. No evidence of neoplastic change was found in 7 of 8 control animals injected with oil alone or in the other 5 experimental animals. The lipogranulomatous reaction observed in four of the latter group was probably due to the combined effect of oil and carcinogen, since no such reaction was found in the controls.

Two cases of lymphosarcoma were found among the 12 animals given methylcholanthrene crystals alone. One animal bore many nodules on the floor of the dorsal lymph sac when it was killed 365 days after crystal implantation. The nodules were composed of lymphocytes, which were also invading the kidney, liver, spleen and rectum. A small, yellow bump in the dorsal skin of the second animal was found to consist of lymphocytes, which had destroyed the glands of the dermis and were invading the epidermis and dermal muscle.

Table 1. EFFECTS OF INJECTING METHYLCHOLANTHRENE IN OIL

Case	Sex	Injection site	Days after injection	Tumour sites	Diagnosis	Total No. of cases
1	F	UAS	263	liver, kidney, spleen	transplantable lymphosarcoma	2
2	F	UAS	384	liver, kidney, oviduct, spleen, bladder		
3	M	UAS	83	lung, liver	lymphosarcoma	2
4	F	AC	384	lung		
5	F	DLS	388	kidney	probable lymphosarcoma	2
6	F	AC control	385	liver, lung		
7–15 plus 7 controls	—	—	78–484	—	negative	16

UAS, Under abdominal skin; AC, abdominal cavity; DLS, dorsal lymph sac.

The induced tumours appeared to be histologically very similar to the spontaneous lymphosarcomas previously described in *Xenopus*[1] and invaded similar sites, particularly the liver and kidney. Small fragments from three of the induced tumours were successfully transferred to other hosts. During the experiments no spontaneous lymphoid tumours became evident in any other of the one thousand adult animals of the colony.

Since most of the positive results in these experiments were found more than 300 days after the introduction of the chemical, it may be that earlier attempts[8,9] using methylcholanthrene with Amphibia have failed because the animals died too soon after injection and long before any neoplastic change had taken place. Furthermore, the proximity of the carcinogen to the host cells would vary with the viscosity of the oil injected with it, the viscosity depending on the temperature of the host itself. Since amphibians live at the temperature of their external environment and their maximum length of life varies from 10 to 40 years, it does not necessarily follow that the results of experiments using these animals would be the same as those using laboratory mammals. Indeed, Leone[3], who induced lymphosarcomas in newts (*Triturus cristatus*), found that lesions developed at the site of methylcholanthrene implantation 1–3 months later, and metastases, usually to the liver and spleen, 3–5 months after implantation. The difference in latent period in Leone's and the present experiments could be an expression of a difference between the urodele and anuran amphibia.

The results of these experiments with the anuran *Xenopus laevis* support the conclusion of Leone[2,3] that the implantation of methylcholanthrene into Amphibia leads to the development of sarcomas.

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Manatees of Guiana

Having recently returned from a conservation survey of manatees in British Guiana, we now put forward our views about the present status of these animals and their suggested deliberate use for the clearance of weeds from artificial waters. More detailed information will be published elsewhere.

American manatees still live, but in small numbers, in many places around the Gulf of Mexico and the Caribbean Sea. The sub-species on the coasts of Central and South America is *Trichechus manatus manatus*, while *T. manatus latirostris* is found on the Florida peninsula. Both sub-species occur in the coastal rivers and in the sea and both grow to a length of about 10–11 ft. and a weight of about 1,000 lb.

In British Guiana the manatees live primarily in the rivers of the coastal plain. Nowhere can they be said to be abundant: nor should they be described as rare, though they are certainly less frequently seen than formerly, suggesting greater scarcity. The increasing use of powered boats, including dug-out canoes with outboard engines, is probably scaring the animals away from many reaches of the rivers and so restricting their distribution. Also they are far from being immune from casual slaughter for food despite the protective legislation so wisely introduced in 1961. But they have not, in recent times, been deliberately hunted to an important degree, though at earlier dates it seems that they suffered an active fishery.

Venezuela probably also contains a considerable stock of these manatees, but information from here and from countries farther west is inadequate. Surinam has fewer than British Guiana and farther east the coastal plain disappears and there are no suitable rivers. In the Amazon Basin the formerly large stock of *Trichechus inunguis* seems to be sadly depleted following regular hunting for meat and hides which probably still continues. This is a smaller animal which seems to be restricted to fresh water.

Little information about the life-history of *T. manatus manatus* is yet available and it is now difficult to obtain without unsuitable and deliberate killing. It seems that the animals are slow breeders: young are very rarely seen. Evidence from captivity suggests longevity. In habits manatees are shy and unobtrusive, unable to leave the water and only appear at the surface to breathe, feed or occasionally lie at rest. They feed entirely on vegetable matter, which may be rooted on the bottom, floating on the surface or growing on the banks close above the water. They are largely unselective in their feeding and will eat any plants which are soft enough to be torn off by the muscular lips. Manatees tend to be gregarious.

In 1960 Mr. W. H. L. Allsopp, British Guiana fisheries research officer, directed attention to the fact that in certain circumstances manatees could be used for keeping water channels clear of both floating and standing vegetation[1]. This revival of interest in these animals received world-wide publicity, for it coincided with the problem of excessive weed growth in the new Lake Kariba. There grew up much exaggerated views on the useful potentialities of manatees as weed clearers if translocated to distant lands. However, for large areas of water the numbers needed to have any significant effect would seem to make the idea quite unrealistic. The picture began to be restored to its proper perspective by the publication of a paper by the Fisheries Division of the U.N. Food and Agriculture Organization in 1961[2].

Yet under particular conditions in British Guiana the local manatees are beginning to prove themselves efficient in weed clearance. They are being so used by the Department of Drainage and Irrigation on an experimental basis; and following a programme initiated by Mr. Allsopp, some seventy individuals have been caught since 1959 and introduced into channels varying in width from about 6 ft. to 60 ft. Reports on their performance are encouraging, and catching on a small scale continues. Further, in the Georgetown Waterworks two large ponds, about 150 and 250 yards by 15 yards, have been kept completely clear since 1950 by three manatees. However, the number of waters where these animals can be used efficiently is very limited, for they are easily disturbed by traffic and vulnerable to illicit human attack in restricted areas. The need to use the water channels for transporting cane makes it impracticable to use manatees in the sugar plantations.

Though potentially economic in a limited number of water ways, this use of manatees is at present all too expensive from the point of view of the stock. Despite improvements in technique, the losses in catching and transport are high. But even more important is the fact that there has been no breeding (apart from occasional births in the Georgetown Botanical Gardens some decades ago). Until this serious deficiency can be overcome, and manatees can be induced to breed regularly in a state of semi-domestication, the large-scale use of these animals for weed clearance cannot be sensibly recommended. Although the British Guiana stock may be able to survive under present pressures, it certainly could not stand the removal of large numbers of expendable individuals.

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Effect of Skeletal Muscle Homogenates on the Differentiation of the Chick Chorio-Allantoic Membrane

The hyperplastic and metaplastic effects of muscle homogenates and muscle extracts on the chorio-allantoic membrane of the chick have recently been the objects of a number of investigations[1-4]. Van Haefton contends that cell-free muscle homogenates pipetted on to the chorio-allantoic membrane can induce the development of non-striated 'myocytes' within the mesoderm of the membrane[3]. Ebert[1] has also reported the induction of occasionally striated 'muscle' cells following the introduction of ribonucleic acid extracted simultaneously from chicken cardiac muscle and Rous sarcoma virus. Ebert recognizes the possibility that van Haefton has observed the proliferation of cells remaining in the 'cell-free' homogenate rather than a response of the membrane itself[5]. Wilt and Stolz[4] have recently repeated the experiments of van Haefton with similar results. They conclude that the non-striated, oriented, elongated cells found within the chorio-allantoic membrane mesenchyme are of host rather than graft origin, thereby representing a genuine response of the chorio-allantoic membrane to components of the muscle homogenate. They further conclude that

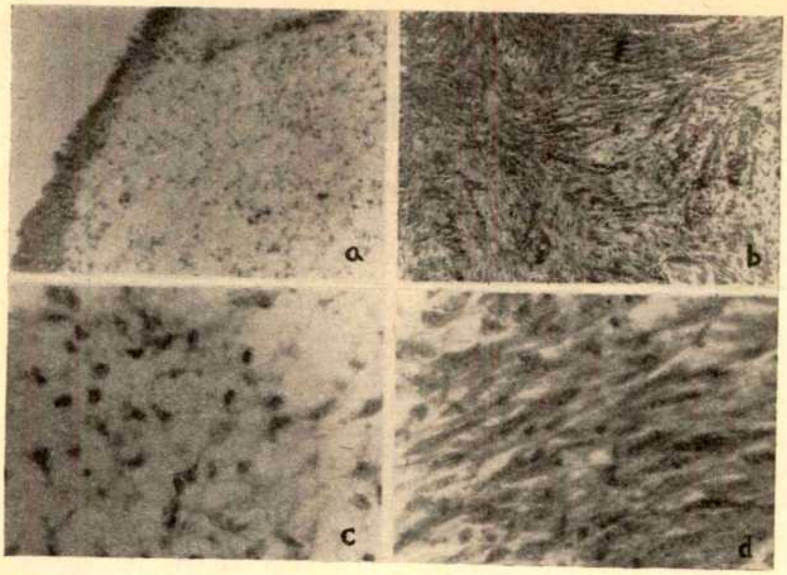

Fig. 1. *a*, Section through the normal chorio-allantoic membrane showing the chorionic epithelium at the left and mesenchyme cells to its right (× *c*. 50); *b*, elongated, oriented cells in the chorio-allantoic membrane mesoderm following treatment with muscle homogenate (× *c*. 50); *c*, mesenchyme cells of the normal chorio-allantoic membrane (× *c*. 200); *d*, induced response in mesoderm of chorio-allantoic membrane seven days after treatment with muscle homogenate (× *c*. 200)

present evidence does not justify statements regarding the nature of the response.

In our experiments homogenization of adult chicken breast muscle for injection on to the chorio-allantoic membrane was as follows: after sterile excision small pieces of muscle were blended with chick Ringer's solution for 25 min in a Waring blender at 4° C. 1 c.c. of this preparation contained 0·09 g of muscle by dry weight. The preparation was alternately frozen and thawed ten times and finally homogenized for 10 min in a motor-driven micro-homogenizer. Microscopical examination of the homogenates revealed the presence of nucleated cell fragments; however, intact cells were not found.

Under sterile conditions, the eggs of White Leghorn chickens were fenestrated on the eighth or ninth day of development. By means of a hypodermic syringe a well-vascularized area of the chorio-allantoic membrane was injected with 0·3–0·5 c.c. of the muscle homogenate. Control embryos were injected with 0·3 c.c. of sterile Ringer's solution. All eggs were sealed with a glass window and re-incubated for 7 days. At the end of this time the eggs were re-opened, the embryos were examined for grossly visible defects, and pieces of the membranes in the region of the homogenate were recovered for histological examination.

Of 36 control embryos, 24 (67 per cent) survived until the completion of the experiment. Histological examination showed that none of these survivors had a chorio-allantoic membrane with elongated, linearly oriented mesoderm cells. Of 70 experimental embryos, 11 (16 per cent) survived for 7 days after treatment with the homogenate. All these exhibited characteristics similar to those described by van Haefton[3] and Wilt and Stolz[4]. 'Non-specific' responses of the experimental membranes generally included vascular invasion of the chorio-allantoic membrane adjacent to the site of the homogenate; proliferation and stratification of both the ectodermal and endodermal epithelia; and metaplastic changes, typically cornification and erosion, in the chorionic epithelium. Mesodermal hyperplasia was also marked and occasional mesenchymal invasion by epithelial 'pearls'[3,6] was noted.

In addition to these 'non-specific', the chorioallantoic membrane of 6 (54 per cent) of the surviving experimental embryos exhibited a mesenchymal metaplastic change comparable with those considered 'muscle-specific' by Ebert[1] and van Haefton[3]. Groups of mononuclear mesenchymal cells became elongated and linearly oriented, lying parallel to the surface of the membrane. Consistent with other experiments of this type[3,4], the presence of cross-striations was never noted. Fig. 1 illustrates the kind of change occurring in the mesoderm of the chorio-allantoic membrane.

Our results differ from those of other investigators[1,3,4] in that the mortality-rate of the experimental animals was very high; also a higher percentage of surviving embryos exhibited linearly oriented, elongated mesoderm cells in the chorio-allantoic membrane. It is possible that the freezing and thawing of the homogenate is responsible for both these differences. However, properly controlled experiments will be necessary to demonstrate this conclusively.

In conclusion, we agree with van Haefton[3] and Wilt and Stolz[4] that cell-free homogenates of adult chicken muscle can elicit a response from the chorio-allantoic membrane of the developing embryo. To define this response, on the basis of present evidence, as the induction of muscle tissue would be premature. We agree with Wilt and Stolz[4] that specific histochemical, immunological and biochemical tests are necessary to demonstrate a relationship between striated muscle cells and the cells found in the mesoderm of the chorio-allantoic membrane of the treated embryos.

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Variation in Pathogenicity in the Cereal Root Eelworm

VARIATION in the pathogenicity of the cereal root eelworm (*Heterodera avenae* Wollenweber, 1924) was first demonstrated in Denmark by Andersen[1] using various barleys as differentials. Four of these barleys, No. 191, No. 14, Drost and Fero, with Rika as control, have been tested here against seven populations of the cereal root eelworm obtained from various parts of Britain.

Table 1. TOTAL NUMBER OF MATURE FEMALES OF THE CEREAL ROOT EELWORM FROM FIVE PLANTS OF EACH OF FIVE BARLEY VARIETIES IN SEVEN SOILS

Barley varieties	Cereal root eelworm populations 1	7	8	4	5	6	9
Rika (control)	796	1,581	2,018	1,390	2,245	1,964	1,949
Drost	0	94	183	860	2,215	1,358	1,501
Fero	0	95	209	1,245	2,068	1,753	1,133
No. 14	0	0	0	6	0	1	3
No. 191	0	0	0	5	0	0	2

The material was sown February 1962 in infested soil in pots in the glasshouse, and the number of mature cereal root eelworm females on the plant roots was counted in June 1962. The results are summarized in Table 1.

Clearly, Rika barley is susceptible to all seven cereal root eelworm populations, while No. 191 and No. 14 can be classed as resistant, although this resistance is not complete since a small number of females was found on some plants when tested against certain eelworm populations; but they never exceeded three on a single plant.

The reaction of Drost and Fero shows that there are differences in pathogenicity between some of the eelworm populations. These two varieties are resistant to population 1 of the cereal root eelworm but completely susceptible to populations 4, 5, 6 and 9, while to populations 7 and 8 their reaction is intermediate.

It also appears that there are genetic differences between the resistances of some of the barley varieties; this is being investigated.

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Influence of Hypophysectomy and of an Adrenocortical Inhibitor (*SU*-4885) on the Stress-Response of the White Blood Cells in the Teleost Fish, *Mollienesia latipinna* Le Sueur

PREVIOUS work has shown that stress by brief immersion in ice-water induces characteristic changes in the number of circulating leucocytes in the male killifish, *Fundulus heteroclitus*: at 20° C, an initial fall (leucopenia) 1 h after stress is succeeded at 2 h by an increase above the normal level (leucocytosis)[1,2]. Single injections of ACTH[3] produced leucopenia at low doses (0·15 I.U./g), and leucocytosis at higher doses[2]. Single injections of cortisol (5 μg/g), while causing leucopenia in regressed males and females, produced leucocytosis after 2 h in both breeding and hypophysectomized fish of either sex[1].

In the warm-water cyprinodont *Mollienesia latipinna*, injections of ACTH into intact adult males at 25° C induced leucocytosis at 4 h at all doses tried, the lowest being 0·075 I.U./g. Maximum leucocytosis (× 6) followed a dose of 0·15 I.U./g; 0·225 I.U. and 0·30 I.U. only doubled the white cell count. Control injections of 0·6 per cent sodium chloride produced mild leucopenia[4].

These earlier results suggested that the stress reaction was mediated by the pituitary-adrenal axis.

Table 1. WHITE BLOOD CELL COUNTS (MEAN NUMBER OF CELLS/MM^3, ± *S.E.*) OF *M. latipinna* FOLLOWING COLD SHOCK

	Group	Unshocked controls	Time after shock 1 h	4 h	6 h
A	Intact males	30,583 ±1,429	2,654* ±233	55,633* ±1,426	22,809† ±1,739
B	Intact males	31,408 ±4,535 (5 fish)	9,970* ±1,575	48,444‡ ±4,706 (5 fish)	24,020 ±2,863
C	Intact males in *tris*	27,746 ±2,241	7,796* ±766	45,320† ±3,280	27,793 ±1,833
D	Intact males in *SU* 4885	21,000 ±1,454	22,716 ±3,096	21,486 ±3,073	18,313 ±1,817
E	Hypophysectomized males	12,246 ±2,033	5,327† ±1,443	8,014 ±639	9,119 ±1,250

Each value is the mean for 7 fish except where indicated. In comparisons with unshocked controls, t tests showed:

* Highly significant, P = 0·001 or less.
† Significant, P = 0·001 – 0·05.
‡ Barely significant, P = 0·05 – 0·1.

In work reported here we have pursued the matter by investigating the response of male *M. latipinna* to cold-shock, using intact fish, hypophysectomized fish, and fish treated with the drug *SU*-4885 (Ciba), which has been shown to inhibit the 11-hydroxylation enzyme system of the mammalian adrenal cortex with little or no toxic or other endocrine effects[5-7,11].

Adult male fish were shipped from Florida through a local dealer, and were acclimatized to laboratory conditions for at least two weeks before being used in an experiment. Since hypophysectomized *M. latipinna* survive for only 2 days in fresh water[8,14], all the fish were kept in dilute sea-water (12 parts per thousand) at 25 ± 1° C, so that results from hypophysectomized fish could be compared with results from the other groups. The fish were stressed by being plunged into ice-water at 0–1° C; after 2 min. they were returned to 25° C in individual flasks and killed at various intervals. At autopsy the fish were anæsthetized in *MS* 222 (1 : 2,000), sexual maturity was confirmed, the pericardial cavity opened and blotted dry, and blood obtained by cardiac puncture from the auricle. The blood was withdrawn into a fine heparinized glass pipette by oral suction, transferred to a measuring pipette[9], diluted with the fluid of Reese and Ecker, and cell counts made in the standard way using a counting chamber.

The arrangement of the experiments is summarized in Table 1. The hypophysectomized fish (group *E*) had been operated two weeks previously, and intact fish of the same dealer's batch served as their controls (group *B*). The drug-treated fish (group *D*) and their controls (group *C*) were set up as follows. *SU*-4885 was available[10] as a solution of the ditartrate (100 mg/ml.), and was administered by adding it to the aquarium water at a rate of 1·5 ml./l. This produced an acid solution which was restored to approximately pH 7·6 by adding 7 ml./l. of a 0·2 M solution of *tris* (2-amino-2-hydroxymethyl-1,3-propanediol). This solution then contained approximately 150 mg *SU*-4885 ditartrate per litre. Assuming equal distribution between the fish tissues, this represented a dose of about 150 μg/g bodyweight. One-tenth of this dose in man promptly reduces the adrenal output of cortisol[11], and doses up to 320 μg/g in the rat have been shown to be without side-effects on the sex organs, thyroid gland, liver and growth, and had no anæsthetic properties[12]. At the dose used in our experiments, 2–4 days treatment was shown previously to result in histological evidence of hyperstimulation in the interrenal cells of *M. latipinna*, in comparison with *tris*-treated controls, presumably a consequence of enhanced ACTH-secretion following reduction in the

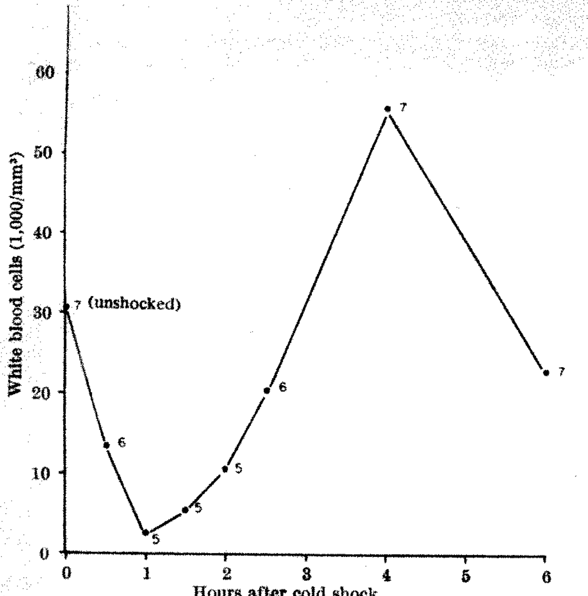

Fig. 1. Effects of cold shock on the white blood cell count of male *M. latipinna*. Number of fish at each point

output of 11-oxy-corticoids[13]. Fish were placed individually in flasks in the *SU*-4885 solution for 24–30 h before cold-shock and were returned to the same solution between cold-shock and autopsy. Control fish from the same dealer's batch were placed for the same period in flasks containing 7 ml. *tris*/l. adjusted to *p*H 7·6 by adding a small amount of tartaric acid.

Results of a detailed run with intact *M. latipinna* are shown in Fig. 1. Repetitions showed that though the actual numbers of leucocytes might differ between experiments, the pattern of the response remains the same: leucopenia followed by slight or marked leucocytosis (Table 1, groups *A*, *B*, *C*). Treatment with *tris* did not alter the response, but *SU*-4885 had marked effects, abolishing both the leucopenic and the leucocytic phases (Table 1). In contrast, and in agreement with the work on *Fundulus*[2], hypophysectomy abolished only the second phase and allowed leucopenia to be expressed at the usual time. It will be seen that hypophysectomized fish have much lower white cell counts than intact individuals. These counts can, however, be elevated by suitable stimulation, as evidenced by the leucocytosis induced in hypophysectomized *Fundulus* by ACTH and by cortisol injections[1].

Collateral effects of *SU*-4885 on pigmentation were also observed. The fish tended to darken, even on a white background, in the drug as compared with controls in *tris*: darkening commenced within a few hours and became more intense in 24 h. These changes coincided with the appearance of lethargy, suggesting mild anæsthesia, and since innervated melanophores of *M. latipinna* are not responsive to intermedin (MSH) or ACTH (ref. 14), the darkening probably depends on direct inhibition of the melanophore-concentrating innervation. However, faded denervated caudal bands[15] became more intensely darkened by treatment with *SU*-4885 than adjacent innervated areas. Since these bands are responsive to ACTH and to intermedin[14], this observation is most reasonably interpreted as indicating elevated ACTH-levels, the intrinsic melanocyte-stimulating hormone-like activity of the ACTH molecule being well known[16].

It is apparent from consideration of these results, together with the previous findings, that stress leucocytosis depends on the release of ACTH and a consequent increased output of 11-oxy-adrenocorticoids. Equally clearly, the leucopenic phase is independent of the pituitary. The abolition of leucopenia by *SU*-4885 but not by hypophysectomy is surprising, and may mean that this phase depends on the release of corticosteroids, presumably at a very low level, by some mechanism independent of pituitary ACTH. However, the slight narcosis observed in *SU*-4885-treated fish emphasizes the possibility that some non-adrenal action of the drug may be paramount in this phase. This matter is at present being investigated.

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Growth Responses of *Avena* Stem Segments to Various Sugars

THE internodal extension which occurs in stems of grasses with onset of reproductive phase is a remarkably striking morphogenetic process[1], the causal basis for which has not been elucidated. We have been attacking this problem from several points of view, one of which is to culture excised stem segments on various substrates to assay by quantitative means the intercalary growth that occurs in internode portions of these segments. We wish to report here on the growth responses of *Avena* segments to a variety of sugars. It is quite possible that several of these may be directly implicated in intercalary growth of internodes of this plant *in vivo*.

Plants of *Avena sativa* L. cultivar Clinton were cultured in the greenhouse at a mean day tempera-

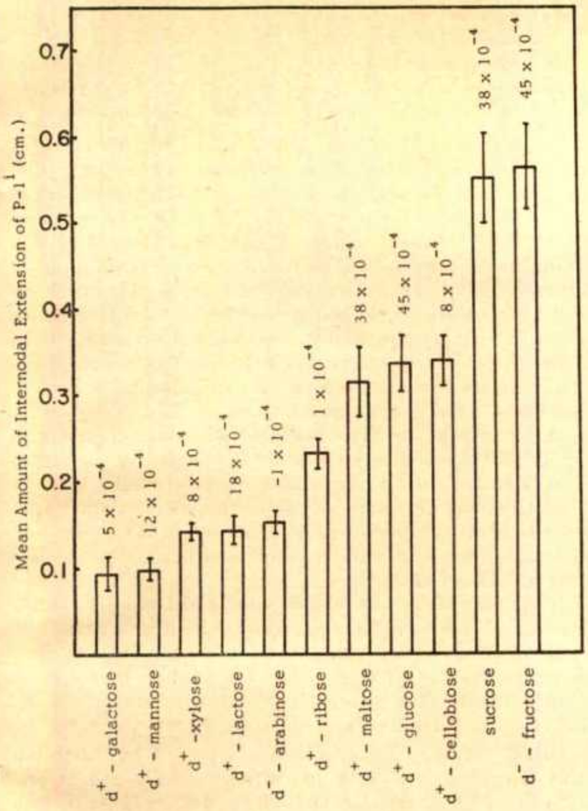

Fig. 1. Amount of internodal extension in $P-1^{n-i}$ segments cultured on various sugars (0·1 M) for 8 days in the dark. Change in dry weight per segment (g) is indicated above each bar of the histogram. The minus sign before net change for arabinose indicates loss in dry weight. *S.E.* for internodal extension data is shown for each sugar

ture of 80° F and a mean night temperature of 70° F under conditions of natural day-length. Plants (42 ± 2 days old) used for excision of stem segments were chosen from a population in which the majority of individuals showed evidence that the lamina of the last leaf had emerged from the sheath of the next oldest leaf. Segments utilized for culture studies were obtained when the internode ($P-1^i$) below the peduncular internode was 1·5–3·0 cm in length. This was done with the intent of incubating segments in which extension growth of this internode has essentially entered the phase of maximum rate of extension. We have found that 1-cm segments obtained when $P-1^i$ is in this length-range give a maximum internodal growth response in culture. Selection of shoots with $P-1^i$ in this length-range is done accurately with *Avena* shoots by observing the position of the peduncular node within the sheath of the leaf that surrounds this node. This node is darker (due to higher chlorophyll density) than the internodes above and below it and thus appears as a dark band when light is allowed to pass through the shoot. The 1-cm stem segments ($P-1^{n-i}$) utilized in our experiments include the base of $P-1^i$ and its associated node ($P-1^n$), an axillary bud, and sheath portion. We have found that the surrounding sheath portion does not elongate appreciably in culture, but the internode portion does. Thus the amount of extension of the latter can be measured to ascertain its growth response in various substrates and environments.

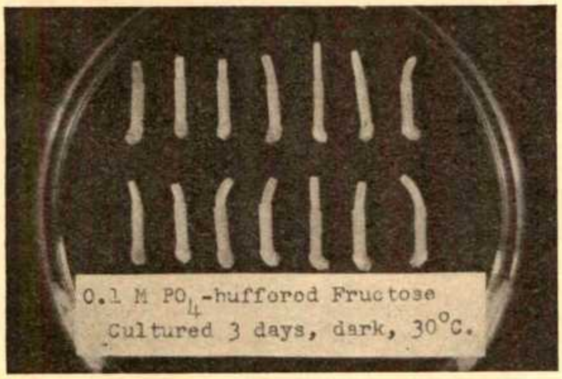

Fig. 2. Growth response of *Avena* $P-1^{n-i}$ stem segments cultured on 0·1 M PO_4-buffered fructose (× *c.* 0·75)

Excised segments (1 cm long) were surface-sterilized *en masse* by immersion in 10 per cent 'Clorox' (sodium hypochlorite) for 5 min. After draining off excess 'Clorox', segments were transferred under sterile conditions to plastic Petri dishes containing 20 ml. liquid medium. 30 segments were used for each treatment. After a 3- to 8-day incubation period at 25° C in the dark, internode-lengths were measured and dry weights of segments obtained.

Fig. 1 illustrates the amount of internodal extension and changes in dry weight for $P-1^{n-i}$ segments cultured on various sugars (0·1 M, phosphate–citrate buffer, *p*H 5·0) for 8 days in the dark. 0·1 M concentration was selected for this comparison of sugars because of superior growth of these segments on both sucrose and fructose at this concentration. Based on internodal extension, we find minimal growth response to galactose, mannose, xylose, lactose, and arabinose; intermediate growth on ribose, maltose, and glucose; and maximal response on sucrose and fructose. No significant difference is seen in linear growth of segments on fructose and sucrose at 0·1 M concentration. Fig. 2 illustrates the growth response of internodes in $P-1^{n-i}$ segments cultured on 0·1 M fructose. In another experiment, we found that *d*-sorbitol, myo-inositol, and *d*-mannitol (also 0·1 M) also induced an intermediate level of growth. Dry weight results for maltose, glucose, sucrose, and fructose indicate that increase in dry weight of the segments is rather substantial in comparison with segments cultured on other sugars.

In a comparison of growth responses of segments on different concentrations of fructose as opposed to sucrose (same *p*H and buffer), we again found no significant difference in growth response at 0·1 M concentration; but at 0·25 M, we clearly found superior growth with fructose (0·69 ± 0·05 cm/segment for fructose, 0·55 ± 0·06 cm/segment for sucrose). The same held true at 0·5 M concentration (fructose = 0·36 ± 0·07 cm/segment, sucrose = 0·14 ± 0·03 cm/segment). That fructose serves as such an excellent carbon source might be explained by its rapid exchange across membranes in excised segments[2] or its ready conversion to sucrose, one of the primary sugars that serves as a precursor in synthesis of polysaccharides[3].

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Type Species of Foraminiferal Genera

IN the course of economic palæontological research we have found it necessary to consult some early nineteenth-century collections of Foraminifera, giving particular attention to those specimens which are the types of type-species of genera. We have examined original material in the A. d'Orbigny, Terquem, Berthelin, Michelin and Munier-Chalmas collections in Paris and, contrary to popular belief, have found that many of the original syntypic specimens are still extant in the authors' collections. The state of preservation as fossils and the state of curation as museum specimens of almost all the material found and listed here is such as to warrant their retention, with due selection of typical specimens, as the authentic types of the nominal species and, consequently, of the genera based on them.

In a number of cases the critical re-examination of the species and the selection and isolation of lectotypes has already been done by A. R. Loeblich and H. Tappan, some of whose selections have already been validated by publication[1,2], and the remainder of which we presume are about to be so validated.

We are now in a position to state that in addition to those specimens isolated and selected by Loeblich and Tappan for the genera which they examined, specimens of the following species have been selected by us as types and are now clearly so marked on the original mountings. We propose, in conjunction with other workers, to validate these selections, in strict accordance with the International Code of Zoological Nomenclature, by publication. In the interim we emphasize that, for the taxa listed here, with the three exceptions noted, no neotypes are required. In these lists, the genera for which the species are type are given in parentheses.

(1) Species published by d'Orbigny in 1826[4] (*Tableau Méthodique de la Classe des Céphalopodes*):

Lectotypes selected by us: *Alveolina quoii* (*Alveolinella*), *Amphistegina quoii* (*Amphistegina*), *Bulimina marginata* (*Bulimina*), *Clavulina communis* (*Martinottiella*), *Dendritina arbuscula* (*Dendritina*), *Lingulina carinata* (*Lingulina*), *Marginulina raphanus* (*Marginulina*), *Planorbulina mediterranensis* (*Planorbulina*). *Quinqueloculina secans* (*Massilina*), *Rotalia dubia* (*Pegidia*) (specimen isolated by Heron-Allen), *Rotalia italica* (*Turbinulina*).

Neotypes have been selected from ideotypic material for: *Truncatulina variabilis* (*Cibicidella*), *Valvulina triangularis* (*Valvulina*).

(2) Species published by d'Orbigny in 1839 (in work on Cuba[5], Canary Islands[6] and Central America[7]):

Lectotypes selected by us: *Biloculina sphaera* (*Pyrgoella*), *Nodosaria quadrilatera* (*Orthocerina*), *Orbiculina compressa* (*Cyclorbiculina*), *Peneroplis proteus* (*Puteolina*), *Rosalina squammosa* (*Cymbaloporetta*).

(Neotype selected from ideotypic material: *Glandulina laevigata* (*Glandulina*.))

(3) Species published by d'Orbigny in 1840[8] (*Belemnitella mucronata* zone Chalk of Paris Basin).

Lectotypes selected by us, in direct consultation with M. Pierre Marie: *Bulimina brevis* (*Eggerellina*), *Cristellaria recta* (*Enantiovaginulina*), *Gaudryina rugosa* (*Gaudryina*), *Globigerina elevata* (*Hagenowella*, designated by P. Marie), *Rosalina lorneiana* (*Pseudovalvulineria*), *Valvulina gibbosa* (*Hagenowella*, designated by J. A. Cushman).

(4) Species published by d'Orbigny in 1846[9] (in work on the Tertiary of the Vienna Basin).

Lectotypes selected by us: *Bulimina pupoides* (*Protoglobobulimina*), *Frondicularia annularis* (*Annulofrondicularia*), *Uvigerina aculeata* (*Euuvigerina*).

(5) We have also been able to select lectotypes for the following: *Annulina metensis* Terquem, 1862 (*Annulina*), *Involutina polymorpha* Terquem, 1874 (*Silicina*), *Rosalina lateralis* Terquem, 1878 (*Poroeponides*), *Triloculina laevigata* Terquem, 1878 (*Miliolina* Williamson), *Frondicularia paradoxa* Berthelin, 1879 (*Berthelinella*), *Pleurostomella barroisi* Berthelin, 1880 (*Pleurostomellina*), *Rhabdogonium liasinum* Berthelin, 1879 (*Tristix*), *Spirillina trochoides* Berthelin, 1879 (*Conicospirillina*), *Orbitolites pratti* Michelin, 1846 (*Orthophragmina*), *Cyclopsina steinmanni* Munier-Chalmas, 1887 (*Cyclopsinella*).

A lectotype of *Frondicularia bicostata* d'Orbigny, 1850 (*Ichthyolaria*) has been selected by us from Terquem collection material, as it is virtually certain that this was Terquem's type of the species, the name of which must, however, be credited to d'Orbigny as the name was first validly published in his *Prodrome*[10] (see also Macfadyen[3]).

Syntypes of *Gyroidina orbicularis* (d'Orbigny, 1826) Parker, Jones and Brady, 1865, exist in Paris, but their nature is such that a lectotype for this species, the type species of *Gyroidina*, has had to be selected from the Parker, Jones and Brady collection in London (British Museum (Natural History)).

(6) The following lectotypes, selected by Loeblich and Tappan, are present in the Paris collections examined by us: d'Orbigny, 1826[4]: *Adelosina laevigata* (*Adelosina*), *Clavulina angularis* (*Clavulina*), *Nonionina sphaeroides* (*Pullenia*), *Planorbulina vermiculata* (*Cyclocibicides*), *Rimulina glabra* (*Rimulina*)[1], *Rotalia turbo* (*Trochulina*), *Sphaeroidina bulloides* (*Sphaeroidina*).

d'Orbigny, 1839[5-7]: *Asterigerina carinata* (*Asterigerina*), *Conulina conica* (*Conulina*), *Cruciloculina triangularis* (*Cruciloculina*)[2], *Faujasina carinata* (*Faujasina*), *Nonionina browni* (*Cushmanella*), *Oolina laevigata* (*Oolina*), *Polystomella poeyana* (*Elphidiononion*), *Sagrina pulchella* (*Sagrina*), *Webbina rugosa* (*Webbina*)[1].

d'Orbigny, 1840[8]: *Flabellina rugosa* (*Neoflabellina*), *Pyrulina acuminata* (*Pyrulinoides*), *Sagrina rugosa* (*Heterostomella*), *Verneuilina tricarinata* (*Verneuilina*).

d'Orbigny, 1846[9]: *Cyclolina cretacea* (*Cyclolina*), *Hauerina compressa* (*Hauerina*)[1], *Lingulina rotundata* (*Ellipsonodosaria*), *Spirolina agglutinans* (*Ammobaculites*).

Terquem, 1866: *Marginulina colliezi* (*Pseudocitharina*).

We thank M. Pierre Marie and Mme. Y. Le Calvez of the Bureau de Recherches Géologiques et Minières for facilities, help and encouragement in locating and working on type material, MM. Lehmann, Fischer and Sornay of the Muséum National de l'Histoire Naturelle, M. Beauvais and Prof. J. Jung of the Laboratoire de Géologie, Faculté des Sciences à la Sorbonne, and M. l'Abbé de Lapparent of the Institut Catholique for their help in enabling us to examine the collections in their care. All the foregoing specimens (with the single exception of *Cyclopsina steinmanni*, which is in the Sorbonne) are deposited in the care of the Muséum Nationale de l'Histoire Naturelle, Paris.

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MICROBIOLOGY

Bacteriolytic Principles of *Myxococcus fulvus*

MEMBERS of the Myxococcaceae are ideal for the examination of some fundamental phenomena unique in the world of bacteria: (1) the mechanism of the gliding movement of the vegetative forms on solid media[1]; (2) the morphogenesis of the fruiting body, following the gathering in of the swarming rods with the simultaneous transformation into microcysts[2]; (3) the production of lytic principles capable of attacking different bacterial and fungal cells on which the formation of fruiting bodies readily takes place[3,4].

This communication deals with some observations made on *Myxococcus fulvus* which may throw some light on the mode of action of the lysis of bacteria by myxobacteria.

Singh, Oetker, Kühlwein *et al.*[3,5,6] have described the lytic activities of myxobacteria. Most of their work was concerned with the breakdown of eubacteria. Nolte[4] also compared the lytic activity of several myxobacteria, for example, *M. fulvus*, *M. virescens*, *M. stipitatus* and *Podangium erectum*, on yeast and mycelial fungi of various orders, as well as algæ and myxomycetes. In most cases growth and formation of fruiting bodies would occur on bacteria and lower fungi, while higher fungi and other thallophyta seemed to be resistant to the attack of the myxobacteria, presumably due to the presence of chitinic substances in the structure of the cell wall[6].

One might conceive three possibilities for the mode of action of myxobacteria on living bacteria:

(1) The lytic principle of myxobacteria is capable of both the inactivation of the organism as well as its lysis by a mechanism similar to that of lysozyme[7].

(2) Living bacteria are susceptible to the action of myxobacteria only if they are primarily attacked by an antibiotic substance or some similar principle, the actual lysis being carried out by some unspecific proteolytic enzyme(s) which would be responsible for the complete dissolution of the bacterial cell contents and/or cell wall.

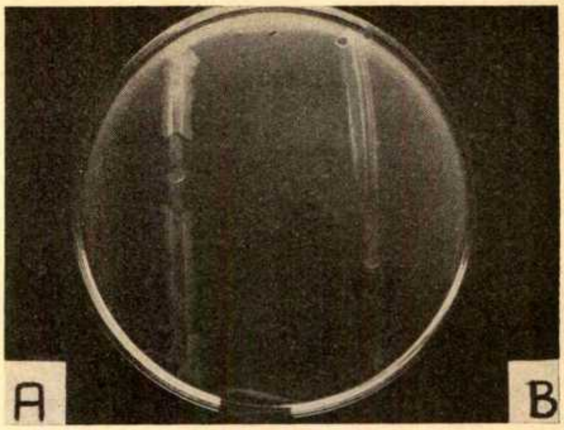

Fig. 1. *Myxococcus fulvus* on *E. coli* (nutrient agar). *A*, Living *E. coli* ; *B*, dead cells

(3) Lysis of bacteria can occur only in the case that as a result of some sort of a trigger action of the myxobacteria on the substrate organism (for example, the deprivation of some vital nutrilite) death would occur followed by rapid autolysis.

Working with a strain of *M. fulvus* isolated from local garden soil the following technique was utilized. The organism was grown on 0·3 per cent casein agar which was prepared by stirring the casein at *p*H 9·0 (sodium hydroxide) until complete dissolution of the protein. The solution was then neutralized before the addition of agar–agar and autoclaved. The inoculum consisted of an agar disk (5 mm diameter) taken from the peripheral, non-fruiting part of a colony. The following observations were made:

(1) There is virtually no difference between the lysis of Gram-positive and Gram-negative bacteria. This can be best demonstrated by comparing the lysis and formation of fruiting bodies on bacteria applied in a thick paste on water agar (2 per cent agar–agar in tap water). Within 3–5 days lysis and fruiting would occur upon Gram-negative bacteria (*Escherichia coli*, *Serratia marcescens*), on non-sporing Gram-positive bacteria (*Lactobacillus arabinosus*, *Micrococcus* sp.); though some difference in the rate of lysis and fruiting can be observed with bacilli (*B. subtilis*, *B. mycoides*) probably due to the formation of endospores as already pointed out by Oetker[5].

(2) There are practically no differences in the lysis or formation of fruiting bodies on living or autoclaved bacteria, when applied on nutrient-free water agar.

(3) When living cells are streaked on nutrient agar, *E. coli* would be lysed by *M. fulvus*, but not to the same extent as autoclaved cells applied similarly (Fig. 1).

(4) Repeating the foregoing experiment on 0·3 per cent casein agar the following picture was obtained. Around every disk of inoculation a distinct halo of proteolysis was formed. While this would correspond to the extent of lysis of autoclaved cells, the zone of proteolysis would be much larger than the lysis of living *E. coli* (Fig. 2).

(5) Although antibiotic activity has been attributed to some myxococci such as *M. virescens* against Gram-positive bacteria[8] and against virus[9], no water-soluble antibiotic could be demonstrated from *M. fulvus*, using *E. coli* as test organism.

From the foregoing observations some theory on the mode of action of *M. fulvus* was attempted. The lytic principle of the *Myxococcus* is most probably

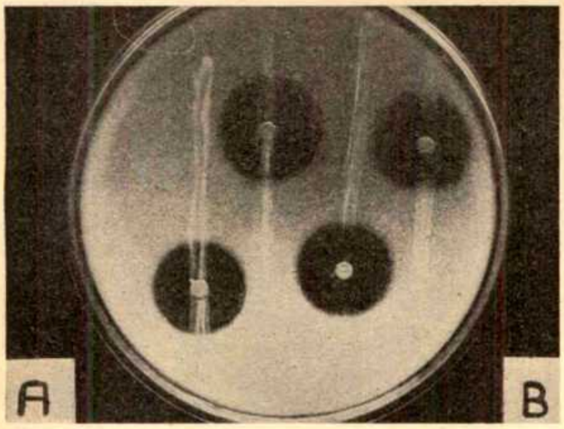

Fig. 2. *M. fulvus* on *E. coli* (0·3 per cent casein-agar). *A*, Living *E. coli*; *B*, dead cells

unlike that of lysozyme in not distinguishing appreciably between Gram-positive and Gram-negative bacteria. The lysis of actively growing cells occurs at a much lower rate than that of dead cells, probably due to metabolic products produced by the former which inhibit the growth of the myxobacterium. Whereas growing cells of *E. coli* are killed by the *Myxococcus* on contact, and not by a diffusable substance, the dead cells are lysed by the proteolytic enzyme(s) practically to the same extent as the breakdown of another protein substrate.

The lysis of living cells seems, therefore, to be the result of the common effort of two principles: a 'killer' and a proteolytic enzyme. So far, practically nothing is known about the nature of the 'killer' although its non-specificity towards its substrate organisms is one of its striking features.

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Binding of Chlortetracycline on the Sporulating Cells of Bacilli and the Atypical Development of Spores

THE affinity of tetracycline antibiotics for divalent metal cations[1-2] led to the hypothesis that the biological effect of these antibiotics is dependent on the presence of these cations and is brought about by complexing with macromolecules through metal ions. The formation of metal bridges was proved, for example, in binding of tetracyclines on serum albumin or deoxyribonucleic acid[3]. Metal-mediated binding of tetracyclines to macromolecules or cells assumes the presence of specific receptors, binding the metal cation.

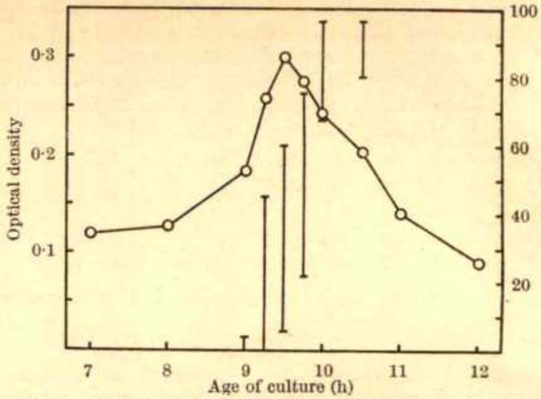

Fig. 1. Binding of chlortetracycline on the sporulating cells of *Bacillus cereus*. Vertical abscissæ stand for the percentage of spores formed during contact with CTC

Sporogenesis of bacilli is connected with the intensive incorporation of calcium into the cells and, therefore, the primary mobilization of binding sites for calcium inside or on the cell surface might be expected. To test the possibility of interference of tetracycline antibiotics with sporogenesis the cells of *B. cereus* (N.C.I.B. 8122) grown in bactopeptone-glucose-salts liquid medium were kept in contact with chlortetracycline for 60-min intervals, chlortetracycline (CTC) being added in the different stages of stationary phase. Concentration of calcium in the medium was 1×10^{-4} M and chlortetracycline ('Aureomycin' Lederle) was added into individual flasks with culture in the concentration 5 µg/ml. of medium. The binding of chlortetracycline on the cell was estimated by measuring the fluorescence of the cells in ultra-violet light. The values (Fig. 1) express the optical density of fluorescent filters with cells measured by a transparometer[4] and the individual values, with regard to the time axis, stand for the moment of addition of chlortetracycline. Simultaneously the percentage of pre-spores and spores was estimated in the moment of addition of CTC and after 60 min contact. Spore formation is expressed by vertical abscissæ, the bottom values standing for the

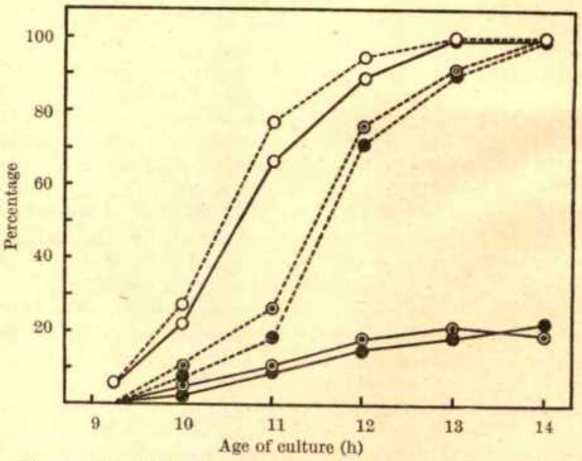

Fig. 2. Sporulation, incorporation of calcium-45 and synthesis of dipicolinic acid in control (dotted lines) and CTC-treated (full lines) culture (values expressed in percentage, final values of control culture being taken as 100 per cent). ○, Sporulation; ●, DPA synthesis; ⊙, incorporation of calcium-45

percentage of spores at the moment of adding CTC, the upper ones for that at the moment of sampling.

Although the method of measuring the fluorescence is only semi-quantitative it shows the relative changes in the amount of CTC bound on cells. The fluorescence tests show that during the period of spore formation the ability of cells to bind chlortetracycline is much increased. Chlortetracycline can be washed out by different washing procedures using *tris* buffer, 0·001 M citric acid, etc. Similar results were obtained with *Bacillus megaterium*.

During my investigations of binding of calcium-45 on the cells in the stage of pre-spores I found that in this period the adsorption of calcium on the cell surface is markedly increased. Also this calcium can be easily washed away and the removal of this 'labile' calcium by different solutions is practically the same as that of chlortetracycline bound during sporulation. It may be assumed that this 'labile' calcium mediates the binding of CTC on the cell surface.

The penetration of calcium inside the cells is accompanied by the synthesis of dipicolinic (pyridin-dicarboxylic) acid (DPA). I therefore followed the influence of CTC on the calcium-45/1 μc./ml./uptake by sporulating cells and the formation of dipicolinic acid. DPA was estimated by the method of Janssen, Lund and Anderson[5]. Chlortetracycline was added (3 μg/ml.) into experimental flasks after formation of about 5 per cent of pre-spores. The results are given in Fig. 2. Chlortetracycline blocks the penetration of calcium inside the cells and the synthesis of dipicolinic acid.

The blockade of uptake of calcium by sporangia and of the synthesis of dipicolinic acid leads to the changes in morphology of spores. The spores formed are less refractive and usually more spherical. Their appearance resembles the so-called 'defective spores' formed in the excess of cystine or its breakdown product, hydrogen sulphide[6]. The post-sporulation autolysis of sporangia treated with chlortetracycline is disturbed, as it was in the case of 'cystine inhibition'.

It is possible that chlortetracycline inhibits some of the processes leading to the synthesis of DPA. On the basis of highly increased binding of chlortetracycline on sporulating cells it may be assumed, however, that the inhibition of calcium uptake and DPA synthesis is more likely brought about by blocking the cell surface. The molecule of tetracycline attached to calcium bound on the cell surface forms a complex which may not penetrate into the cell and/or is unable to induce DPA synthesis.

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Effect of Multi-site Mutation to Colicine Resistance on Recombination and Segregation in *Escherichia coli*

SOME phage-resistant mutants of *Escherichia coli* are resistant to some colicines, and vice versa[1,2]. Among mutants selected for resistance to colicine *B* four different classes result from mutation near the *try* (tryptophan-requirement) locus: (1) resistant to colicines *B* and *I*; (2) resistant to colicines *B*, *I* and *V* and to phage *T*1; (3) like (2), but tryptophan-exacting, responding also to indole; (4) like (2), but tryptophan-exacting, not responding to indole. The phenotypes of these four classes and genetic analysis by transduction using phage *P*1 and several tryptophan-exacting mutants[3] of *E. coli K*12 suggest that mutants of classes (2), (3) and (4) arise by multi-site mutations (presumably deletions) of variable extent (Fig. 1), in class (3) involving the *try* 2 locus, with resulting inability to convert indole glycerophosphate to indole, and in class (4) involving both *try* 2 and *try* 1, with resulting inability to convert indole glycerophosphate or indole to tryptophan. Yanofsky and Lennox[3] similarly concluded that tryptophan-exacting phage-*T*1-resistant mutants, which include classes (3) and (4), arose by deletion.

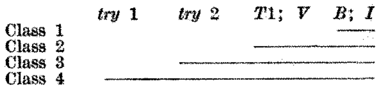

Fig. 1

In crosses, by conjugation or transduction, with phage *P*1, between strains one of which carried a 'deletion' mutation of types (2), (3) or (4), minute colonies containing persistent heterozygotes, at least for the *try* region, were observed; delayed recombination led to production of stable *try*$^{+}$ segregants. Clones heterozygous for several loci not closely linked to *try*, including *gal*, *met*, *azi* and *str*, segregated the parental and various recombinant types. The following results indicate that these effects result from the deletion, rather than merely from mutation involving the *try* region.

In a control $F+$ by $F-$ cross an $F+$ streptomycin-sensitive strain requiring anthranilic acid (or indole or tryptophan) through point mutation in *try*4 was crossed with a streptomycin-resistant F-strain tryptophan-exacting through point mutation in *try*1; on a casein–hydrolysate–streptomycin medium *try*1^{+} *try*4^{+} recombinant colonies of normal size developed with a frequency of about 2×10^{-5} per $F+$ bacterium. In one experimental cross the $F-$ parent was likewise tryptophan-requiring, but because it carried deletion (4) which includes *try*1. The number of large colonies obtained was less than in the control cross and there also appeared numerous small colonies, each consisting largely of *try*4 *str-r* cells fed by some tryptophan precursor, probably anthranilic acid, excreted in large amount by the deletion-4 parent. After several days faster growing sectors of *try*1^{+} *try*4^{+} recombinants developed in many of the small colonies, which must therefore have contained clones heterozygous for *try*4. Enumeration of *try*$^{+}$ bacteria by re-spreading at intervals after plating showed an abnormal delay, of variable duration, before such recombinants began to multiply. In another experimental cross the $F+$ parent was *try*4^{-}, as in the control cross, but also carried deletion (2), conferring resistance to colicine *B*, *I* and *V* and phage *T*1; the $F-$ parent was *try*1^{-}, as in the control cross. The number of colonies obtained on casein–hydrolysate–streptomycin medium was much less than in the control cross. Some additional colonies, of variable size but mostly small, appeared on prolonged incubation. These correspond to the fast-growing *try*$^{+}$ sectors developing in the small, mainly *try*4^{-}, colonies seen in the other experimental cross; the expected $try4^-$ component could be recognized on replication to medium supplemented with anthranilic acid. In this cross also re-spreading experiments showed that the number of bacteria able to produce try^+ colonies did not begin to increase until after a variable abnormal delay. Thus in $F+$ by $F-$ crosses the presence of a deletion mutation in either parent causes: (1) delay in the production of try^+ recombinants, a class which requires crossing-over near the deletion; (2) the development of small colonies apparently through multiplication of cells heterozygous for the *try* region, since they produce both $try4^-$ and $try4^+$ progeny.

The presence of a deletion in either parent also modified the outcome of *Hfr* to $F-$ crosses, as follows. In crosses of *HfrH* (met^-, transmitting *thr* and *leu* early and *try* later[4]) with F-strains marked by thr^-, leu^- and $try4^-$, the presence or absence of a deletion in the *Hfr* parent did not affect the yield of recombinants selected for thr^+ leu^+ from the *Hfr* parent. But when $try4^+$ was also selected for, the presence of deletion (2) in the *Hfr* parent greatly reduced the number of colonies obtained, which were of variable size. Testing of clones derived from 20 such colonies showed them to be heterogeneous, indicating delayed segregation at several loci, including *thr*, *leu* and *try*4. The presence of deletion (4) in the $F-$ parent also caused aberrations, though less apparent ones. Colonies selected on tryptophan-supplemented medium for possession of thr^+ and leu^+ from the *Hfr* parent and found by replication to contain some try^+ cells showed delayed segregation for try^+ and other characters derived from the *Hfr* parent. The number of recombinants was not reduced when thr^+, leu^+ and try^+ from the *Hfr* parent were selected for, but re-spreading experiments showed a delay of 4 h before they began to multiply, compared with two h for thr^+ leu^+ recombinants in the same cross and for thr^+ leu^+ try^+ recombinants in a control cross in which neither strain carried a deletion. When the *Hfr* parent was $try4-$ instead of try^+ and the $F-$ parent was tryptophan-exacting because of deletion (4) small colonies, made up in large part of $try4-$ recombinants, were obtained on a medium selective for thr^+ leu^+ try^+; only large colonies, of try^+ recombinants, were obtained in a control cross with an $F-$ parent carrying $try1^-$ instead of deletion (4). Thus in *Hfr* by $F-$ crosses a deletion in either parent, but especially in the donor, causes failure or delay in recombination and a variable persistence of heterozygosis for part of the chromosome, but only among those F-cells which receive the *try* region transmitted by the *Hfr*.

When the *try* or *his* regions were transduced by phage $P1$, a deletion in either donor or acceptor strain resulted in persistent heterozygosis among the *try* but not the *his* transductants, the effect being greater when the donor carried the deletion.

Thus on mating or transduction, the presence in either parent of a deletion causing resistance to certain colicines and phage $T1$ inhibits or delays recombination in the adjacent *try* region and causes a variable persistence of partial heterozygosity among the progeny of bacteria which receive the *try* region of the donor. The effect of mutation at other loci controlling sensitivity to colicines and phages, and the relation of the present observations to the 'Het' factor inferred by Lederberg[5,6] from experiments with a mutant resistant to phage $T1$, are being investigated. Examination of persistent heterozygotes has now shown that sensitivity to colicine B, V, I and K, like sensitivity to phage $T1$, $T5$ and $T6$, is dominant to resistance.

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ENTOMOLOGY

Neurosecretion and the Transport of Secretory Material from the Corpora Cardiaca in Aphids

THE development of differential staining techniques for neurosecretory material in animals made it theoretically possible to trace secretions from their source to their point of release or utilization. In most cases, however, insufficient secretory material is present to enable more than a very incomplete picture to be obtained. In adult aphids which are several days old large amounts of material tend to accumulate in the axons of some neurosecretory cells and it has been possible to follow its course.

The following account is based on studies of serial sections of several species of aphids, principally *Aphis fabae* Scop. and *Drepanosiphum platanoides*. The most effective stain was found to be Gomori's paraldehyde fuchsin, and this stains the secretion of only a few of the neurosecretory cells present in the brain and other ganglia.

Two large neurosecretory cells are situated near the mid-line on the dorso-anterior surface of the protocerebrum. Secretory granules can be traced from the cell bodies along their axons, each of which passes through the brain and then branches so that

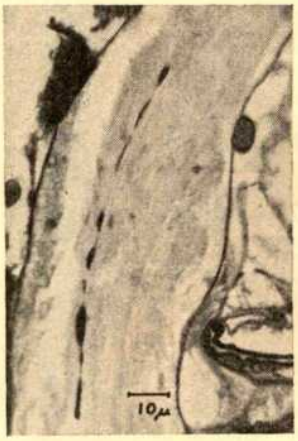

Fig. 1. Photomicrograph of a longitudinal section of the sub-œsophageal ganglion of an old virginopara of *A. fabae* Scop. showing extensive deposits of neurosecretory material along one of the axons which pass from the brain neurosecretory cells to the hindgut. (Stained with Gomori's paraldehyde fuchsin after permanganate oxidation)

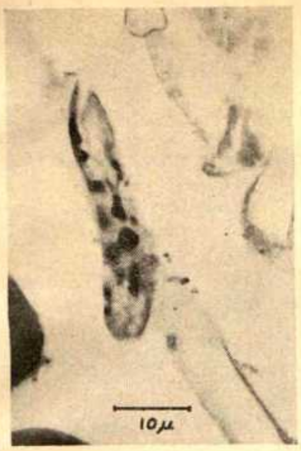

Fig. 2. Photomicrograph of the lateral nerve from the corpus cardiacum of a virginopara of *D. platanoides* as it passes over a trachea in the prothorax. The dark staining inclusions are secretory material from the corpora cardiaca. (Stained with Gomori's paraldehyde fuchsin after permanganate oxidation)

one branch of each axon passes along the nervus corporis cardiacus while the other goes into the circum-œsophageal connective and continues along the ventral nerve cord. Similar cells with axons containing neurosecretory granules passing along the ventral nerve cord have recently been described in larvæ of a mosquito *Corethra*[1].

The branches of the axons which pass along the nervi corporis cardiaci appear to terminate in the corpora cardiaca where copious amounts of fuchsinophilic material sometimes accumulate. This material differs in character from that found in the axons of the neurosecretory cells in that it is not granular and it occurs in relatively large drops. In aphids in which large amounts of this secretion are present, it can clearly be followed from the corpora cardiaca into two pairs of nerves, a lateral and a medial pair. In cross-section these nerves can each be seen to consist of a single giant axon which appears to function as a duct transporting secretion from the corpora cardiaca. The nerves are not of uniform thickness and in *Drepanosiphum* individual axons vary in width from 3μ to 10μ. They have no nuclei except where they branch.

The medial pair of nerves pass over the centrally placed fused corpus allatum and join together to form a single nerve which continues along the ventral surface of the aorta; attached to this nerve are the pericardial cells. The lateral pair of nerves, which are larger and contain more secretory material, follow tracheæ to the vicinity of the prothoracic spiracles and then pass downward and backward into the abdomen. Branches have been traced to various muscles in the prothorax and near the metathoracic and first abdominal spiracles; another branch from each nerve passes upward and rejoins the medial nerve in the abdomen. Although the lateral nerves pass close to the ecdysial glands, which are situated near the prothoracic spiracles, they do not appear to have any direct tissue connexion with them.

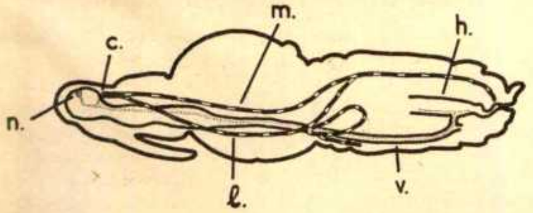

Fig. 3. Pathways of secretory material along nerve axons from the brain neurosecretory cells and corpora cardiaca in *Drepanosiphum platanoides*. *n*, Neurosecretory cell; *c*, corpus cardiacum; *m* and *l*, medial and lateral nerves from the corpus cardiacum; *v*, ventral nerve cord; *h*, hindgut

The neurosecretory axons which pass along the ventral nerve cord were also traced. After continuing along it for some distance into the abdomen they pass into two nerves which go to the ventral surface of the hindgut. Adhering to the hindgut each of the axons then divides again: one branch of each going forward, the other backward, the two axons running parallel to one another and extending over about one-third of the hindgut. The secretory material is distributed along the axons on the hindgut in a series of bead-like swellings. In some aphids vacuoles have been found in the swellings.

Secretions from the corpora cardiaca in insects have been shown to influence a number of different functions. These include moulting[2], water metabolism[3], rate of heart-beat[4], contractions of parts of the intestine[5] and oviducts[6], physiological colour change[2] and protein synthesis[7]. In some of these the corpus cardiacum extract is known not to act directly but to stimulate an intermediate system. Rate of heart-beat and hindgut contractions have recently been shown to be influenced by an indolalkylamine which is released by pericardial cells and cells of the colon when they are activated by corpus cardiacum extracts[4,5]. In aphids both these tissues appear to receive secretory material which originates from the same neurosecretory cells in the brain and which is transported to them along nerve axons.

Secretions from the corpora cardiaca are generally believed to be released directly into the blood[8] although direct evidence of this is lacking. The fact that in aphids large amounts of secretory material appear to leave the corpora cardiaca along the lateral and medial nerves suggests that this may be the main method of release and that the material does not normally enter the blood at all but is transported directly to the tissues it influences. The presence in aphids and *Corethra* larvæ of neurosecretory material extending right along the ventral nerve cord further indicates that the neuroendocrine system of insects may be more of a closed system than has previously been thought.

This work was done during my tenure of a Nuffield Dominion travelling fellowship. I thank Prof. G. C. Varley for allowing me to work in his department.

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GENETICS

A Gene synthesizing Deoxyribonuclease in the Polyhedral Pre-viral Genome

IT has been reported[1] that in the chromosome of larvæ of *Bombyx mori* there is a pre-viral genome containing a gene synthesizing protease. We have now shown that the genome contains a gene controlling the synthesis of an alkaline deoxyribonuclease.

Our previous work[2] showed that the pupa of *B. mori* possesses no alkaline deoxyribonuclease. About 700 young pupæ of P_{21} strain were each injected with 0·02 ml. of polyhedral solution (0·1 mg/ml.), and polyhedra were collected from diseased individuals[3]. Virus particles were then isolated from the polyhedral crystals according to the method previously described[4]. To measure the deoxyribonuclease action of polyhedral virus, 1 ml. of enzyme solution was mixed with 1 ml. of deoxyribonucleic acid (from cattle spleen) solution (0·5 mg/ml.), 1 ml. of 0·2 M sodium carbonate + orthoboric acid + potassium chloride buffer and 0·3 ml. of 0·01 M magnesium sulphate solution. The enzyme solution was prepared by homogenizing thoroughly 5 mg of viral particles with 1 ml. of 0·02 N sodium carbonate. The *p*H of reaction mixtures was appropriately regulated by adding 0·2 N hydrochloric acid or sodium hydroxide. In the control, 1 ml. of water was added instead of the substrate. The reaction temperature was 40° C and the time 3 h. After the reaction, the solutions were each mixed with 1 ml. of 1 M perchloric acid, centrifuged at 3,000 r.p.m. for 10 min and further filtered. The enzymatic activity was determined spectrophotometrically and expressed in increase of absorption (log T) at 260 mμ. Some of the results obtained are given in Table 1.

Table 1. DEOXYRIBONUCLEASE ACTION OF POLYHEDRAL VIRUS

*p*H of reaction mixture	7·0	8·0	8·8	9·6	10·5	11·1	11·8	12·6
Log T	0·017	0·043	0·152	0·291	0·382	0·432	0·446	0·410

It is thus obvious that the polyhedral virus forms a deoxyribonuclease which displays the maximum activity at about *p*H 12. As this virus is of a deoxyribonucleic acid nature and produced only in the nucleus, it can be concluded that in the virus a gene exists which synthesizes the alkaline deoxyribonuclease.

Our previous investigations[3,5] also indicated that the *B. mori* larva has an alkaline deoxyribonuclease and produces polyhedra containing a deoxyribonuclease of the same nature. The white cuticle of the P_{44} strain and the black cuticle of the P^S strain respectively were taken in the same way as before[1]. The two strains were then crossed and the cuticle was further taken from the descendants in the F_1- and F_2-generations. To estimate the deoxyribonuclease action of cuticle, 0·5 ml. of enzyme solution was mixed with 1 ml. of deoxyribonucleic acid solution (2 mg/ml.), 1 ml. of 0·4 M sodium carbonate–orthoboric acid–potassium chloride buffer of *p*H 9·6 and 0·3 ml. of 0·01 M magnesium sulphate. The enzyme solution was prepared by centrifuging the suspension obtained after grinding 50 mg of cuticle with 1 ml. of 0·5 M sodium chloride for 30 min at 37° C. The reaction temperature was 30° C and the time 30 min. The enzymatic activity was determined viscosimetrically and expressed in velocity constant, k, of monomolecular reaction. The results are given in Table 2.

TABLE 2. DEOXYRIBONUCLEASE ACTION OF LARVAL CUTICLE

Generation	P_{44}	PS	$P_{44} \times PS - F_1$	$P_{44} \times PS - F_2$	
Colour of cuticle	White	Black	Black	White	Black
$k \times 10^4$	65	128	133	56	111

It is thus evident that there is a correlation between the activity of nuclease and colour of the skin. As stated before[1], the colour of P_{44}- and P^S-cuticles is governed by the genes in the second chromosome in the larval cell and the black of P^S is the dominant. Accordingly, it may be concluded that the gene synthesizing the alkaline deoxyribonuclease is located in the chromosomes, possibly in the second chromosome.

It is supposed that the genome containing the genes synthesizing deoxyribonuclease and protease is highly resistant, can independently multiply and may cause the decomposition of the other chromosomal portion consisting of deoxyribonucleic acid and protein. As the metabolic processes in silkworm tissues proceed near the neutral reaction, it is surmised that the genes producing the alkaline enzymes might have been formed by a mutation in the past. The production of the polyhedral virus can thus be formulated as in the following scheme.

Cell containing normal chromosome
↓ mutation
Cell containing pre-viral genome
↓ induction
Cell containing viral particle

The induction is caused by the metabolites which appear as intermediates in the cyclic reaction established by us[6]. It is possible that the mutation also might have been caused by some of the metabolites such as nitrous acid, hydroxylamine and hydrogen peroxide, which can act as mutagens[7-9].

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Immunogenetics of the Domestic Mink: Blood Group Factors A, B_1 and B_2

SEROLOGICAL differentiation of red blood cells, especially in such species as man, cattle and the chicken, is well known[1,2,5]. It has been established that red blood cells have antigenic properties on their surfaces; further, these antigenic properties are hereditary characters, their structures being determined by genes. Blood group investigations have proved helpful in elucidating the nature of the chain of events from gene to character, as well as of practical use in animal breeding[3,4,6]. So far as we are aware, nothing on blood groups in the domestic mink (*Mustela vison*) has been published.

Table 1. ANALYSIS OF ISO-IMMUNE AND HETERO-IMMUNE SERA AGAINST MINK RED CELLS

Species	No. injected	No. responding	Type antibody	Titre	No. of samples of red blood cells used for absorption of each serum	No. of specific reagents
Mink	76	15	Agglutinin	1 : 2–1 : 16	12–53	3 Anti-A 4 Anti-B_1 and B_2
erret	8	8	Agglutinin Hæmolysin	1 : 32 1 : 8	24	2 Anti-B_1 —
bbit	12	12	Agglutinin Hæmolysin	1 : 1,000–1 : 4,000 1 : 500–1 : 2,000	15–18	1 Anti-A 1 Anti-B_1 —
Chicken	12	12	Agglutinin	1 : 16–1 : 64	12	1 Anti-A 1 Anti-B_1
Guinea pig	14	14	Agglutinin Hæmolysin	1 256 1 . 256	15	— —

The 612 mink used in this investigation were of various colour phases from the herd of the Fur Animal Research Laboratory, University of Wisconsin. 8–15 ml. of blood were drawn from each animal by cardiac puncture into an isotonic solution of sodium citrate (2·3 per cent) and sodium chloride (0·4 per cent). Blood samples were kept at 4° C for use within 10 days, or stored at $-20°$ C for later use. Additional details regarding the techniques used will e described elsewhere.

Neither normal agglutinins nor hæmolysins were found in the sera of the initial group of 53 animals ested. An agglutinin was manifest in a single individual among 121 examined six months subsequent to the first test: from the results of comparing this serum with reagents prepared from isoimmune sera, it appears that this one mink has antibodies against a mink blood group tentatively designated B_2. Sera of all mink lacking the antigen B_2 were checked, but in no case were antibodies against this blood type found.

Agglutinins or hæmolysins for mink red blood cells, or both, were found in the sera of the chicken, marten, rabbit, guinea pig, cattle and man: of the seven species tested, only the ferret failed to show cross-reacting antibodies with the mink. Consequently, fresh sera from rabbits and most guinea pigs cannot be used as complement. The sera of two guinea pigs of 18 used showed agglutinins of very low titre (1 : 2), and could be used as complement in dilutions of 1 : 14; however, results from such tests may be questionable.

122 individuals were immunized against mink red cells to get iso- and hetero-immune sera: 76 mink, 12 rabbits, 12 chickens, 14 guinea pigs and 8 ferrets. The immune sera were prepared as follows: 2–4 ml. of a mixture of equal parts of mink erythrocytes (washed three times) and saline solution (0·85 per cent) were injected 5–6 times into each recipient, the animals bled 7–10 days following the last injection, and the sera stored at $-20°$ C. Paired recipients of each species used were given red cells from a single donor. The results are shown in Table 1.

Of the 76 mink injected, 30 were young (six months); only one of the young group responded (weakly), while among the adults 14 produced antibodies. The red blood cells used for absorption were taken from mink chosen at random. At least seven different reagents were detected from the 14 immune sera, leading to the recognition of seven blood factors. Since the mink is so small, only three reagents were obtained in quantities sufficient for extensive tests; four reagents from single individuals were expended for absorptions.

Each hetero-immune serum reacted with the blood cells of all mink tested as shown in Table 1. By absorption, it was possible to distinguish reagents from the hetero-immune sera which seems identical with certain reagents obtained from the iso-immune sera. All reagents functioned as agglutinins; consequently, saline agglutination tests were utilized in additional studies. Reagents used in these investigations have given consistent and clear-cut (+ + +, + + + +) results.

The symbols assigned to the three blood factors are A, B_1 and B_2: their frequencies in this population were, respectively, 64, 55 and 77 per cent. Five phenogroups, A_1, B_1B_2, AB_1B_2, AB_2 and B_2, were found in the sample of 612 mink tested, and each individual was identified as belonging to one or another of these groups. Pedigree analysis of this population suggests that the three blood factors are members of a single system, and are determined by alleles or closely linked genes. Investigations of inheritance will be presented in a report to follow.

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AGRICULTURE

Low-Cost Weighable Lysimeters

Glover and Forsgate[1] have recently proposed a method for weighing a soil-filled lysimeter by noting pressure variations in a water-filled bag on which the lysimeter tank rests. Lysimeters working on this principle have been operating here during the past year.

Field results obtained with four such replicate instruments are shown in Fig. 1. Soil moisture deficit estimated daily by a method based on that of Penman is included for comparison, and the response of the lysimeters to rainfall is clearly seen.

Glover and Forsgate mention the possibility of errors arising from ambient temperature fluctuations; but at Wellesbourne it has been found that, with the lysimeters buried so that their soil surfaces were at

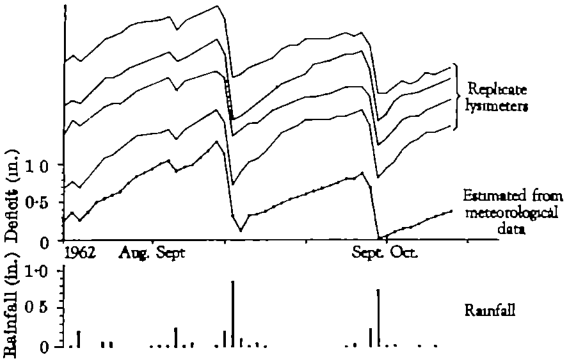

Fig. 1. Records from four replicate grassed pressure-weighing lysimeters in the open at Wellesbourne during 1962, compared with soil moisture deficit estimated from meteorological records and rainfall collected in a standard gauge. For clarity, the lysimeter records have been displaced vertically, but all the data shown are on the same scale

ground-level, natural air and soil temperature variations had a negligible effect on the manometer readings. It has not been found necessary to keep the manometer itself under thermostatic conditions as shown in the illustration accompanying the communication of these authors.

As a routine check on the correct functioning of each lysimeter, at the time when the daily reading is made, a special weight equivalent to 1/10 in. of water spread over the exposed lysimeter surface may be added temporarily. With our instruments this results in a vertical manometer deflexion of about 2/10 in.

A full description of these lysimeters is in the press[2].

The use of lysimeters for direct measurement of water use by plants growing in different soils under varying conditions of soil moisture stress has been limited in the past by the high cost of the necessarily large number of weighable instruments. The lost cost and absence of need for precision engineering which are features of the pressure-weighing equipment referred to here, will enable more detailed investigations of plant water use to be carried out than hitherto.

I wish to acknowledge the help of Miss Christine Down in building and maintaining these lysimeters, and in recording the results.

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SOIL SCIENCE

Effect of Freezing of Soil on Denitrification

A MAJOR difficulty in investigations of biological processes in soils is the preservation of the biological activity from the time of collection of the soil sample in the field until commencement of the laboratory investigation. Freezing of soil samples could feasibly be the means of preserving this biological activity since microbial growth ceases and enzymatic actions are retarded at low temperatures. Gasser[1] has suggested that the nitrifying activity in freshly sampled soil can be retained in this way. Since then, other workers have shown that when soils frozen at temperatures of −78° C and −196° C are thawed and incubated, increased nitrogen mineralization and aerobic respiration result[2,3].

Table 1. COMPARISON OF DENITRIFICATION WITH DIFFERENT FREEZING AND INCUBATION PRE-TREATMENTS

Treatment*		µg nitrogen/g soil Time (h) 36	 72	 108	 144
Initial sampling, 30/8/60	(i)	36	69	106	149
	(ii)	40	77	117	159
	Mean	38	73	111	154
(1) Frozen, −30° to −78° C	(i)	106	153	159	161
	(ii)	101	147	160	164
	Mean	104	150	160	[illegible]
(2) Frozen, −5° to 0° C	(i)	66	135	152	[illegible]
	(ii)	68	138	150	[illegible]
	Mean	67	137	151	[illegible]
(3) Frozen, 0° to 2° C	(i)	77	125	139	[illegible]
	(ii)	53	119	147	156
	Mean	65	122	143	150
(4) Incubated, 30° to 31° C	(i)	28	55	82	114
	(ii)	31	58	87	119
	Mean	30	56	85	117
(5) Air dried, 15° to 19° C	(i)	41	90	117	129
	(ii)	48	102	139	154
	Mean	45	96	128	142

* Nitrate nitrogen was added to give a level of 177 µg nitrogen/g soil to all treatments.

Present investigations of the denitrifying potential of soils utilized for wheat growing require freshly sampled soil from areas distant from our laboratories. The efficacy of various freezing treatments on the preservation of denitrifying activity in soil was therefore examined.

A black earth top soil (0–3 in., *p*H 6·3) was sampled from a wheat field adjacent to the laboratories. It contained 2·895 per cent organic carbon, 45·0 per cent moisture, and 2 p.p.m. nitrate nitrogen. The denitrifying activity was determined immediately after sampling, and the remainder of the sample was divided into five portions, which were placed in covered, but not airtight, containers; each of th portions was subjected to one of the following treatments.

(1) Frozen with acetone–solid carbon dioxide mixture and maintained in the range −78° C to −30° C.

(2) Frozen in a deep freeze cabinet and maintaine in the range −5° C to 0° C.

(3) Frozen in a refrigerator and maintained in th range 0° C to 2° C.

(4) Placed in an incubator and maintained in th range 30° C to 31° C.

(5) Air dried and maintained in the range 15° C t 19° C.

The stated temperatures were maintained from th date of sampling, August 30, 1960, until Septembe 27, 1960. Treatments (1)–(4) showed only a slight loss of water (1–3 per cent) while the content of wate in the air-dried sample fell to 11·4 per cent. After 28 days, duplicate 20-g samples of soil aggregates (2 3 mm) from each treatment were brought to saturation, and the denitrifying activity was determined by the manometric technique[4], using the production of nitrogenous gases from potassium nitrate substrate added to the anaerobic soil as a measure of this activity. Gas evolved from soil during 144 h incubation at 30° C has been calculated as micrograms of nitrogen per gram of oven dry soil (µg nitrogen/g) and results are given in Table 1. At the end of the incubation an average of 98 per cent of the added nitrate was recovered as gas (calculated as nitrogen) plus nitrite- and nitrate-nitrogen.

An analysis of variance of these results indicates that differences in means between treatments, between times, and treatment × time, are all significant at the 0·1 per cent level of probability.

All the freezing treatments increased the denitrifying activity as measured by gas production, so that neither quick freezing using dry ice nor freezing in the refrigerator (and maintenance of low temperatures) preserved the denitrifying activity found at the time of sampling the soil from the field. The lower the temperature maintained for the 28 days after the initial freezing of the soil, the greater the denitrifying activity on subsequent anaerobic incubation. Air drying also increased the denitrifying activity, an observation substantiated by other experiments not reported here.

Evolution of measurable quantities of nitrogenous gases began after the same length of time for all treatments as determined by the manometric technique; the rate of gas evolution also remained constant until the nitrate substrate was exhausted. These observations suggest that any changes in activity or numbers of the denitrifying organisms between treatments are relatively small. The increase in rate of denitrification following soil freezing therefore indicates that the denitrifying organisms are more able to utilize oxidizable substrate, and this could arise from a reduction in numbers of other micro-organisms resulting in an increased nutrient supply for surviving denitrifiers, or from an increase in the availability of the soil organic matter itself. An analogy can be drawn between these results for what is essentially anaerobic respiration in soils and those of Soulides and Allison[2], who found that the effects of freezing and drying of soils were to increase aerobic respiration.

Freezing and drying, therefore, cannot be recommended as a universal means of preserving the denitrifying activity of freshly collected soil. A corollary to this work is that the denitrifying activity of soils in the field, particularly surface soils, could well be expected to change seasonally due to natural freezing and drying. This latter aspect is at present being investigated.

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VETERINARY SCIENCE

A Reciprocal Relationship between the Urinary Excretion of Magnesium and Phosphorus in Wether Lambs

In a series of investigations[1] into the cause and prevention of ovine phosphatic urinary calculi, a reciprocal relationship between the urinary excretion of phosphorus and magnesium was observed in wether lambs. Also, lambs which developed visible uroliths in the bladder or kidney excreted more phosphorus and less magnesium than did the non-affected animals.

This pattern was found to hold regardless of the dietary treatment. While the magnitude of excretion changed from year to year and study to study, the decrease in urinary magnesium and the increase in urinary phosphorus in affected wethers were observed in all instances. Table 1 presents the average excretion results from our most recent test. The coefficient of correlation between the excretions of phosphorus and of magnesium was found to be negative and highly significant.

Table 1. Excretion of Magnesium and Phosphorus in 24 h Urine Samples

	No. of samples	Magnesium (mg/100 ml. urine)	Phosphorus (mg/100 ml. urine)
Diet *A*. Average daily intake: 13·8 g Ca, 3·8 g P, 2·9 g Mg (No calculi)			
First 28 days	7	84	2
Second 28 days	7	85	8
Third 28 days	4	64	8
Fourth 28 days	7	69	2
Diet *B*. Average daily intake. 6·7 g Ca, 8·6 g P, 4·9 g Mg (88 per cent calculi)			
First 28 days	6	133	5
Second 28 days	6	99	16
Third 28 days	5	124	16
Fourth 28 days	7	53	34
Diet *C*. Average daily intake. 6·0 g Ca, 5·0 g P, 4 4 g Mg (80 per cent calculi)			
First 28 days	6	31	29
Second 28 days	6	8	66
Third 28 days	3	7	67
Fourth 28 days	8	16	50

$\rho = -0{\cdot}798$, $P < 0{\cdot}01$ (Groups *A*, *B* and *C*). $\rho = -0{\cdot}919$, $P < 0{\cdot}01$ (Groups *B* and *C*). $\rho = -0{\cdot}962$, $P < 0{\cdot}01$ (Groups *A* and *C*).

A comparison of the excretory-levels in animals which developed calculi with those lambs not affected revealed a higher urinary phosphorus and a lower urinary magnesium excretion by affected lambs during the course of the 112–140 day feeding periods on a sorghum grain, cottonseed hull and meal, and molasses based ration. These averages of excretory levels, which were obtained from selected animals in each treatment group, could be used to forecast those treatments which would be calculogenic prior to the development of any serious number of clinical cases of urinary calculi.

The reciprocal relationship between phosphate and magnesium excretions by the kidney may mean that urinary calculi represent one end of a spectrum of metabolic interactions of minerals bounded at the other end by hypomagnesæmic tetany – grass tetany and related maladies.

The accumulation of magnesium in the blood sera of lambs affected with urinary calculi has been previously reported[2].

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PSYCHOLOGY

Relations between Excitatory and Inhibitory Retinal Responses as determined by a Revised Apparatus for the Study of Induced Negative After-Images

Induced negative after-images are of interest in that they reflect relations between inhibitory and excitatory retinal function. In 1897, Bidwell[1] reported that when a coloured, a white, and a black stimulus are repeatedly presented in that order for certain durations and at certain intensities, only the complement of the coloured stimulus is perceived. This effect facilitates certain after-image investigations as decisions as to reversal of colour are made more readily than are estimates of after-image duration or intensity. Suitable durations for coloured, white, and 'black' stimuli are 0·017, 0·10, 0·083 sec respectively; the matter of suitable brightness is the principal concern of this communication.

In 1950, Lehmann[2] reported that if the coloured stimulus is maintained at a lower level of illumination than is the white stimulus as the illumination of the system is varied there is a threshold below which only the colour of the stimulus and above which only its complement is perceived. Kaplan[3] also devised an apparatus in which the coloured stimulus was maintained at a fixed fraction of that of the white stimulus as both were varied. The equipment of both these investigators could determine only one point on a curve of brightness of coloured versus brightness of white stimulus at which reversal of perception took place; it seemed indicated to more completely determine this curve, and modifications of the apparatus of Lehmann and Kaplan made this possible. The observer views a coloured stimulus through an eyepiece with an aperture smaller than the meiotic human pupil so that changes in the diameter of the observer's pupil will not affect retinal illumination. An episcoster disk slotted for 30° to expose the coloured stimulus, white for 180° (this constitutes the white stimulus), and black for 150° (this constitutes relative absence of stimulus), rotates between the eyepiece and the coloured stimulus. The disk rotates at a speed of 5 r.p.s., which presents coloured, white, and 'black' stimuli for 0·017, 0·10, 0·083 sec, respectively. A diffuse light source illuminates the episcoster from considerably above and slightly in front (observer's side is front); a diaphragm between this source and the disk alters the illumination of the disk and, thus, the brightness of the white stimulus. The coloured stimulus, a section of opal glass trans-illuminated from the rear, is situated considerably behind the episcoster. Filters between its trans-illuminant and the opal glass determine the chromacity and intensity of the coloured stimulus. A horizontal partition extending from just above the episcoster does not shade the episcoster but effectively shades the coloured stimulus from the light incident on the episcoster. The brightness of the stimuli were calibrated by photo cells; differences in brightness are reliable and consistent. The system, as seen through an artificial pupil in the eyepiece, consists of a coloured spot intercepting 1½° of a 10° white field.

Fig. 1 demonstrates the curves reported by two observers experienced in the use of the apparatus when the brightness of a red stimulus (the light passing through 620 m.μ. interference filter) was set at the values indicated, and the brightness of a white stimulus (colour temp. 2,860° K) increased until green rather than red was perceived. Brightness is expressed in terms of millilamberts rather than in terms of trolands as the latter would depend on details of construction of the apparatus, particularly that of the eyepiece. It is expected that if another apparatus were constructed curves of similar slope and shape but differing in absolute values would be determined. Curves of like configuration were reported by 8 of 10 observers; the absolute values of points on the curve vary among observers and for the same observer over time. Immediately consecutive determinations of points on the curve or of the curve by the same observer are consistent. At and below the lower end of the curve decisions are complicated by transitional colours and whites; at and above the upper end of the curve, colour and complement are alternately perceived. The limits at which reversal is perceived vary among observers. Similar results are obtained using stimuli of other colours but colour reversal is most clear with long wave-length stimuli.

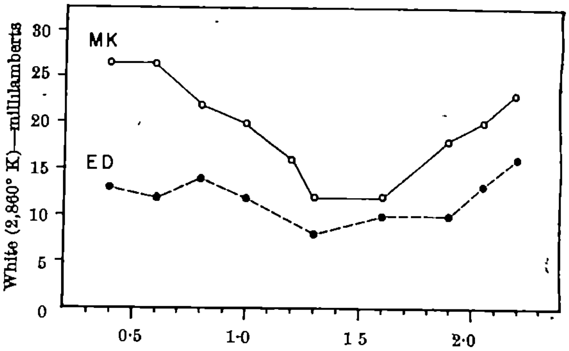

Fig. 1. Brightness of red and white stimuli at which, when the brightness of the red stimulus is held constant and that of the white stimulus increased, green rather than red is first perceived. (Red, white and 'black' stimuli presented repeatedly in that order for 0 017, 0·10, 0·083 sec respectively)

When the brightness of white is decreased until the colour of the stimulus rather than its complement is reported reversal occurs at lower brightness-levels of white. Colour reversal can be observed when the brightness of the white stimulus is held constant and the brightness of the coloured stimulus altered, but the transition is slow and endpoints are uncertain. (Reversal occurs at virtually the same brightness of the white stimulus at a wide range of brightness values of the coloured stimulus.)

That an induced negative after-image of a stimulus can suppress perception of the stimulus demonstrates that under certain conditions a stimulus can have greater inhibitory than excitatory effect. Reversal can be observed, in the conditions given, at a limited range of brightness of the white stimulus. This, as well as the fact that the reversal is perceived at different brightness levels of white when white is increased than when white is decreased, suggests that light adaptation, as determined by the brightness of the white light, plays a part in the phenomenon. The existence of ascending and descending limbs indicates that in this range of illumination, definitely photopic, there is not a constant relation between inhibitory and excitatory response as the brightness of the stimulus varies. This effect, besides being of theoretical interest, may be of use as a parameter of the relation between inhibitory and excitatory retinal function in a variety of physiological and pharmacological circumstances.

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[1] Bidwell, S., *Proc. Roy. Soc.*, **61**, 263 (1897).
[2] Lehmann, H. E., *Science*, **112**, 199 (1950).
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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Wednesday, January 2

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 10 a.m.—*Symposium on "Masers and Lasers"*.

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. A. J. Sutcliffe: "Caves—their History and Exploration" (Dr. Mann Juvenile Lecture).

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. I. R. H. Crail, Mr. J. S. Elliott, Mr. D. C. Pearman and Mr. G. H. J. Simmons: "The Effect of Polymeric Dispersants on Engine Sludge".

Thursday, January 3

Institute of Refrigeration (at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, *London*, S.E.1), at 5.30 p.m.—Dr. F. L. Levy: "A Review of Methods of Refrigerating Road Vehicles".

Institute of Metals (at 17 Belgrave Square, London, S.W.1), at 7 p.m.—Dr. J. S. Clarke. "Why Cannot Metallurgists Provide the Materials Required by Engineers?".

Friday, January 4

British Mycological Society (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 11 a.m.—General Papers.

Plastics Institute (at the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Mr. J. J. P. Staudinger: "Plastics in the World Today" (Young People's Lecture).

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. M. G. J. Beets. "Odour and Molecular Constitution".

Saturday, January 5

London County Council (at the Horniman Museum, London Road, Forest Hill, *London*, S.E.23), at 3.30 p.m.—Mr. Geoffrey Kinns: "Photographing Wild Life".*

Monday, January 7

British Institution of Radio Engineers, Research Committee (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 2 p.m.—Symposium on "Radio and Electronics Research in Great Britain".

University College, London (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Prof. Harland G. Wood (U.S.A.): "The Problems of Determining Pathways of Carbohydrate Metabolism Using Labelled Glucose". *(Further lecture on January 10.)**

Institution of Mechanical Engineers, Steam Plant Group (at Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Testing of Modern Large Steam Generating Plant".

Royal Institute of Chemistry, London Section (joint meeting with the London Section of the Society of Chemical Industry, at the Society of Chemical Industry, 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. Alexander King: "Scientific Research in Europe in Relation to Economic Development".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned·

Head (with a higher degree, and substantial teaching and industrial experience) of the Department of Electrical Engineering (Grade IV), Enfield College of Technology—The Chief Education Officer (F.3), 10 Great George Street, Westminster, London, S.W.1 (January 4).

Teaching Fellow (with an honours degree or equivalent qualifications) in the School of Chemistry, University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W.C.2; and the Appointments Section, The University of New South Wales, P.O. Box 1, Kensington, New South Wales, Australia (January 11).

Biologist (pre- or post-doctoral appointment) to assist in Chromosomal Research *with a leaning to leukaemia—The Professor of* Haematology, King's College Hospital Medical School, Denmark Hill, London, S.E.5 (January 12).

Lecturer (with interests in (a) the instrumental methods for control of semi-technical or pilot-plant processes and equipment and/or (b) *modern methods of analytical chemistry, particularly instrumental* methods) in Chemistry—The Registrar, The Manchester College of Science and Technology, Manchester, 1 (January 12).

Chair of Clinical Chemistry—The Secretary, The University, Edinburgh (January 14).

Research Assistant (*with qualifications in mathematics, physics* or chemistry) for Electronic Computing in the Department of Inorganic and Structural Chemistry and in the Electronic Computing Laboratory, to help in the development of programmes for crystal structure analysis on the KDF9 computer—Dr. M. R. Truter, School of Chemistry, The University, Leeds, 2 (January 15).

Senior Lecturer or Lecturer in Oceanography (Biological or Physical) at the University of Natal, South Africa—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, January 15).

Curator or Assistant Curator (honours graduate) of Fishes at the Australian Museum, Sydney, New South Wales—The Agent General for New South Wales, 56 Strand, London, W.C.2 (January 18).

Assistant Experimental Officer (preferably with a Higher National Certificate or a degree in a biological subject) for research in the Nematology Section—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (January 19).

Physical or Organic Chemist (*with a university degree or a* diploma or equivalent qualifications with physical chemistry or organic chemistry as a major subject, together with some years of appropriate chemical experience, preferably involving instrument methods) in the Division of Coal Research, Commonwealth Scientific and Industrial Research Organization, North Ryde, *New South Wales, Australia, to take part* in investigations into the development of physical methods of organic analysis, particularly in the field of gas chromatography—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 480/422 (January 19).

Scientific Officer or Senior Scientific Officer (preferably with an honours degree in chemistry or biochemistry, or a biologist with sound experience of pesticides research) for research in the Crop Husbandry Department—The Secretary Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex (January 26).

Lecturer in Pharmaceutical Chemistry (Physical Chemistry) at *the University of Sydney, Australia—The Secretary,* Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, January 29).

Chair of Philosophy in the University of the Witwatersrand, *Johannesburg, South Africa—The Secretary, Association of Universities* of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, January 31).

Computation Director (with experience of electronic computation) to organize and run a small computation laboratory, operating in conjunction with a quantum theory group—Dr. R. McWeeny, The University, Keele, Staffs (January 31).

Lecturer in Chemistry at Fourah Bay College (The University College of Sierra Leone)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 31).

Research Assistant (with an honours degree in chemistry) in the Department of Oceanography *to assist in research in chemical* oceanography—The Registrar, The University, Liverpool, quoting Ref. No. CV/225/N (January 31).

British Nylon Spinners Postdoctoral Fellowship in Physics—The Registrar, The University, Bristol 8 (February 1).

Lecturer in Biochemistry in the Department of Physiological Chemistry—The Registrar (Room 22, O.R.B.), The University, Reading (February 1).

Chair of Agricultural Economics at Monash University, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or The Registrar, Monash University, P.O. Box 92, Clayton, Victoria, Australia (Australia and London, February 4).

Official Fellow and Tutor in Philosophy—The President, Corpus Christi College, Oxford (February 9).

Veterinary Parasitologist (with a degree in veterinary science or equivalent qualifications, and some years of appropriate post-*graduate research training)* in the McMaster Animal Health Laboratory, Commonwealth Scientific and Industrial Research Organization, Sydney, to participate in specific aspects of the programme of research concerned with the epidemiology and control of helminth infections of sheep with particular reference to infections with *Ostertagia* sp. in the first instance—*Chief Scientific Liaison Officer,* Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 202/200 (February 9).

Chair of Chemistry; Chair of Zoology; and Chair of Botany at Fourah Bay College (The University College of Sierra Leone)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 15).

Official Fellow and Tutor in Pure Mathematics—The Master, St. Catherine's College, Oxford (February 15).

Official Fellow in Mathematics (pure or applied)—The Principal, Hertford College, Oxford (February 19).

Assistant Physicist (with a good honours degree in physics) in the Regional Medical Physics Department of the Sheffield Regional Hospital Board—The Secretary, Sheffield Regional Hospital Board, "Broom Cross", Tree Root Walk, Sheffield 10.

Biochemist (Basic grade)—The Superintendent, The Children's Hospital, Western Bank, Sheffield 10.

Experimental Officer (male, graduate in horticulture, botany *or forestry, keenly interested in the scientific aspects of practical* fruit culture and in people, and preferably some knowledge of languages) in the Scientific Liaison Section—The Secretary, East Malling Research Station, Maidstone, Kent.

Lecturer in the Department of Medical Microbiology at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Lecturer (with an interest in physical metallurgy) in Chemical Engineering at the University of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

Research Assistant (with an honours degree in zoology), for work on the reticuloendothelial system of lower vertebrates—Prof. G. E. H. Foxon, Biology Department, Guy's Hospital Medical School, London Bridge, S.E.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1050, Vol. 255, (25 October, 1962): Studies of the Jahn-Teller Effect. 4. The Vibrational Spectra of Spin-Degenerate Molecules. By M. S. Child. Pp. 31–53. 7*s.* 6*d.* No. 1051, Vol. 255 (25 October, 1962): The Band Structure and Fermi Surface of Magnesium. By L. M. Falicov. Pp. 55–83. 9*s.* (London: The Royal Society, 1962). [8010

The Future of Optical Astronomy. By Prof. D. E. Blackwell. (An Inaugural Lecture delivered before the University of Oxford, 12 June 1962.) Pp. 19. (Oxford: Clarendon Press; London: Oxford University Press, 1962.) 3*s.* 6*d.* net. [111

Department of Scientific and Industrial Research. Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the year 1961, with Report of the Geological Survey Board. Pp. iii+83. (London H.M. Stationery Office, 1962.) 5*s.* 6*d.* net. [111

Department of Technical Co-operation. Technical Co-operation under the Colombo Plan: Report for 1961–62 of the Council for Technical Co-operation in South and South-East Asia. Pp. 92. (London: H.M. Stationery Office, 1962). 5*s.* net. [111

The Tropical World. By Prof. D. L Linton. (An Inaugural Lecture delivered in the University of Birmingham on 9th May 1961). Pp. 18 (Birmingham: The University, 1962.) 2*s.* 6*d.* [711

The Chest and Heart Association. Annual Report 1961–1962. Pp. 8 (London: The Chest and Heart Association, 1962.) [711

Ciba (A.R.L.), Ltd. Technical Notes, No 238 (October, 1962): Mouldings from Wood Chips and Aerolite. Pp. 11. (Duxford: Ciba (A.R.L.), Ltd., 1962.) [711

British Broadcasting Corporation. BBC Engineering Monograph No. 44: A Band V Signal-Frequency Unit and a Correlation Detector for a VHF/UHF Field-Strength Recording Receiver. Part 1: A Band V Signal-Frequency Unit By Dr. C J. Phillips and P. T. W Vance. Part 2: A Correlation Detector. By R. V. Harvey Pp 24. (London: British Broadcasting Corporation, 1962) 5*s.* [711

International Association for the Exchange of Students for Technical Experience. Report of IAESTE in the United Kingdom for year ending September 1962. Pp. 18. (London: International Association for the Exchange of Students for Technical Experience, 1962). [711

British Education. By H. C. Dent New Edition. Pp. v+70+17 plates. (London: Longmans, Green and Co., Ltd., 1962.) 2*s.* 6*d.* [711

Association of Universities of the British Commonwealth. United Kingdom Postgraduate Awards, 1962–64: Fellowships, Scholarships, Grants, etc. tenable at Universities in the United Kingdom; Short List of Awards tenable outside the United Kingdom. Pp. 149. (London: Association of Universities of the British Commonwealth, 1962.) 5*s.* [711

Cement and Concrete Association. An ABC of Prestressed Concrete. By M. R. Hollington. Pp 56. (London: Cement and Concrete Association, 1962.) [711

Scottish Marine Biological Association. Annual Report, 1961–62. Pp 37. (Millport, Isle of Cumbrae: Scottish Marine Biological Association, 1962.) 4*s.* [711

National Union of Teachers. Revalue the Teacher! Pp. 16. (London: National Union of Teachers, 1962.) [911

Privy Council. Medical Research Council Special Report Series No. 302. Tables of Representative Values of Foods Commonly Used in Tropical Countries. By B. S. Platt (Revised edition of Special Report, No. 253.) Pp v+46. (London: H.M. Stationery Office, 1962.) 7*s.* net. [911

The Royal Society. British National Committee on Space Research. United Kingdom Report for 1961 to the the I.C S.U. Committee on Space Research, submitted to the Fifth Meeting of Cospar, Washington, 30 April—9 May, 1962. Pp. 23. (London The Royal Society, 1962.) [911

Minerva: a Review of Science, Learning and Policy, Vol. 1, No. 1, (Autumn 1962). Edited by Prof. Edward Shils. Pp. 1–144. Published under the auspices of the Committee on Science and Freedom Subscription rate: 20*s.* or 5 dollars a year. Single issues 5*s.* (London: C.F.S. Publications, Ltd., 1962.) [911

The Health of the School Child: Report of the Chief Medical Officer of the Ministry of Education for the years 1960 and 1961. Pp. xi+248+14 photographs. (London: H.M. Stationery Office, 1962.) 15*s.* net. [911

Meteorological Office. Scientific Paper No. 16: An Experiment in Operational Numerical Weather Prediction. By E. Knighting, G A. Corby and P. R. Rowntree. Pp. iii+28. (London: H.M. Stationery Office, 1962.) 3*s.* net. [911

Building Research Station Digests, (New Series), No. 28. Factory Building Studies. Pp. 6. (London: H.M. Stationery Office, 1962) 4*d.* [911

The Chester Beatty Research Institute Serial Abridged Life Tables. England and Wales, 1841–1960. Part 1: Tables, Preface and Notes. Compiled by R. A. M. Case, Christine Coghill, Joyce L. Harley and Joan T. Pearson. Pp. xxvi+87. (London: The Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, 1962) [911

University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1960–1961. (Cmnd. 1855). Pp xxiv+48. (London. H.M. Stationery Office, 1962.) 5*s.* 6*d.* net. [1411

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